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AGING

1 DAILY HIGH-FREQUENCY STIMULATION DIFFERENTIALLY PROLONGS SYNAPTIC ENHANCEMENT IN MIDDLE-AGED AND SENESCENT RAT HIPPOCAMPUS.
C. A. Barnes and B. L. McNaughton. Dept. Psych., Dalhousie Univ., Halifax, Nova Scotia, CANADA, B3H 4J1.

The perforant path-granule cell synapse in the hippocampus has been shown to undergo an enduring enhancement of its efficacy after brief high-frequency bursts of stimulation (Bliss & Lømo, JP, 1973, 232, 331-356). Barnes (JCPP, 1979, 93, 74-104) found that the magnitude and decay time constant of enhancement following a single stimulus session were equivalent in middle-aged and old rats. The decay time constant, however, was extended from 4 to 29 days in the young animals by repetition of the enhancing stimulus at 24 hour intervals for three days. The time constant for the old rats failed to show an increase. In the present study we sought to determine whether the decay time constant of enhancement in old rats could ultimately be extended given a sufficient number of repetitions of the enhancing stimulus. A secondary goal was to determine whether the time constant in young animals tended towards some asymptote or whether it was extended indefinately with further repetition.

Thirty-six middle-aged (10 mo) and 36 senescent (29 mo) male Long Evans rats were bilaterally implanted with electrodes for chronic stimulation and extracellular recording of perforant path granule cell synaptic response as described in Barnes (1979). Animals with electrodes that failed to remain stable for the full period of observation were eliminated from the final data analysis. The stimulus current was set just above the threshold for granule cell discharge and was kept constant throughout the experiment. After baseline control responses were obtained, 12 high-frequency stimulation sessions (15, 20 msec bursts of 400 Hz stimuli) were given at 24 hour intervals. The response amplitude was measured prior to and following each high-frequency session, and at daily intervals for three weeks after the last (12th) session.

Synaptic enhancement reached an asymptotic value of approximately 60% in both age groups. The decay time constants reached maximal values of 37 and 17 days for the young and old groups respectively. The rate of growth of the time constant was a decreasing function of the number of stimulus repetitions.

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

2 DIFFERENCES IN RETENTION OF INHIBITORY AVOIDANCE TASK AND BRAIN BIOCHEMISTRY IN MIDDLE-AGED MICE: EFFECTS OF CHRONIC CHOLINE-ENRICHED AND CHOLINE-DEFICIENT DIETS.
R. T. Bartus, J. Coupet*, R. L. Dean*, J. A. Goas, V. A. Szucs-Myers* and C. E. Rauh*, American Cyanamid Company, Med. Res. Div., Pearl River, NY 10965.

Considerable research indicates that cholinergic mechanisms play an important role in the expression of learning and memory. More recent evidence suggests that alterations in some of these same cholinergic mechanisms may be critically involved in the memory declines observed in aged human and non-human mammals. At the same time, it has been demonstrated that systemic administration, or dietary manipulation, of choline, the acetylcholine precursor, can significantly influence cholinergic function. For example, there have been reports of significant changes in levels or amount of brain choline, acetylcholine, choline acetyltransferase and nicotinic receptor binding following choline administration to young rodents and humans. These data collectively suggest that it might be possible to alter the development of age-related cognitive impairments by manipulating cholinergic function through proper precursor control.

The following study addressed this question directly by placing retired breeder C56Bl/6j mice (8.5 months old at the start of this study) on purified diets which were either deficient (0.5 - 1.0 mg/gm feed) or enriched (15.0 mg/gm) with free choline. The diets were given ad lib for a period of 4.5 months, after which time the mice were tested for effects on psychomotor coordination, closed field activity and retention of an inhibitory avoidance task (at 13 months old). All animals were then sacrificed and biochemical assays performed to assess possible differences in choline levels, as well as changes in receptor binding and enzymatic levels in the central cholinergic and dopaminergic systems.

Although little or no differences were observed on psychomotor coordination or general activity, a striking effect was observed on retention of the inhibitory avoidance task. In fact, mice fed the choline-enriched diet performed as well as young, 3 month old mice, whereas mice fed the choline-deficient diet performed as poorly as senescent mice (23 months and older). Preliminary biochemical analyses of the mouse brains demonstrated that significant differences in specific pre- and post-synaptic markers existed between the choline-enriched and choline-deficient groups. More detailed results will be presented. These data demonstrate that chronic manipulation of the cholinergic system, via precusor control, can produce significant changes in brain and behavior in middle-aged mice. Further, these changes are similar to those which occur naturally with old age, and suggest that certain debilitating effects of age may, therefore, be modulated through appropriate precusor control.

3 SHORT-TERM MEMORY AND AGING: LOCALIZATION OF CELLULAR CHANGES WITHIN MULTIMODAL SENSORY REGIONS OF THE CEREBRAL CORTEX AND LIMBIC SYSTEM IN THE RHESUS MONKEY.
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According to recent studies, some of the more prominent manifestations of aging include short-term memory impairment, cell loss, and lipofuscin pigment accumulation in the brain. Although nonhuman primates are of the same taxonomic order as man, they have not been used extensively for studies of age-related short-term memory impairments in relation to cellular changes in specific neuronal circuits that may be involved in the mediation of short-term memory within limbic and cerebral cortical centers. Using an automated delayed response test, and 0, 15, and 30 second retention of information intervals, significant impairments were observed in short-term visual memory at 15 and 30 second delays in aged (18-24 years) compared to young (3-5 years) rhesus monkeys ($p<.01$). Although declines in sensory function and age-dependent cellular changes, particularly loss of cells, dendritic spines, and lipofuscin age pigment accumulation, have been observed in modality-specific sensory pathways, considerable interest has been focused recently on age-related cellular changes in such convergent multimodal sensory regions as the entorhinal cortex, the hippocampus, thalamus, and frontal cortex in relation to the acquisition, storage, and retrieval of multimodal information designated as short-term memory. The aims of this study were to compare age differences in neuron numbers, in two of the major convergent multimodal sensory centers, the hippocampus of the limbic system and the gyrus principalis of the frontal cerebral cortex between young adult and aged short-term memory impaired rhesus monkeys. Morphometric studies in the lamina pyramidale of the hippocampus revealed that the mean number of neurons in the CA-1 zone, per transverse section, was significantly lower ($p<.01$) in aged animals than in young adults. The total dorsoventral depth of the hippocampal strata, in the CA-1 zone was also significantly less ($p<.05$) in aged than in young adult monkeys. In the gyrus principalis the mean cortical depth was significantly less in aged than in young adult animals ($p<.05$). The mean number of neurons in "core" samples of cerebral cortex, extending from the pia mater to the deepest part of lamina VI, was also significantly less ($p<.02$) in the old than in young adults. These studies indicate that significant age-dependent cellular changes occur in multimodal sensory pathways involved in the mediation of short-term memory. Supported by NIH Grant RR00164 and a grant from Lederle-American Cyanamid Corp.

4 AGE-RELATED CHANGES IN PAVLOVIAN CONDITIONING IN RABBITS: CNS CORRELATES.
Shirley L. Buchanan*, Linda Hernandez, and D. A. Powell, VA Medical Center, Columbia, S. C. and N.S. Shah, William S. Hall Psychiatric Institute, Columbia, S. C.

"Old" (mean age = 40 mos.) and "young" (mean age = 6 mos.) New Zealand albino rabbits were exposed to Pavlovian conditioning training in which corneoretinal potential (CRP), heart rate (HR), and electromyographic (EMG) CRs were assessed. Three experiments were performed in which 75 db tones served as CSs and a 300 msec., 3 ma paraorbital electric shock served as the US. Two experiments involved simple conditioning in which a .5 sec. 1216 Hz tone served as the CS. A third experiment involved differential conditioning in which 1 sec. 304 and 1216 Hz tones were CSs. At the end of conditioning, (a) free field activity, (b) paraorbital shock thresholds, and (c) HR URs were determined in selected animals. Regional CNS concentrations of norepinephrine (NE), serotonin (5HT) and dopamine (DA) were also determined in selected animals. DA receptor binding in the caudate nucleus was also studied in selected "old" and "young" Ss.

In all three experiments, CRP CR frequency was decreased and the rate of CRP acquisition impaired in the "old" Ss. However, CRP discrimination was unaffected by age. Data from two experiments suggest that these deficits were more pronounced in males than in females. HR CR magnitude was enhanced in old as compared to young Ss in all three experiments, although the HR orienting response to unreinforced tones was smaller in the old Ss. Sex differences again appeared, however; females revealed faster baseline HR and smaller bradycardiac CRs than males regardless of age. Free field activity, paraorbital shock thresholds, and HR URs in response to shock were unrelated to either age or sex. Regional differences in CNS monoamine distribution were, however, related to both age and sex in several instances. Moreover, hippocampal and cortical NE and 5HT concentrations were correlated with HR CR magnitude. NE concentration was directly related to HR CR magnitude; 5 HT concentration was inversely related to HR CR magnitude. Striatal DA concentration was also decreased in old as compared to young males, and decreased DA receptor density was also observed in the old animals. Previous experiments suggested that the striatum was related to Pavlovian CRP conditioning (D. A. Powell, et al., Physiology & Behavior, 1978, 20, 143-150). Thus age-related presynaptic and/or post-synaptic neostriatal changes may underlie the Pavlovian CRP conditioning deficits obtained in the old animals.

*Indicates nonmember of the Society for Neuroscience.

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

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

AGE IN DAYS	1	4	10	23	38	61	200
Pur. Cells	1706	1493	3036	----	3398	4558	5606
Nuc. Glom.	2199	2447	2886	4651	4620	7017	9153
D.R.G. Cells	330	372	667	670	433	910	1258
V.H. Cells	966	980	921	----	1090	1375	1640

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

6 EFFECTS OF ENVIRONMENT AND SOCIAL INTERACTION BETWEEN YOUNG AND OLD RATS ON VISUAL CORTEX AND DENDRITIC BRANCHING. James R. Connor, Marian C. Diamond, and Ruth Johnson*. Department of Physiology-Anatomy, UC Berkeley, Berkeley, CA 94720.

The present study was designed to investigate whether the interaction between young and old rats in a specific environment influences the cortex and cortical structures. Seven 600 day old Long Evans male rats were separated into 2 conditions: 4 into an "enriched" environment (EC) and 3 remained together in a standard colony cage (SC). Eight 60 day old rats were placed in the enriched cage with the 4 old rats. Nine 60 day old rats were housed 3 to a standard colony cage. All animals were given access to food and water ad lib. The Mann-Whitney U and Students-t tests were used to analyze the data.

After 30 days, the brains were removed and placed in Golgi-Cox fixative. Dendritic branching was investigated on basal branches of pyramidal cells in layers II and III. Alternating sections were counterstained with thionine for determining cortical depth. The cortical thickness did not differ significantly between the 2 conditions when observed within the same age group. In compering between age groups (SC), the young animals had significantly thicker cortices in all areas but one (areas 18, 18a; $p < .01$, area 17; $p < .001$; area 39, NS). In the EC the significant difference between the age groups decreased in each case. All areas of the old rats' visual cortex showed some increase in the EC whereas in the young rats, 3 of the 4 areas increased (18, 18a, 17) while one (area 39) decreased.

Investigation of dendritic branching in 600 day old animals showed a near significant increase of 9% in the number of dendritic branches in the EC ($p = .057$, $\alpha = .05$). Although insignificant, the branching order of dendrites showed a tendency to be higher in the EC in all but one order (4°). The fifth order was most nearly significant ($p = .057$, $\alpha = .05$). The 60 day old animals did not differ significantly in either of the 2 conditions. The 60 day old animals in the SC had 9% more branching segments than the 600 day old animals (NS). When the enriched animals were compared, the 60 day old animals had 3.2% fewer branching segments than the 600 day old animals (NS).

The inability of the enriched environment plus old animals to increase the cortical thickness in young animals is puzzling. In previous investigations, using animals the same age, the visual cortex has increased in depth with enrichment. Possibly the tendency for old rats to show increased responsivity to the enriched environment is due to the loss of an inherent inhibitory regulatory system for dendritic growth. Thus, environmental stimuli may result in a random increase of dendritic projections. This may be related to the "lawless" growth reported by Scheibel & Tomiyasu (1978) in Alzheimer's disease.

7 SYNTHESIS OF MONOAMINE OXIDASE IN MICE AS RELATED TO AGING. Lucien J. Côté, Leon T. Kremzner,* and Angelika Huebner,* Departments of Rehabilitation Medicine and Neurology, College of Physicians and Surgeons, Columbia University, NY, NY 10032.

The rate of synthesis of both A and B forms of monoamine oxidase (MAO) was studied in mice as related to age, using pargyline as an irreversible inhibitor of the enzyme. The return of MAO activity after pargyline treatment depends solely on the de novo synthesis of the enzyme, providing a way to assess the rate of synthesis of MAO. Three groups of mice (Jackson C57BL/6J), 70, 416, and 730 days old were given a single dose of pargyline (50 mg/kg) i.p. and MAO-A and MAO-B activities were assayed at 1, 2, 5, 9, 15, and 26 days after treatment. Five to eight mice were used for each point. Non-treated mice of comparable age served as controls. MAO-A and MAO-B activities were 85% and 95% inhibited, respectively, at one day after treatment. There was a gradual return of activities; the pattern was similar for both forms of the enzyme. At nine days about 40% of the activity had returned. At 26 days after pargyline treatment, 75% of the basal activity had returned. There was no significant difference in the rate of recovery of activities, and thus synthesis, between young, middle age, and old mice. Our findings indicate that old mice can synthesize both forms of MAO at the same rate as young mice.

Studies of both acetylcholinesterase and nonspecific cholinesterase activities indicate that there is no apparent difference in the rate of return of enzyme activity in young versus old mice. However, studies with ornithine decarboxylase and S-adenosyl methionine decarboxylase, enzymes with extremely short half-lives (15-30 minutes), indicate that relative to young animals, old animals show a delay in the initiation of enzyme synthesis.

(Supported by NIH Grant AG00276.)

8 COMPARATIVE EFFECTS OF PROTEIN SYNTHESIS INHIBITION ON LEARNING AND MEMORY IN SENESCENT AND MATURE MICE. Dennis D. Crady* and Elton E. Quinton. Dept. Psych., Univ. of Louisville, Louisville, Kentucky 40208.

The processes of learning and memory are believed to be dependent upon protein synthesis. Senescence has been hypothesized to result from a chronological decrement in effeciency of protein synthesis and repair. If these theories are true one might expect to see a decrement in learning and memory abilities in senescent animals as well as an exaggerated impairment following protein synthesis inhibition. This study examined this possible interaction between protein synthesis inhibition, learning and memory processes, and aging.

Three age groups of C57Bl/6J mice (7-10 months, 28-31 months, and 35-36 months) were trained on a simultaneous five-choice visual discrimination active avoidance task. Animals were given 10 consecutive trials per session, with 10 daily sessions. Anisomycin (5 mg./kg.) or water was administered 5 minutes prior to the start of each daily session. Animals completed the session within 25 minutes of the drug administration.

All groups demonstrated a high degree of acquisition over the 10 sessions. The performance of the mature control group was superior to that of the senescent control group. Anisomycin significantly impaired the performance of the mature mice, but had no apparent effect on the performance of the senescent mice (compared with the control group).

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

9 CHANGES IN BEHAVIOR AND RECEPTOR BINDING IN AGED VS. YOUNG MICE. R. L. Dean*, J. A. Goas*, A. S. Lippa*, R. T. Bartus (SPON: R. Schoenfeld), American Cyanamid Co., Medical Research Division, Pearl River, NY 10965; and, R. Pelham* and J. K. Marquis*, Tufts-New England Medical Center, Dept. of Neurology and Biochemistry, Boston, MA 02111.

Interest in defining the behavioral impairments associated with age, and the biochemical sources of these impairments, has accelerated rapidly in recent years. It is clear from the data accumulated that although age-related deficits in performance do occur in human and non-human mammals, not all types of behaviors are impaired. Similarly, although many different biochemical changes have been reported, it remains unclear which changes are primarily responsible for the behavioral impairments. Because of the differences in the behaviors measured, biochemical assays performed and neurological regions evaluated, comparisons across studies have been difficult, while comparisons across species have been next to impossible. Yet, the ability to make comparsions such as these would seem to be an important pre-requisite for the development of effective animal models of aging.

One approach that might be useful would involve a comprehensive evaluation of the behavioral and biochemical changes occurring within the adult lifespan of a single species. For this reason, we tested four age groups of C57Bl/6J mice (3, 9, 23 and 31 months old) on a number of procedures. Highly reliable age-related behavioral differences were observed on retention of an inhibitory avoidance task. Smaller, but still significant, differences in degree of exploratory behavior (closed field activity) and behavioral rigidity (position reversal learning) were also observed. In contrast, no age-related differences were observed in tests intended to measure tonic reflexes, pain threshold and acquisition and retention of a simple position habit.

Following behavioral testing, all mice were sacrificed and their brains removed and frozen for receptor binding studies. Each brain was dissected into the medulla, pons, hippocampus, diencephalon, striatum and cortex. Binding of ^{3}H-quinuclidinyl benzilate (^{3}H-QNB) to muscarinic cholinergic receptors, of ^{125} I-alpha bungarotoxin (^{125} I-BTX) to nicotinic cholinergic receptors, of ^{3}H-strychnine to glycine receptors and ^{3}H-haloperidol to dopamine receptors was performed. Several age-specific, regional variations in receptor binding were observed. Most impressive among these were a decrease in strychnine binding in the medulla, an apparent increase in haloperidol binding in the striatum and a more general decrease in muscarinic receptor binding in multiple subcortical areas. These data will be discussed as they relate to possible neurochemical etiologies for the behavioral impairments observed with advancing age.

10 CONTINUING GROWTH IN THE AVIAN CILIARY GANGLION.A QUANTITATIVE LIGHT AND ELECTRON MICROSCOPIC STUDY. Mario G. Fiori* and Enrico Mugnaini. Lab. of Neuromorphology, Dept. Biobehavioral Sciences, Univ. of Connecticut, Storrs, CT 06268

Previous studies pointed out that synaptic contact between preganglionic oculomotor fiber and ciliary neuron in the chicken ciliary ganglion undergoes several changes during first 18 months of life. In particular, at the late embryonic stages and in the young chick, the fiber presents a large calyciform nerve ending embracing one pole of the egg-shaped neuron; during growth of the animal, the size of the ciliary neurons increases somewhat and the calyx is changed into a cup of closely apposed boutons. The present study was undertaken to ascertain whether this peculiar synapse shows additional changes during aging. In adult chickens 6-8 months old, an increasing number of ciliary neurons sprout a few short dendrites which intrude among the boutons; at the same time the perikaryon tends to flatten at the synaptic pole, so that the postsynaptic surface enlarges. On its turn, the preganglionic fiber forms an increasing number of synaptic, terminal and preterminal branches, some of which invaginate more or less deeply into the ciliary neuron. The number of neurons displaying invaginations increase with age, reaching the maximum at about 2 years (25 to 28% of ciliary neurons are invaginated at this time) and then decreasing slowly but progressively: in 7-year-old chickens only 9-10% of the ciliary neurons have invaginations.

Moreover, neurochemical studies (Giacobini et al., abstract, this meeting) indicate that ACh content in the ciliary ganglion increases during adulthood reaching a maximum at about 2 years of age and then decreasing progressively up to 7 years. Since in the chicken the body weight continues to increase beyond the first six months of age and, on the other hand, morphological data indicate regressive changes in the eyes of old birds, it is presumed that the changes at the ciliary ganglion cell accompany changes in the target tissue (sphincter iridis and ciliary body).

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11 EFFECT OF DIETARY RESTRICTION ON LIPOFUSCIN ACCUMULATION IN RAT CEREBELLAR PURKINJE CELLS. Wm. B. Forbes. Worcester Fndn. for Exptl. Biol., Shrewsbury, MA 01545.

Severe dietary restriction throughout adult life has been reported to significantly enhance the longevity of rodents in the laboratory. In order to evaluate the effect of longevity-promoting dietary regimens on aging-related changes in rat brain, lipofuscin accumulation in cerebellar purkinje cells was measured in three dietary treatment groups: rats reared in small litters (8 pups) and fed rat chow ad lib following weaning at 21 days of age (group HH); rats reared in large litters (16 pups) and fed ad lib following weaning (group LH); and rats reared in small litters and fed restricted amounts of chow throughout post-weaning life (group HL). Group LH exhibited a permanent body weight deficit in comparison with group HH of about 10%. Mean body weight of group HL was held at 200 gm. To date, group survivorship curves are consistent with the expectation that the HL animals will exhibit a mean life-span of about 150% that of group HH, while group LH will exhibit only a small increase in life-span.

At 220 days of age, 10 animals of each group were sacrificed for lipofuscin determination. In each animal, 20 individual purkinje cells in the cerebellar vermis were studied at 1560X magnification using unstained paraffin sections 6 μ thick. Lipofuscin volume within the perikaryon was estimated by counting the number of intersections of a grid reticle which overlay autofluorescent lipofuscin granules in a single plane of focus. Perikaryal volume was estimated in a similar manner using phase contrast elements. In comparison with group HH, groups HL and LH exhibited significantly less lipofuscin/cell ($t < .05$, one-tailed). However, group HL exhibited slightly and group LH significantly lower perikaryal volumes than group HH. Consequently, there were no significant group differences in lipofuscin volume/unit perikaryal volume. If these preliminary results are confirmed by additional data, it would imply that restricted rats are undergoing aging-related changes in brain at a normal rate. By virtue of its much greater life-span, the restricted rat may be a superior model for the study of senescence.

(Supported by NIA grant AG00779.)

12 LOSS OF AXON TERMINALS IN THE DENTATE GYRUS OF SENESCENT RATS. Y.Geinisman, K.D.Bennett* and M.E.Yates*. Dept. Anat., Sch. Med., Northwestern Univ., Chicago, IL 60611.

A loss of axo-dendritic and axo-somatic synapses was described by us earlier in the dentate gyrus of senescent rats (Neurosci. Abstr., 1977, 3: 106; 1978, 4: 113). This loss of synapses was determined in relation to the postsynaptic elements remaining in senescence, and it could, therefore, be due to a disappearance of synaptic densities or to a loss of presynaptic terminals. In the present study, an attempt has been made to elucidate the question whether a loss of presynaptic terminals underlies age-related synaptic loss.

Male rats of the Fischer-344 strain were used. A group of five senescent rats of 25 months of age was compared with a group of five young adult rats of 3 months of age. After perfusion of animals with Karnovsky's fixative, the rostral portion of the right dentate gyrus was embedded in Araldite. Coronal ultrathin sections were prepared to include the region of the dorsal blade of the dentate gyrus, which is opposite the free, lateral end of its ventral blade. These sections, stained with uranyl acetate and lead citrate, were used to obtain electron micrographs of granule cell bodies in the dorsal-most part of the granule cell layer and of the neuropil in the supragranular zone of the molecular layer. In the micrographs, axon terminals contacting neuronal soma membranes in the granule cell layer and axon terminals contacting membranes of longitudinally cut dendrites in the supragranular zone of the molecular layer were examined. The number of axon terminals was counted and expressed per unit length of neuronal plasma membrane. The membrane length was estimated with the help of a map-measurer.

Examination of axon terminals in contact with neuronal soma membrane showed that their number was significantly decreased in the group of senescent rats relative to the young adult group. The decrease in the number of axon terminals was found not to be associated with any significant change in the length of neuronal soma membrane in senescence. These results, taken together, indicate that an absolute loss of axon terminals contacting the neuronal soma membrane occurs in the dentate gyrus of senescent rats. The loss of axon terminals can account for the observed age-related loss of axo-somatic synapses, and further work is in progress to determine whether the same is true for the age-related loss of axo-dendritic synapses.

The loss of axon terminals in senescence may be considered as evidence of a primary impairment of presynaptic elements in the process of age-related synaptic loss.

Supported by NIH BRSG Grant RR 05370; animals were purchased by NIA Grant AG 00383.

13 AGING OF CHOLINERGIC SYNAPSES IN AUTONOMIC GANGLIA AND IRIS OF THE CHICK. Ezio Giacobini, Mario Marchi*, and Douglas Hoffman*. Lab. of Neuropsychopharmacology, Dept. of Biobehavioral Sciences, Univ. of Connecticut, Storrs, Ct. 06268.

Information of the aging process of cholinergic synapses in the peripheral nervous system is slight. We have followed the pattern of variation in the endogenous levels of acetylcholine (ACh) and choline (Ch) in sympathetic (lumbar) ganglia, a parasympathetic (ciliary) ganglion and its target organ, the iris, from 1 to 7 years of age in the chick. The radioenzymatic micromethod of McCaman and Stetzler (J. Neurochem. 26: 669, 1976) was used. The data are expressed as pmoles per ganglion or iris. In the iris, ACh levels remain constant between 1 and 1.7 yrs. (402 ± 42 to 488 ± 29). Following this period ACh levels decrease continuously from 289 ± 50 at 2 yrs. to 198 ± 40 at 5 yrs., and to 68 ± 11 at 7 yrs. At this latter age the amount of ACh is less than in the iris at 14 days of incubation (d.i.). The ACh content in the sympathetic ganglion is unvaried between 1 and 2 yrs. (182 ± 47 to 259 ± 42) and decreases slightly at 5 yrs. (130 ± 29) with no further decrease up to 7 yrs. (155 ± 48). The ACh levels of the ciliary ganglion show a different pattern. The ACh content increases > 2 fold (161 ± 40 to 384 ± 89) between 1 and 2 yrs., then declines at 3 yrs. (236 ± 60) with the value at 5 yrs. being the same as at 1 year of age. This slight fall continues up to 7 yrs. (96 ± 13). The Ch content parallels the ACh pattern in all three organs. The V_{max} for Ch uptake follows the same pattern as ACh levels. This is the same trend we observed during development (Marchi et al., 1979, abstract, this meeting). In conclusion: 1) Unexpectedly, the ACh content of the ciliary ganglion continues to increase during the first part of aging (1-2 years); whether or not these biochemical changes correlate with morphological changes in the maturing synapse remains to be seen. 2) On the contrary, ACh levels in the iris decrease during aging, reaching embryonic levels at 7 years. If these values are calculated per protein the ACh level of the iris is lower at 7 years of age than at 5 d.i., suggesting that these low levels of ACh may be sufficient for synaptic function at this age or that decline of function occurs. 3) Ch uptake seems to be a limiting factor in regulating ACh synthesis during aging as it is during development. Changes in V_{max} closely parallel changes in ACh levels.

14 SPONTANEOUS DIURNAL AND NOCTURNAL PROLACTIN SURGES IN AGED FEMALE RATS. D. P. Gilman, D. A. Damassa*, K. H. Lu*, H. L. Judd*, and C. H. Sawyer. Depts. of Anatomy and Obstetrics and Gynecology, UCLA Medical School, Los Angeles, CA 90024.

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

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

PD females which were free of pituitary tumors and other gross pathology exhibited two distinct prolactin surges each day. The diurnal surges were relatively small with mean peak prolactin values of 90 ± 9(SE) ng/ml at 1400-1700 hr. The nocturnal surges were larger, showing peak titers of 385 ± 56 ng/ml at 0200-0500 hr. CE females without signs of pathology also showed both diurnal and nocturnal prolactin surges. In contrast to findings in PD rats, however, the diurnal surges were larger (434 ± 179 ng/ml) than the nocturnal surges (174 ± 29 ng/ml). In PD and CE animals which had evidence of pituitary tumors, plasma prolactin values were elevated and did not show any consistent patterns.

These findings reveal that aged female rats exhibit daily prolactin surges which are comparable in timing, and for PD rats also in magnitude, to those seen in young pseudopregnant rats. The prolactin surges in the aged rats, however, differ from those of pseudopregnancy in that they appear to be generated spontaneously and do not require cervical stimulation for activation. (Supported by grants from NIH and The Ford Foundation).

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

Recent studies indicate that there are cerebral vasculature changes with increasing age. There is loss of capillary endothelial cells, and increased capillary length and volume with age (Bar, Adv Neurol 20: 1, 1978). However, the functional integrity of the blood-brain barrier (BBB) in old animals has not been characterized. Rapoport, et al (J Gerontol 34: 162, 1979) did show minimal increased permeability of the BBB with age; however, no information is available in aged animals on the integrity of the BBB carrier systems characterized by Oldendorf (Crit Rev Toxicol 3: 159, 1975). This study tests the hypothesis that the function of the choline transport system at the BBB decreases with age.

Sprague-Dawley rats obtained from the NIA were used at 10 mo., 18 mo., and 26 mo. of age. The brain uptake index (BUI) was measured by the method of Oldendorf, using $^{3}H_2O$ as the diffusible substance and $^{99m}TcO_4$-Sn-EDTA as the indiffusible substance. In ten brain regions there were no age-related differences found in the choline chloride BUI, although in seven regions the mean BUI was lower in the older rats. The overall trend for decreased BUI of choline was significant ($p<.05$, Kendall Coefficient of Concordance).

Marked regional differences were found in the choline chloride BUI. The regions investigated in order of increasing choline BUI are striatum, septum-nucleus accumbens, midbrain, diencephalon, hippocampus, hypothalamus, cerebellum, cortex, pons, and medulla. The BUI in the medulla was about fourfold higher than in the striatum.

These findings indicate that there is some indication of decreased functional capacity of the BBB choline transport system in aged rats. The physiological significance of this observation needs further evaluation.

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

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

Male Sprague-Dawley rats, 4 mos and 28 mos of age, and female Sprague-Dawley rats 9 mos and 23 mos of age, were sacrificed by aldehyde perfusion and the pineals processed for electron microscopy.

With advancing age it was found that the pineal capsule thickness increased and severe collagen infiltration was noted. Accompanying the collagen infiltration were patches of granular material and filaments. Definitive light, dark and intermediate density pinealocytes were present, and no consistent change in their relative frequency was found with increasing age. The number of pinealocytes with deep nuclear invaginations increased with advancing age in the male rats but no pattern was found in the females. Nuclear inclusions, possibly the "Kernkugeln" reported by Dimitrova in 1901, were identified in the pinealocytes. These inclusions consist of filaments 10nm in diameter and increased in frequency with advancing age. Cytoplasmic dense bodies increased in frequency as a function of age. The dense bodies have the structural characteristics of lipofuscin. The maximum diameter of lipid droplets tended to increase with age suggesting the possibility that the rate of secretion may decline in older animals. Occasional cells contained reticulated mitochondria and some cell processes had a similar appearance to neuroaxonal dystrophy seen in the dorsal columns of the brainstem in aging animals of several species.

17 AGING AND MINIATURE ENDPLATE POTENTIALS IN MICE. S.S. Kelly*, R.A. DeRosa*, and Norman Robbins. (SPON: A.C. Breuer) Dept. Anat. Case West. Res. Schl. Med., Cleveland OH 44106.

The frequency and amplitude of miniature endplate potentials (MEPPs) exhibit large changes between young and old (30-33 mo) rats (Gutmann et al., J.Physiol. 216:331,1971; Vyskocil & Gutmann, Experientia 28:280,1972). Those experiments, conducted at 20° C, showed that the changes (increased amplitude, decreased frequency) were greater in soleus than in diaphragm. In the present study, MEPPs were recorded in C57BL female mice age 7-8 or 31-33 months. Recordings were made from the extensor digitorum longus (EDL) and soleus muscles in vitro at 30°C. The amplitudes, corrected to a standard resting membrane potential of -80mV, and frequencies were calculated, and the results from 3 young and 4 old mice were compared using the Mann-Whitney nonparametric test.

In the EDL, mean amplitude of MEPPs increased significantly ($p<2\%$) from 0.46mV in the younger group to 0.62mV in the older group. Mean frequency decreased from 7.1Hz to 5.4Hz ($p<2\%$). In the soleus, MEPP amplitudes slightly increased from 0.56mV to 0.69mV ($p<7\%$), but there was no significant change in frequency from the value of 5.2Hz in younger mice.

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

(Supported by NIH grant AG-00795)

18 QUANTITATIVE ANALYSIS OF SYNAPTIC JUNCTIONS, AXON PRETERMINALS AND ASTROGLIAL PROCESSES IN THE HIPPOCAMPUS OF YOUNG AND OLD RHESUS MONKEYS. C.A. Knox*, S.K. Jirge*, K.R. Brizzee, J.M. Ordy and R.T. Barthus* (SPON: W. Sewell).Depts. Anat/Neurosurg. & Delta Regional Primate Research Center, Tulane University, New Orleans and Covington, LA and Lederle Laboratories, American Cyanamid, Pearl River, New York, 10965.

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

Quantitative analysis of electron micrographs was performed in two young (approximately 6-8 years of age) and three old (approximately 20 years of age) rhesus monkeys. Fifty random electron micrographs at a final magnification of 25,000 times were prepared from each animal. Micrographs were quantitatively analyzed for numerical and volume density (random hit method) for synaptic junctions, axon preterminals and astroglial processes. The numerical density of synaptic junctions and axon preterminals was found to be decreased by 25% and 41%, respectively, in aged animals. In a similar manner, the decrease in volume density of these parameters was 33% and 30%, respectively. In contrast to the loss of synaptic junctions and axon preterminals, astroglial processes demonstrated a marked age-related increase in numerical density (225%) and in volume density (567%). These results confirm and support similar findings which have been reported in the dentate gyrus of the rat and in the human cerebral cortex during aging. This study also demonstrates that marked age-related ultrastructural alterations occur in the hippocampus, one of the primary convergent multimodal sensory centers in the brain concerned with the mediation of short-term memory.

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19 MARKED ALTERATIONS IN RESPONSIVITY TO PSYCHOSTIMULANT AND CHOLINERGIC DRUGS ASSOCIATED WITH SENESCENCE IN THE FEMALE MOUSE. Harbans Lal*, Robert Gianforcaro* and Kalidas Nandy* (SPON: Robert Hill): Department Pharmacology & Toxicology, University of Rhode Island, College of Pharmacy, Kingston, RI, 02881, and GRECC, V. A. Hospital, Bedford, MA, 27710.

Elderly patients are known to respond differentially to a variety of psychotropic and other drugs but the mechanism underlying these differences are not known. Although significant pharmacokinetic changes have been reported in the elderly, overwhelming evidence suggests that pharmacodynamic factors play a more important role in determining their responsivity to the drugs. With a view to define in vivo changes in the pharmacodynamic factors, we studied selected cholinergic and dopaminergic drugs in the senescent (15-30 months old) female mice of C57BL6 strain. These mice did not show any deficit of spontaneous or exploratory motor activity related to senescence. However, there was a marked reduction in responsivity to amphetamine, a catecholaminergic stimulant drug, and to dexetimide, a specific anticholinergic drug, with respect to locomotor stimulation. In contrast, the responsivity to oxotremorine, a cholinergic agonist, was enhanced with respect to locomotor depression. Senescent mice also showed enhanced lethality due to oxotremorine. Because the changes in drug responsivity were not all in one direction, they are not likely to be due to pharmacokinetic factors. The observed changes in the amphetamine action suggest alterations of catecholaminergic systems in the senescence. Previously a reduction in the number of haloperidol and spiroperidol binding sites has been shown in the senescent animals. Enhanced activity of a cholinergic agonist and reduced sensitivity to a cholinergic antagonist is difficult to intepret but is probably due to hypersensitivity of the central cholinergic functions.

20 LONG-TERM ADRENALECTOMY REDUCES SOME MORPHOLOGICAL CORRELATES OF BRAIN AGING. P.W. Landfield[1], C. Wurtz[2]*, J.D. Lindsey[3]* and G. Lynch[4]. Dept. Physiol. & Pharmacol., Bowman Gray Sch. Med., Winston-Salem, NC 27103[1]; Dept. Psychobiol., Univ. Calif., Irvine, CA 92717[2-4]; Dept. Neurosci., UCSD Sch. Med., La Jolla, CA 92093[3].

In previous reports we noted that the hypertrophy of hippocampal astrocytes is a consistent correlate of brain aging in Fischer rats (Landfield et al., 77, J. Gerontol.). We also found evidence that this astroglial hypertrophy is related, in part, to adrenocortical hormones (Landfield et al., 78, Science; Soc. Neurosci. Abstracts, 78). To date, our findings are consistent with the hypothesis that endocrine systems, in particular the adrenocortical system, modulate the rate of development of some asepcts of brain aging (Landfield, In Parkinson's Disease II, Finch et al. (eds.), Plenum, 1978).

We report here the initial results from a study in which aging animals were adrenalectomized (Adrx.) at 14 mo. of age and maintained for 5½ mo. on 0.9% NaCl behind an air barrier system to prevent airborne infection. These Adrx. animals (low dose) received 10 µg/ml replacement glucocorticoid in the drinking saline. A control group was Adrx. at the same age and maintained in the same manner. However, these control animals received 250 µg/ml cortisol in the drinking saline (high dose). This dose was, however, adjusted on several occasions because of weight loss and some deaths in the high dose group. Final dose was 175 µg/ml. An intact control group was also included. Plasma glucocorticoids were analyzed after the animals were perfused.

Analysis of semithin brain sections appears to indicate that the low dose animals exhibit reduced numbers of hippocampal microglia and a reduced depth of the hippocampus. Both increased microglia and increased depth are correlates of aging in the hippocampus of rats (Landfield et al., Proc. Gerontol. Soc. 1978; Diamond et al., Behav. Biol. 1975; Lindsey et al., J. Gerontol. 1979). Lipofuscin appears unaffected by these treatments. Astrocyte analyses are still underway, as are more complete analyses of all variables. To this point, however, it appears as if adrenalectomy can reduce at least some correlates of brain aging in rats. Of course, whether these reductions are relevant to functional changes still remains to be determined.

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21 REINSTATEMENT OF BRAIN STIMULATION REWARD IN AGED RATS BY AMPHETAMINE. M.J. Lewis, Dept. Psychology, Howard University, Washington, D.C. 20059.

A single low dose (0.2-0.35 mg/kg) of d-amphetamine sulfate reinstated lever-presses(l-p) for brain stimulation reward(BSR) in rats who prior to injection no longer l-p for BSR. These animals ranged in age from 400-550 days, but were implanted at 200-250 days with bipolar platinum electrodes in the medial forebrain bundle in regions of the lateral hypothalamus. All showed vigorous l-p after implantation and subsequently received drugs, but were drug-free at least 60 days prior to amphetamine injection. Food deprivation, increased stimulating current, frequent priming stimulation and reshaping failed to reinstate stable l-p. Amphetamine injected 5 minutes prior to session reinstated l-p in 7 of 11 rats with minimal priming and the remaining animals after one shaping session. BSR parameters threshold current and mean resistance to the stimulating current increased over initial values while rate of l-p remained unchanged. All animals exhibited l-p on two subsequent sessions without additional amphetamine injection. Three animals continued to l-p without further injection on five subsequent trials over a 1 month period. These data suggest that amphetamine may rejuvenate degenerating reward systems in aged animals.
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22 ELECTROPHYSIOLOGICAL AND BIOCHEMICAL EVIDENCE FOR AGE-RELATED ALTERATIONS IN HIPPOCAMPAL CHOLINERGIC FUNCTIONING. A. S. Lippa*, D. J. Critchett*, R. T. Bartus (SPON: C. Latimer), Medical Research Division, American Cyanamid, Pearl River, NY 10965; and, W. Harrington* and R. W. Pelham*, Tufts-New England Medical Center, Dept. of Neurology, Boston, MA 02111.

While central cholinergic (ACh) mechanisms have recently been implicated in the cognitive disorders associated with old age, no direct evidence for age-related functional alterations in ACh mechanisms has been reported. Since hippocampus has also been implicated in memory functions, we have investigated muscarinic ACh receptors in dorsal hippocampus in male, albino rats aged either 6-9 months (YOUNG) or 26-29 months (OLD). All rats were anesthesized with chloral hydrate (400 mg/kg, intraperitoneally) and allowed to breath spontaneously. Microiontophoresis and recording were performed with stereotaxically placed 5-barrelled glass micropipettes of approximately 6 μM overall tip diameter. The center recording barrel was filled with 1.5 M NaCl and generally had a resistance of 3-12 Megohms. Three of the four side barrels were filled with either ACh (.5 M, pH 3.4) or L-glutamic acid (GLU; 1 M, pH 8.5) which were passed as cation and anion, respectively. The fourth barrel contained 4 M NaCl to balance drug ejection and holding currents. Dorsal hippocampal pyramidal cells were tentatively identified by their low firing rate and bursting firing pattern. At the end of each electrophysiological recording session, brains were removed and stored frozen for the subsequent measurement of ^{3}H-quinuclidinyl benzilate (^{3}H-QNB) binding and choline acetylase activity. Both ACh and GLU stimulated the firing of pyramidal cells in a dose-related manner (1, 2, 5 and 10 nA). However, the responses to ACh were significantly reduced ($p < .05$, analysis of covariance) in the OLD group with an approximately 50% reduction in the maximum amount of stimulation. No age-related differences in the responses to GLU were observed ($p > .05$, analysis of covariance). Scatchard analysis of ^{3}H-QNB binding in dorsal hippocampus revealed a significant reduction ($p < .004$, t test) in the number of binding sites (YOUNG B_{max} = 1580 + 50 fmol/mg protein; OLD B_{max} = 1330 + 40 fmol/mg protein) with no significant change ($p > .05$) in affinity (YOUNG Kd = .274 + .016 nM; OLD Kd = .294 + .035 nM). Choline acetylase activity, a marker of ACh neurons, was also unchanged. Since the responses to iontophoretically applied GLU were dose-related, but not age-related, the present results document a selective age-related functional impairment of hippocampal ACh mechanisms.

23 AGE DIFFERENCES IN CONDITIONED TASTE AVERSION: POSSIBLE ROLE OF VASOPRESSIN. M. Colleen McNamara and Ralph L. Cooper.* Center for the Study of Aging, Duke University Medical Center, Durham, North Carolina 27710.

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

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

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

24 MORPHOLOGICAL CORRELATES OF AGING IN THE MONKEY BRAIN - A LIGHT AND ELECTRON MICROSCOPIC STUDY. Ronald Mervis, Robert D. Terry* and Douglas Bowden. Dept. Pathol (Neuropathol), Ohio St. Univ. Col. Med., Columbus, OH 43210, Albert Einstein Col. Med., The Bronx, NY 10461, and Dept. Psychiat., Univ. Washington Sch. Med. Seattle, WA 98195

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

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25 BRAIN PROTEINS IN SCHIZOPHRENIA AND AGED CONTROLS AS ANALYZED BY COMPUTERIZED DENSITOMETRY. Gary D. Miner and Susan Maurer*. Dept. Biology, Northwest Nazarene College, Nampa, ID 83651.

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

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

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

Circadian rhythms in locomotor activity and drinking behavior were monitored in all rats (Long Evans, hooded) in both entrained (L:D, 14-10) and free-running (constant dim illumination, 1.0 lx) conditions. The results indicate that circadian rhythmicity in both behaviors declines with increasing age. In diurnal lighting, YC, OCE and ORPP rats restrict 72.7 $\pm$ 2.4%, 67.1 $\pm$ 1.7% and 58.9 $\pm$ 2.2%, respectively, of their drinking behavior to the 10 hrs of darkness. This percentage decrease in aged rats is primarily the result of fewer drinking events during the dark phase. Power spectral analyses of drinking events in both entrained and free-running conditions reveal a progressive flattening of the spectrum and a diminished circadian peak with advanced age. YEA rats, in contrast, exhibit normal rhythmicity in activity and drinking.

Our findings indicate that the amplitude of circadian functions declines significantly with advancing age in rats. Aging of central circadian rhythm generating mechanisms is implicated. Dampened circadian functions in old age could contribute to the normal pattern of reproductive senescence observed in female rats. The observation that YEA rats exhibit normal circadian rhythms may indicate that different mechanisms underlie the CVE states of the YEA rat and rat with suprachiasmatic nucleus ablation.

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

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

Intraparenchymal brain microvessels consisting of arterioles, capillaries, and venules were isolated from the forebrains of Sprague Dawley rats by isopycnic sucrose density gradient centrifugation and microsieving techniques. The microvessel preparations were free of contaminating neurons and glia, as shown by light and electron microscopy and by the absence of radioactivity within microvessels prepared after the addition of radiolabelled synaptosomes and cytoplasmic proteins to the whole brain homogenates. We also examined the protein patterns of these blood vessels by isoelectric focusing and by sodium dodecyl sulfate polyacrylamide gel electrophoresis, and found that they differ considerably from those of synaptosomes and brain homogenates. The isolated microvessels were incubated at 37°C in the presence of Eagle's minimal essential medium (devoid of methionine) and 35S-methionine under 95% O_2 / 5% CO_2 conditions. The incorporation of radiolabelled methionine into trichloroacetic acid-precipitable protein appeared proportional to the concentration of vascular protein at ranges of 1-2 mg/ml. The microvessels incorporated methionine at a linear rate up to at least 30 minutes. The newly synthesized proteins, resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis and by isoelectric focusing, exhibited some differences between animals of 4 and 21 months. Such studies appear useful as models for examining the biochemical activity of cerebral blood vessels during aging and other pathological states.

28 AGE-RELATED BEHAVIORAL, PHYSIOLOGICAL, AND ANATOMICAL CHANGES IN APLYSIA CALIFORNICA. B. Peretz,* R. Papka,* K. Rattan,* and J. Becker* (SPON: D. T. Frazier) Depts of Physiology and Anatomy, Univ. of Kentucky Med. Ctr., Lexington, Ky. 40536

The gill withdrawal reflex to gill stimulation and its neural correlates are age-dependent. The reflex and the physiology and anatomy of L_7 and R_2, central neurons in the parietovisceral ganglion were examined in old Aplysia, 500 gm and heavier, and in mature Aplysia, 100-250 gm. No less than 50 days of age separated the two groups. The reflex in old Aplysia was elicited by a stimulus of 1.5 gm or greater whereas in mature animals 0.2 gm was threshold. Habituation of the reflex, to 1.5 gm, was twice as fast in old Aplysia (n=7) as in mature animals (n=7). L_7 spiking did not reliably dishabituate the reflex in old animals as it did in mature ones. EPSP's, evoked by gill stimulation, in L_7 from old Aplysia, were correlated with reflex habituation but were 1/8 the size of those in L_7 from mature animals. Rin, of L_7 was 0.7 M in the old group as compared to 4.8 M in the mature one; decreased Rin can explain reduced PSP size. Time constant, T, in old animals was twice that in mature animals. Size of L_7 in living ganglia from the two groups were not significantly different. Rin, T, and size comparisons of R_2 were similar to those found for L_7. LM and EM studies of L_7 and R_2 revealed that with aging the number of glial cells increased, and there was increased membrane infolding probably accounting for decreased Rin and greater T. Age-pigment, lipofuscin, in close proximity to the infoldings and to trophospongiun, increased as a function of age and exhibited autofluorescence similar to that seen in vertebrate neurons (Brizee et al., 1969). In old Aplysia, EM showed that lipofuscin was a membrane bound amorphous body containing vacuoles and lamellated structures. The bodies attained sizes of about 5 μm in length. In mature Aplysia and those 60 days younger (20 gm or less), the bodies were round, had fewer or lacked lamellated structures, and were 0.5 - 1 μM. Behavioral and physiological signs of aging relate closely to the anatomical signs. The Aplysia nervous system is appropriate to study the life history of individual neurons, esp. those mediating behavior (NIMH; Sanders-Brown Ctr., U.K.)

29 DENDRITIC DYING BACK, NEUROFIBRILLARY TANGLES, AND LEARNING AND RETENTION IMPAIRMENT FOLLOWING ALUMINUM ADMINISTRATION: IMPLICATION FOR BRAIN AGING. Ted L. Petit and Gerald B. Biederman*, Div. Life Sci. & Dept. Psychol., Univ. Toronto, West Hill, Ont., Canada.

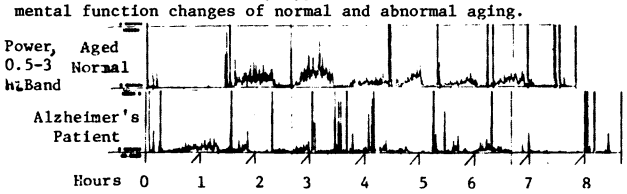
Senile dementia of Alzheimer's type is accompanied by a number of brain changes, including an accumulation of neurofibrillary tangles in the neuronal cytoplasm, and a progressive dying back of the dendritic tree. Elevated levels of aluminum cause a somewhat similar accumulation of neurofillamentous tangles in some species, and as such has been used as a model for the human condition. Crapper has found elevated levels of aluminum in the brains of senile patients and has hypothesized that this element may play a critical role in the etiology of the disease. To further determine the possible role of aluminum in that disease, it is important to systematically discern the consequences of increased brain aluminum content. Aluminum tartrate (5μM in 100μl water) or physiological saline was infused into the left lateral ventricle of adult male New Zealand white rabbits. All rabbits were trained 10 days post-operative in a step-down active avoidance task, and retested on post-operative day 13. At the first onset of neurological symptoms (14-21 days post-op), or at 21 days post-op for control animals, the rabbits were sacrificed. Sensori-motor neocortex was examined with electromicroscopy for evidence of aluminum induced neurofibrillary tangles. For light microscopy, brains were stained with the Golgi-Cox stain, layer V giant pyramidal cells were drawn from the dorsal sensorimotor cerebral cortex, and dendritic morphology analyzed by the use of the Scholl method. Aluminum treated animals were deficient on both original learning on day 10 ($p<.01$) and retention on day 13 ($p<.05$) of the step-down active avoidance task. Electron microscopic analysis indicated the presence of neurofibrillary tangles in the soma and dendrites of neurons of aluminum treated animals. The Scholl analysis revealed that there was a sharp and progressive drop off in the number of dendritic branches with increasing distance from the cell body in the aluminum animals. This progressive reduction of dendritic branches as one proceeds toward the periphery of the dendritic field would be the pattern expected from a dying back process. Westrum reported a loss of dendritic spines, dendritic beading and degneration after topical alumina cream application. Such dendritic degeneration may represent the resulting course of the dendritic dying back reported here, and may be homologous to that observed in brains from the senile human.

30 EEG AND SLEEP CHANGES IN NORMAL AGING AND ALZHEIMER'S DISEASE. Pat Prinz, Murray Raskind* and Carl Gerber.* GRECC, Seattle/Tacoma VA Hospitals and Dept. Psychiatry, U. W., Seattle, Wa.

Sleep and waking EEGs from 12 healthy elderly and from 12 inpatients (drug free) diagnosed as having senile dementia of the Alzheimer's type* (i.e., primary neuronal degeneration) were monitored in the subject's typical home or ward environment whenever the subject sat or lay down. As compared with young adults (age 20-30) aged normals (age 55-80) had less stage 3 and 4 and stage REM sleep, and more wakefulness. Napping was similar to young adults. Alzheimer's type patients (aged 55-80) had even less stage 3 sleep, no stage 4 sleep, very little REM sleep and fragmentation of diurnal sleep-wake rhythms, with frequent daytime naps and nighttime awake periods (data are based on the last 48 hours of 72 consecutive hour studies).

The degree of sleep pattern change correlated with mental function measures (Wechsler Intelligence Scale, Tomlinson Dementia Scale). Correlative neuropathological data will be available for both normal and Alzheimer's subjects to determine whether the sleep and mental function relationship is based on a common factor like neuronal degeneration. Aside from sleep pattern changes, parallel changes were also seen in the sleep and waking EEG waveforms: All-night continuous power spectra analysis on a PDP 11-34 revealed a waxing and waning of power in the delta frequency band (.5-3 Hz) that corresponded with periods of slow wave sleep (Stages 2, 3 and 4). The amount of this delta activity associated with sleep was greatest in young adults, less in healthy elderly, and least in Alzheimer's patients (see below). In addition, there appeared to be a slowing of median delta wave frequency in Alzheimer's patients, and to a lesser degree in aged normals.

Changes were also observed in spindle (11-17 Hz) and in the dominant occipital rhythm (6-11 Hz) frequency bands. These changes in rhythmic EEG activity differ from focal paroxysmal EEG activities in that they appear to better correlate with the mental function changes of normal and abnormal aging.



* Clinical diagnostic criteria: see Katzman, R. Postgrad Med, 64, 119-125, 1978.

31 AGE RELATED CHANGES IN RAT CEREBELLUM: PURKINJE CELL ELECTROPHYSIOLOGY AND CORRELATIVE ANATOMY. Joseph Rogers, Michael A. Silver*, William J. Shoemaker, and Floyd E. Bloom. The Salk Institute, P.O. Box 1809, San Diego, CA 92112.

Sprague-Dawley rats (N=16) aged 3,10,20, and 28 mos were prepared for recording under halothane anesthesia. Several penetrations of cerebellar folia 6 and 7 near the vermis with 3 M NaCl filled glass pipettes (tip diameter = 1 μ) provided extracellular recordings from at least 11 Purkinje neurons per rat. On-line computer programs generated interspike interval (ISI) histograms and extracted information about firing rates, climbing fiber evoked bursts, modal ISIs, and number of long (>75 msec) ISIs. Overall, the 3,10, and 20 mos cells appeared relatively normal. The 28 mos cells, however, had significantly lower mean discharge rates, longer modal ISIs, and greater numbers of long ISIs. In the face of this diminished activity, climbing fiber evoked bursting remained remarkably normal. Inspection of individual records from the 28 mos rats revealed that approximately 1/2 the cells fired relatively normally while the rest were highly aberrant with little activity other than climbing fiber doublets and triplets. Occasionally these cells would display more normal patterns, usually for a few seconds within 2-3 min recording epochs.

Following 3-4 hours of electrophysiological assessment, rats were perfused transcardially with 2.0% glyoxylic acid-0.5% paraformaldehyde and cerebellar samples were dissected. These tissues are presently being evaluated for catecholamine varicosities, small granular vesicles, and a number of ultrastructural correlates of aging (e.g., lipofuscin) using histofluorescent and electron microscopic methods.

Since Purkinje cells provide the final common efferent pathway of the cerebellum, their electrical activity represents a plausible test system for gauging the relevance of basic neurochemical and anatomical anomalies occurring in this structure during senescence. (Supported by a grant from the Aetna/BW Price Foundation.)

32 EFFECTS OF CHRONIC HYPOXIA ON LEVELS OF CATECHOLAMINES IN BRAIN REGIONS FROM AGING RATS. Isaac F. Roubein, Larry J. Embree and David W. Jackson*. Veterans Administration Medical Center and Department of Neurology, Louisiana State University Medical Center, Shreveport, Louisiana 71130.

Oxygen deprivation affects neural tissues earlier and with a greater degree of severity than it does other tissues, and behavioral changes may be induced by hypoxia in man and animals. While there are published studies concerning the effect of hypoxia on monoamine levels in mature mammalian brain, information is lacking on the effect of chronic hypoxia on catecholamine levels in aging mammalian brain; hence this study was undertaken. Further, in an attempt to correlate neurotransmitter levels, functional anatomy and the aging process, seven discrete brain regions were selected for investigation.

Male Sprague-Dawley rats at early senescence (21-22 months old) were exposed to a mixture of 10% oxygen - 90% nitrogen in a sealed plastic cage for 36 hr. The gas mixture was passed through the cage at a rate of 3.5 l/min. Simultaneously, control animals of identical age received air at the same rate. At the end of this period, the rats were decapitated, the brain excised, and immediately dissected into the following regions: cerebral cortex, hippocampus, striatum, hypothalamus, midbrain, cerebellum, pons and medulla.

Spectrofluorometric determinations of norepinephrine (NE) in cerebral cortex, hypothalamus, midbrain, cerebellum, pons and medulla, and of dopamine (DA) in striatum indicated that the levels of these monoamines in the regions examined did not differ significantly from controls.

Gross observation of the animals after 13, 19, and 35 hr showed that the animals in the hypoxic environment were less active as compared with the controls, and the hypoactivity was at its maximum at 13 hr. Whether this observed reduction in animal activity and responsivity could be related to alterations in the levels of NE or DA in the specified brain regions is currently under investigation and will be reported.

Supported by the Medical Research Service of the Veterans Administration.

33 DIFFERENCES IN STRIATAL DOPAMINE RECEPTORS IN SEVERAL MOUSE STRAINS AS A NOVEL MODEL FOR STRIATAL AGING. J. A. Severson*, P. K. Randall* and C. E. Finch* (SPON: D. F. Lindsley). Andrus Gerontology Center, USC, Los Angeles, CA 90007.

Male CBA/J mice have 25 to 50% fewer substantia nigra neurons than male BALB/cJ mice (Ross et al., Nature 264:654, 1976). Losses of nigral neurons are a possible mechanism involved in nigrostriatal aging and are undoubtedly involved in Parkinson's disease. Genetic deficits or subclinical lesions of nigral neuron number, coupled with an age-related loss of nigral neurons has been suggested as a mechanism for the age-related onset of Parkinsonian symptoms. We viewed the CBA/J mouse as having a "genetic nigrostriatal lesion" through which we could examine the effect of early losses of nigral neurons on catecholaminergic function, particularly on dopamine receptors.

Male CBA/J and C57BL/6J mice had 50% fewer specific striatal spiroperidol binding sites than male BALB/cJ mice. Binding affinity for spiroperidol was similar (Kd= 0.5 nM). CBA/J mice failed to develop striatal dopamine receptor supersensitivity following chronic haloperidol treatment, while haloperidol treated C57BL/6J and BALB/cJ mice showed 25 to 30% increases in spiroperidol binding. CBA/J mice also failed to develop supersensitivity as measured by apomorphine stereotypy.

Decreased spiroperidol binding and an inability to develop behavioral and biochemical supersensitivity of the striatal dopamine receptor in the CBA/J mouse are similar phenomena as those observed in the aged C57BL/6J mouse striatum. The CBA/J mouse may represent a novel model for striatal aging.

34 AGE RELATED DECREASE IN CNS β-RECEPTOR ACTIVATION OF THYROTROPIN SECRETION. Michael A. Silver[1] and Shirley A. Joseph.[2] (SPON: George Siggins) [1]A.V. Davis Ctr., Salk Inst., La Jolla, CA 92037 and [2]Dept. of Anatomy, Univ. of Rochester, Rochester, N.Y.

Recent studies have implicated central noradrenergic processes in the neuroendocrine regulation of thyrotropin (TSH). We recently reported that pharmacological activation of central β-adrenoreceptors, through ephedrine-HCl administration, resulted in a significant elevation of plasma TSH levels. In this study, we have examined this β-receptor mediated neuroendocrine response in rats at three different ages: 3 weeks, 4 and 19-22 months (n=8). Two hr. after ephedrine administration (10 mg/kg i.p.), plasma TSH was significantly elevated to over 500% of control values in the 3 week and 4 month old rats. In the aged rats, however, plasma TSH levels were not significantly elevated.

To clarify whether this inadequate neuroendocrine response was caused by either a decreased pituitary secretory response or by a CNS β-receptor-associated deficit, the quality of pituitary responsiveness to thyrotropin releasing hormone (TRH) was examined. TRH (30 pg/100g) was administered intravenously to rats at each age and the animals sacrificed after 2 hrs. Plasma TSH levels were found to be similarly elevated from 300% to 395% in each age group, suggesting that pituitary responsiveness was not impaired in the aged rats.

These findings indicate that the lack of neuroendocrine response to ephedrine administration in the aged rats was due to a β-receptor associted deficit. It is not known whether this effect is due to an age-related decrease in β-receptor affinities or binding sites or a loss of β-receptor containing cells.

35 AGE-RELATED ALTERATIONS IN PERIKARYAL MONOAMINES IN NON-HUMAN PRIMATES. John R. Sladek, Jr. and Celia D. Sladek. Depts. of Anatomy and Neurology, Univ. of Rochester Sch. of Medicine and Dentistry, Rochester, NY 14642.

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

Nine monkeys (M. nemestrina); 3 each at 4, 10, 20 years old (y.o.) were prepared together for formaldehyde-induced histofluorescence. Quantitative microspectrofluorometric analysis (MSF) was performed on individual neurons of select, monoaminergic, cell groups; i.e. the locus coeruleus (LC), substantia nigra (SN), and nucleus raphe dorsalis (Rd). The relative intensities were recorded; means and standard deviations were calculated and plotted as histograms. Intensity levels were highest in the 4 y.o., and declined progressively in the 10 y.o. and 20 y.o. Norepinephrine fluorescence intensity was depressed further in neurons of the LC which also contained autofluorescent pigment granules. Monoamine content as reflected in relative fluorescence intensity was depressed as much as 75% in Rd and SN, and 50% in LC.

These data indicate that monoamine levels are depressed in perikarya of origin of major aminergic systems. It is not known whether this is reflected also in the terminal fields supplied by these neurons; our preliminary analysis in these animals suggests that terminal fields in brain stem and hypothalamus are comparable to the 4 y.o., but quantitative MSF has not been performed. The present findings of depressed perikaryal stores might be related to decreased synthetic capabilities and/or heightened turnover of central monoamines during aging and may correlate with altered functional capabilities.

Supported by NIH grants AM-19761, NS-00259, AG-00847, AG-01456 and NSF grant BNS 78-11153.

36 AGE-RELATED DECREASE IN MOUSE BRAIN CHOLINERGIC MUSCARINIC RECEPTOR BINDING. R. Strong*, L. Hsu*, P. Hicks*, and S. J. Enna (SPON: K. Campbell). Univ. Texas Med. Sch. and Texas Res. Inst. for Mental Sciences, Houston, Tx. 77025

Recent reports have indicated a number of pre- and postsynaptic alterations in neurodegenerative disorders. Of the various neurotransmitters, the brain cholinergic system appears to be particularly vulnerable with cholinergic changes reportedly occuring in Huntington's, Parkinson's and Alzheimer's diseases. In the present investigation, pre- and postsynaptic cholinergic markers were studied in the brains of mice aged 6 to 30 mos. in an attempt to determine whether alterations in this neurotransmitter system may also occur during the normal aging process.

For the study, brains of young (6 mos.) middle-aged (12 mos.) and senescent (30 mos.) male C57Bl/6J mice were removed, rapidly dissected on ice and frozen (-20°) until assayed. Cholinergic muscarinic receptor binding was studied using ^{3}H-QNB as a ligand. Brain glutamic acid decarboxylase (GAD) activity, an enzyme marker for GABAergic neurons, and choline acetyltransferase (ChAc) activity, a presynaptic marker for cholinergic neurons, were measured using standard biochemical techniques.

The results indicate that in the brains of the older (30 mos.) animals there was a significant 30% reduction in ^{3}H-QNB binding in the corpus striatum compared to either the 6 mos. or 12 mos. animals. Receptor saturation experiments revealed that the decrease in muscarinic receptor binding was due to a reduction in receptor number, with no change in binding affinity. No significant differences were detected in either GAD or ChAc activity in this brain region. While there was also a tendency for a reduction in cholinergic muscarinic receptor binding in the hippocampus, this decrease was not significant in preliminary experiments.

These findings suggest that there may be an age-related decline in striatal cholinergic function due to loss of receptors for this neurotransmitter. Furthermore, the absence of a change in GAD or ChAc activity in this brain area suggest that the GABAergic and cholinergic neurons are intact, indicating that the decrease in muscarinic receptors is not associated with a loss in either of these neuronal cell types. This loss of striatal cholinergic receptors may contribute to, or be the result of, the motor impairments that often accompany old age. (Supported in part by USPHS grants NS-13803, an RCDA NS-00335 (S.J.E.) and a TRIMS predoctoral fellowship).

37 OPIATE RECEPTOR SUPERSENSITIVITY IN SOME BRAIN REGIONS OF AGED MALE RATS. Beatriz J. Vasquez, Rita B. Messing, Robert A. Jensen, Joe L. Martinez, Vina R. Spiehler*, Bernardo Samaniego*, and James L. McGaugh. Dept. of Psychobiology, University of California, Irvine, CA 92717, USA.

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

	24 Month Old		3 Month Old	
Brain Region	K_D(nM)	B_{max} (fmol/mg prot.)	K_D(nM)	B_{max} (fmol/mg prot.)
		Whole Homogenates		
frontal poles	1.83**	41.3	7.1	136.0
anterior cortex	3.84	123.1	3.47	131.9
amygdala	1.73	54.1**	3.93	138.4
striatum	1.11**	72.2	2.80	139.0
	7.73	220.2		
hippocampus	3.59	83.2	4.59	78.0
		Washed Membranes		
frontal poles	1.62*	58.3	4.7	123.0
anterior cortex	3.13	152.8	2.77	155.5
amygdala	1.14	56.0*	3.11	148.1
striatum	2.76	145.7	3.13	157.0
hippocampus	3.39	83.7	3.75	89.1

*$p < 0.05$. **$p < 0.01$ different from young.

Increased affinity for DHM accompanied by decreased receptor concentrations were observed in whole homogenates of frontal poles, amygdala, and striatum of aged as compared to young rats. In washed membranes, however, no difference between young and old rats was apparent in striatal tissue. The results suggest that receptor supersensitivity observed in brain regions of old rats may compensate for decreases in receptor number. Further, these receptor alterations may be related to *in vivo* differences in the effects of opiates in aged rats. [Supported by USPHS AG00538 and MH12526, NSF BNS 76-17370, and a grant from the McKnight Foundation (all to JLMcG)].

38 CULTURED EXPLANTS FROM AGING BRAINS: CHARACTERISTICS OF OUTGROWTH, NEUROFILAMENT PROTEIN, AND CYTOLOGY. P. L. Witte*, J. W. Fuseler*, W. S. T. Griffin and J. W. Shay* (SPON: R. M. Stewart). The University of Texas Health Science Center at Dallas, Dallas, TX 75235.

In a number of pathological disorders, the neurofilaments are tightly packed within the cellular processes of brain cells. Our hypothesis was that aging could cause similar effects. In order to test this hypothesis, we cultured brain explants from 24 month old rats and compared outgrowth, 100Å filament protein (neurofilament protein) characteristics and general cytoplasmic morphology between aged explants and brain explants from much younger animals, *viz.*, three days and three months of age. All rats were killed by decapitation and cerebral cortex was excised under sterile conditions. Explants were mechanically dissociated on coverslips which were flooded with CMRL 1066, containing 10% horse serum and 5% fetal calf serum. After 7 days, the coverslips were checked for outgrowth of cells. We found that cellular outgrowth is possible although the time of cell spreading may be prolonged. After 2 weeks in culture, the coverslips were fixed by washing with 4% neutral buffered formalin, incubated with rabbit-anti-bovine neurofilament antibody and fluoresced with FITC goat- and anti-rabbit globulin at a dilution of 1:6. Neurofilament fraction for antibody preparation was isolated from bovine spinal cord white matter and characterized by a molecular weight of 51,000. The cytological differences included an increase in the number of lipofuscin granules in cells from aged brains as well as accumulations of vacuoles which were bounded by neurofilaments. Even though cells from aged brains appeared to contain less neurofilament, that which was present was tightly packed and in tortuous bundles both in the cell periphery and surrounding the nucleus; whereas, young brain cells, especially from neonate explants, contained a filamentous network of thread-like neurofilaments in the periphery when compared by immunofluorescence.

Supported by USPHSHL20, HL0042.

AUDITION

39 CYTOLOGY OF PERIOLIVARY CELLS. Joe C. Adams . LNO, NINCDS,NIH Behtesda, Md. 20205.

Clusters of auditory cells located around the superior olivary nuclei are referred to as periolivary cell groups rather than nuclei because of their lack of well defined borders or neuropil. These cell groups are heterogeneous with respect to their anatomical inputs and outputs, as well as in their physiological properties. As an initial step towards a comprehensive analysis of these cells in the cat a cytological study was undertaken employing Nissl, Golgi, and reduced silver preparations. Some seventeen classes of cells were identified in Nissl material. Some cell classes were found predominantly within limited regions of the olivary complex. Cells similar to globular cells of the cochlear nucleus constitute the bulk of the medial trapezoid nucleus. Similar cells are also found in a large group caudal to the lateral superior olive and extending rostrally in a region ventro-lateral to the medial superior olive, with a few cells of the same type extending rostrally in a region dorsolateral to the dorsal tip of the medial superior olive. Multipolar cells with abundant large, dark clumps of Nissl substance are concentrated close to the caudal surface of the lateral superior olive. Cells of this type extend rostrally for a short distance dorsal to the lateral olive. Ventral to the lateral olive they extend rostrally for almost its entire extent. Pale cells are found caudally, predominantly dorso-medial to the medial olive. Ventral to the medial trapezoid nucleus many cells have only a few large, dark clumps of Nissl substance. Many cells in the lateral trapezoid region have only a few small Nissl clumps. Concentrations of large pale cells are found caudal and rostral to the medial olive and are connected by a sparse line of these cells that runs ventro-medial to the medial olive. Anterior to the olivary nuclei there are many large multipolar cells with diffuse Nissl substance. Large cells with dark Nissl clumps are scattered throughout the olivary complex. The morphology of several of these cell types as seen in Golgi and reduced silver preparations will be described. This study will provide information necessary for future studies of the ultrastructure, anatomical connections, and physiology of individual classes of periolivary cells.

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

Cell bodies of neurons containing met-enkephalin have been noted in general mapping studies of the rat brain (Hokfelt et al. 1977). Fibers containing met-enkephalin have been localized in the dorsal cochlear nucleus (DCN) and in the ventral cochlear nucleus (Sar et al. 1978). In the present study the cochlear nuclei of rats were examined to determine the category of cells that contain met-enkephalin. Rats were pre-treated with colchicine to inhibit axonal transport. Indirect fluorescence immunohistochemistry was performed on 10 micron cryostat sections to detect met-enkephalin. Nissl stains on adjacent, or the same sections after quenching of fluorescence, were used to identify cell types and regions. A second adjacent section was used for an absorption control to test for specificity.

Met-enkephalin positive neurons in the DCN fell into the category of small cells. They were seen predominantly in the deep layer of the DCN. Only a small percentage of the small cells were met-enkephalin positive. This suggests it may be possible to subdivide the heterogeneous population of small cells on the basis of positivity for met-enkephalin. Cell bodies positive for met-enkephalin were also seen in the posteroventral cochlear nucleus near the border of the dorsal cochlear nucleus. These neurons were also categorized as small cells.

Many positive fibers were coursing through and often terminating in the DCN. Fewer fibers were seen in the postero- and anteroventral cochlear nucleus. Fibers were also seen in the dorsal and intermediate acoustic striae.

In conclusion, met-enkephalin containing cell bodies were localized in both the dorsal and posteroventral cochlear nuclei. In both cases these neurons were small cells, though only a portion of the total population of these cells.

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

Previous studies have shown that the utricle and lagena are innervated by afferent axons whose diameters range from 1 to 10 μm (Dunn, R., J. Comp. Neurology 182: 621-636, 1978), that these axons can be divided functionally into several classes based on regularity of resting discharge and response to constant-velocity tilt (Blanks, R. and Precht, W., Exp. Brain Res. 25: 379-390, 1976), and that the hair cells innervated by these afferents can be divided into several classes based on surface morphology (Lewis, E. and Li, C., Brain Res. 83: 35-50, 1975).

It has been proposed that each unit with irregular resting discharge and phasic response to tilt corresponds to a thick afferent originating from a small number of hair cells in the striolar region of the sensory macula, while each unit with regular resting discharge and tonic response to tilt corresponds to a thin afferent originating from a large number of hair cells in the extrastriolar region of the macula (Fernandez, C. et al., J. Neurophysiol. 35: 978-997, 1972). To test this proposition directly, intracellular recordings were made from primary afferents in the anterior and posterior branches of the bullfrog VIIIth cranial nerve medial to the intact otic capsule. After functional characterization of each unit, Lucifer Yellow (Stewart, W., Cell 14: 741-759, 1978) was injected iontophoretically in order to trace the axon to its site(s) of termination on the sensory macula.

So far the results indicate that all hair cells contacted by a particular fiber are of the same morphological polarization and are either striolar or extrastriolar but not both. that large-diameter fibers contact greater numbers of hair cells than do small-diameter fibers, and that afferents innervating only one hair cell may exhibit regular resting discharge with a high mean firing rate, whereas afferents innervating many hair cells may exhibit irregular resting discharge with a low mean firing rate. These observations are contrary to the propositions that thick fibers tend to innervate smaller numbers of hair cells and that fibers with regular resting discharge innervate large numbers of hair cells. It is expected that this line of research will soon reveal how the branches of afferent fibers relate to the specific hair cell types defined by Lewis and Li, and whether the response properties of an afferent fiber correspond to the types of hair cells it contacts or simply the number and morphological polarizations of those hair cells.

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42 CYTOARCHITECTURE IN THE TORUS SEMICIRCULARIS IN THE GOLDEN SKINK MABUYA MULTIFASCIATA. Alice M. Baruch and Robert H. Browner. Dept. Anat., New York Medical College, Valhalla, NY 10595.

The cytoarchitecture in the torus semicircularis (TS) was examined in 14 brains. After perfusion the brains were prepared for cresyl violet staining or Golgi-Kopsch impregnated Araldite-embedded sections 120um thick. The 15um cresyl violet sections were studied in the three standard planes; the Golgi-Kopsch material was analyzed in transverse and horizontal planes. The TS is a mesencephalic nucleus which is dorsally and superficially placed caudally and extends rostrally, laterally and ventrally to end at a mid-optic tectal level. Three nuclei can be seen within the TS: A central nucleus (CN), a laminar nucleus (LN) and a caudal superficial nucleus (SN).

The CN consists of concentric rings of small (6-12um) ovoid cells surrounding a core of large ovoid-triangular (14-28um) and fusiform (20-30um) cells. The ovoid neurons have scant cytoplasm and possess 2-5 dendritic trunks. Most of these processes are directed around the periphery of the CN and often dendrites can be traced into the CN. The average dendritic field covers approximately 100 x 100um mediolateral by dorsoventral. The large central cells have clumped darkly-staining Nissl, abundant cytoplasm and central nuclei. The dendritic fields have a mediolateral orientation and average 188 x 154 x 270um for the triangular cells and 285 x 183um for fusiform cells. Spine population increases distally.

The laminar nucleus is found caudally just ventral to the CN and at successively more rostral levels is medial and finally dorsomedial to it. This nucleus consists of a laminated group of fusiform (20-40um) and ovoid (8-12um) cells with scattered large ovoid-triangular cells (18-28um). Fusiform cells have pale Nissl, a central nucleus and medially and laterally directed dendrites, covering an average field of 278 x 288um. The ovoid cells have sparse cytoplasm and an eccentric or central nucleus. The arborization of both these cells is often skewed to present an orientation of caudomedial and rostrolateral. The large ovoid-triangular cells have dense clumped Nissl and at least two dendritic trees with a medial and lateral orientation, covering average fields of 325 x 355 x 268um. The rostral limit of the LN consists of two fusiform layers orthogonally arranged.

The SN is dorsal, lateral and caudal to the CN. As it extends ventrolaterally around the CN it becomes confluent with the LN, ensheathing the CN except medially. Present are fusiform (18-30um), ovoid (8-14um) and a few ovoid-triangular cells (14-24um). Their average dendritic fields measure 389 x 133 x 100um, 138 x 138 x 200um and 100 x 88 x 200um respectively. Both cell morphology and orientation of dendrites resemble those in the LN.

43 OBSERVATIONS ON UNIT ACTIVITY IN MONKEY AUDITORY CORTEX AND DORSOLATERAL FRONTAL CORTEX DURING A SOUND LOCALIZATION TASK. Dennis A. Benson, Robert D. Hienz*, and Moise H. Goldstein, Jr. Dept. Biomedical Engineering, Johns Hopkins School of Medicine, Baltimore, Md. 21205.

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

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

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

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44 EFFECT OF INTRACOCHLEAR KAINIC ACID ON COCHLEAR POTENTIALS IN GUINEA PIGS. Sanford C. Bledsoe, Jr., Richard P. Bobbin and Dorothy N. Morgan*, Kresge Hear. Res. Lab., Dept. Otorhinolaryngol., L.S.U. Med. Ctr., New Orleans, LA 70119.

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

45 ACTION OF GLUTAMATE AND RELATED SUBSTANCES ON THE SPONTANEOUS ACTIVITY OF AFFERENT NERVES IN THE TOAD LATERAL LINE. Richard P. Bobbin, Dorothy N. Morgan* and Sanford C. Bledsoe,Jr., Kresge Hear. Res. Lab., Dept. Otorhinolaryngol., L.S.U. Med. Ctr., New Orleans, LA 70119.

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

46 DYNAMIC VERSUS STATIC CHARACTERISTICS OF SINGLE AUDITORY-NERVE FIBERS. M. L. Brachman* and R. L. Smith. Institute for Sensory Research, Syracuse University, Syracuse, NY 13210.

Although the operating range of an auditory-nerve fiber can be defined as the range of intensities over which changes in intensity produce changes in response, the observed operating range depends to some extent on the experimental paradigm. Firing rates averaged over intervals of 10 msec or more appear to reflect an inherently static operating characteristic with a small operating range (Smith, R. L., J. Acoust. Soc. Am. 65, 166-179, 1979). For example, in response to stimuli of constant sound intensity, a maximum firing rate occurs at the onset followed by a decay to a quasi steady-state rate within a few hundred milliseconds. The steady-state firing rate typically increases monotonically with sound intensity, and asymptotically approaches saturation within 30 dB of threshold. After subtraction of spontaneous activity, the ratio of onset to steady-state response is independent of sound intensity so that the onset and steady-state responses have the same operating range. In contrast, when the onset firing rate is measured using intervals of 2 msec or less, the ratio of onset to steady-state response increases with increasing sound intensity. In some units the increase continues into the steady-state saturation region, producing a dynamic operating characteristic with a range that exceeds the static range by more than 20 dB. Amplitude modulation by sinusoids reveals a similar increase in operating range. For modulation frequencies of about 200 Hz, period histograms synchronized to the modulating wave form have a sinusoidal shape and provide a measure of response modulation. Response modulation is a nonmonotonic function of average intensity, and the shape and location of the response modulation function can be more accurately predicted from the dynamic than from the static operating characteristic. Hence the use of sufficiently small time windows and/or sufficiently rapid changes in intensity reveals a dynamic operating range that can exceed the static operating range by several orders of magnitude.

47 RESPONSE PROPERTIES OF EI UNITS IN THE SUPERIOR OLIVARY COMPLEX OF THE DECEREBRATE CAT. William E. Brownell, Paul B. Manis*, and Louis A. Ritz. Depts. of Neuroscience and Surgery (ENT), University of Florida, Gainesville, Florida, 32610.

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

Discharge rate could be a monotonically or nonmonotonically increasing function of intensity for ipsilateral BF stimulation. Contralateral BF stimulation produced a monotonically decreasing rate-intensity function as inhibition became more pronounced. Binaural rate-intensity functions were always non-monotonic. For some units it was possible to have a peak of excitation flanked by inhibition for intensities both above and below the peak.

We have recorded from some units before and after administration of anesthetic doses of sodium pentobarbitol. In the few EI units for which we carried out this procedure the most dramatic response to anesthetic was the almost total disappearance of spontaneous activity. Anesthetic also produced an increased response latency. Post-stimulus time histograms showed chopper responses, which were not observed in unanesthetized preparations. Interspike interval distributions became more symmetric. Ipsilaterally driven rate-intensity functions became monotonic, if they were not already so, and binaural rate intensity functions displayed no inhibition. Responses measured after administration of anesthetic were similar to those previously reported for SOC units in anesthetized intact preparations. Quantification of these results will be presented.

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48 AN ACOUSTIC FOVEA IN THE COCHLEA OF THE HORSESHOE BAT. Volkmar Bruns* (SPON: J. A. Simmons). Dept. of Biology, Frankfurt Univ., FRG.

The greater horseshoe bat, Rhinolophus ferrumequinum, emits echolocation cries that have a long (10-100 msec) constant frequency component of about 83 kHz. The auditory system of this bat is very sharply tuned to about 83 kHz, the frequency corresp-ding to the emitted CF component. Of particular importance, the sharp tuning is created in the periphery and correlates with a number of specialized features within the cochlea. The innervation pattern of the cochlear region where the 80-86 kHz frequency band is analyzed is comparable to other mammals. 85% of all afferent fibers run to the inner hair cells and only 15% of the afferent innervation is devoted to the outer hair cells. Sixteen radial fibers converge onto one inner hair cell and one spiral fiber innervates several outer hair cells. Frequency mapping shows that the small frequency band between 80-86 kHz is widely expanded on the basilar membrane (4.1 mm of the total length of 16 mm). These frequencies are represented by 25% of the receptors and 21% of the neurons in the spiral ganglion (3,400 of the total number of 16,000). This means that dispite the normal innervation density per mm of basilar membrane the frequencies between 80 and 86 kHz are highly overrepresented (35,000 neurons/octave). This peripheral expansion of the behaviorally important narrow frequency band bears a striking analogy to the retinal fovea in the visual system. A study of the vibration pattern of the cochlear partition in the "foveal region" revealed an antiphasic motion between the inner and outer segment of the cochlear partition which supports the inner and outer hair cells respectively. Scanning EM studies of this "foveal region" demonstrated enlarged steriocilia and a spacing of the cuticular plates of the inner hair cells. This is interpreted as an additional improvement of the hydromechanical frequency analysis. The data from the studies of the vibration pattern of the basilar membrane and the fine morphological features of the cochlear partition are integrated into a model to account for the mechanical sharpening of the 80-86 kHz frequencies which respresent the "acoutic fovea".

49 GLUTAMATE AND ASPARTATE: EFFECTS ON THRESHOLD AND RESPONSE PATTERNS IN THE COCHLEAR NUCLEUS. D.M. Caspary and D.C. Havey. Division of Neurobiology, Dept. Medical Sciences, Southern Illinois University School of Medicine, Springfield, IL 62708

Recent evidence suggests that glutamate and/or aspartate may be the neurotransmitter(s) at acoustic nerve endings in the cochlear nucleus (CN). Godfrey et al. (J. Histochem. Cytochem. 25:417, 1977) have produced a map of the quantitative distribution of concentrations of these excitant amino acids in the CN. Wenthold (Brain Res. 143:544, 1978) has shown that glutamate and aspartate levels decrease in conjunction with the degeneration of auditory nerve terminals and has also described a calcium dependent release of these amino acids (Brain Res. 162:338, 1979). Bird et al. (Science 202:1087, 1979) have reported that kainic acid, which may be specifically neurotoxic to cells receiving a glutamatergic input causes degeneration of CN neurons.

The response patterns, at best frequency, of CN neurons in chinchilla were examined using poststimulus time and interspike interval histograms. Control histograms were obtained before, during, and subsequent to the application of putative neurotransmitters. Microiontophoretic application of glutamate and aspartate onto neurons in the posterior ventral cochlear nucleus (PVCN) and the dorsal cochlear nucleus (DCN) can induce changes in the response thresholds of these neurons to tone-burst stimulation at best frequency. With the iontophoretic application of glutamate or aspartate some PVCN neurons displaying "on" patterns become responsive to stimulation as much as 15 dB below control threshold. At near-threshold stimulus levels the "on" or onset pattern is observed to change from a brief, phasic response to a sustained response over the duration of the stimulus. At higher iontophoretic currents spontaneous activity may be induced in these neurons which display little or no control spontaneous activity. In response to glutamate or aspartate application, some PVCN "chopper" neurons display threshold shifts and a disruption of the chopper pattern as well as an increase in spontaneous activity. DCN "build-up" and "pauser" neurons also display threshold shifts and some changes in response pattern. These neurons display either a generalized increase in the number of responses during the early portion of the histogram or, for certain "build-up" neurons, the induction of an initial "pauser" peak. For many neurons which are sensitive to glutamate and/or aspartate, the iontophoretic application of these substances mimics the effects of increasing stimulus intensity as well as increasing the spontaneous activity. These findings in conjunction with the previously noted studies lend support to the hypothesis that glutamate and/or aspartate may be the transmitter(s) at acoustic nerve endings in the CN. (Supported by the Deafness Research Foundation and SIU-MS CRC Funds)

50 ASCENDING PROJECTIONS TO THE SUPERIOR OLIVARY COMPLEX OF RAT. J. Coleman, P. O'Connor, A. Wells*, B. Blatchley* and C. Brown*. Depts. of Psychol. and Physiol., Univ. of South Carolina, Columbia, SC 29208.

Basic connectional patterns between various divisions of the rat ventral cochlear nucleus and the superior olivary nucleus have been resolved by anterograde degeneration and Golgi techniques (Harrison and Irving, J. Comp. Neurol., 1966, 126, 51; Harrison and Feldman, Contrib. Sens. Physiol., 1970, 4, 95). The present study was undertaken to examine aspects of neuronal systems providing input to the superior olivary complex of the albino rat using the horseradish peroxidase (HRP) transport technique. Unilateral microiontophoretic injections of HRP into portions of the superior olivary complex were followed by 18-24 hr survival and processing of tissue for HRP histochemistry.

Fibers transporting label could be traced from sites of injection to the ipsilateral and contralateral cochlear nuclei. Although most injections were not confined solely to a single nucleus of the superior olivary complex there was evidence of some specificity of projections. Injections of the medial superior olive resulted in bilateral labelling of large spherical cells of the anteroventral cochlear nucleus (region III of Harrison and Irving). Within the densest foci of labelling, most, but not nearly all of the large spherical cells exhibited reaction product. Little or no reaction product was observed in this area and in other divisions of the cochlear nuclei after control HRP injections in nonauditory areas or in tissue processed without HRP injection. Injections of the medial nucleus of the trapezoid body produced labelled globular cells in contralateral region II of Harrison and Irving. Many globular cells within the most densely labelled clusters of these cells were void of reaction product. In addition, medium sized spherical cells of region I were labelled after injections in the vicinity of the medial nucleus of the trapezoid body. Larger injections of the superior olivary complex resulted in extensive labelling of multipolar neurons of region IV; no labelled cells appeared in region V following any injection of the superior olivary complex. (Supported by NIH Biomedical Research Support Grant 5 S07 RR07160 and the Deafness Research Foundation.)

51 HEARING SENSITIVITY IN AN OSTEOGLOSSOMORPH FISH. S. Coombs. Dept. Anat., Georgetown Univ. Sch. Med. & Dent., Washington, D.C. 20007.

Auditory sensitivity as a function of frequency has been determined using shock avoidance techniques for several specimens of clown knifefish, Notopterus chitala (Family: Notopteridae). The frequency range of hearing is from 100 Hz to over 1500 Hz, with best sensitivity occurring around 500 Hz, where the threshold reaches -50 dB re: 1 dyne/cm^2.

Thresholds as low as -50 dB have been reported for only a few other fish in the teleostean infraclass: several species (including the goldfish, Carassius auratus) all belonging to the superorder Ostariophysii and one other species, Myripristis kuntee, from the squirrelfish family Holocentridae. Best thresholds reported here are at least 20-30 dB lower than those reported for most other teleost fishes. While the frequency range of hearing for N. chitala also extends beyond the high frequency end reported for most teleosts (800-1000 Hz), it does not extend as high as those reported for M. kuntee (3000 Hz) or for ostariophysan species (2000-5000 Hz).

Fish from these three taxonomically unrelated groups, Notopterus, Myripristis, and Ostariophysii, all have in common a non-homologous specialization of the peripheral auditory system: an anatomical connection between the swimbladder and the inner ear. Since for many teleost species the swimbladder has a known sound amplification function, the exceptional threshold sensitivity of fishes from these groups can be attributed to the functional relationship between the swimbladder and inner ear. It is not clear at this time, however, what determines the frequency range of hearing in these fishes. It is possible that characteristics of the swimbladder coupling system and/or properties of the inner ear may play a role in this determination. (Supported by an NIMH predoctoral fellowship and NINCDS Grant NS-15090).

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

The unusually high K^+ concentration of the endolymph facing the apical surfaces of virtually all vertebrate hair cells suggests that K^+ carries the receptor current in these cells (Russell and Sellick {1976}, J. Physiol. 257: 245). Yet large intracellular receptor potentials can be recorded in frog saccular hair cells in vitro when a standard saline solution bathes both apical and basal cell surfaces, and thus when the electrochemical gradient for K^+ is near zero (Hudspeth and Corey {1977}, Proc. Natl. Acad. Sci. U.S.A. 74: 2407). Calcium ion has also been proposed as carrying the receptor current (Sand {1975}, J. Comp. Physiol. 102:27), yet its concentration is extremely low in the endolymph of some species, including the bullfrog.

We have measured the voltage dependence of the receptor current in frog saccular hair cells by voltage-clamping single cells with two microelectrodes, while mechanically stimulating individual hair bundles. The reversal potential for the receptor current, with standard saline on both cell surfaces, is - 2 ± 4 mV. This suggests that the transducer channel permits both Na^+ influx and K^+ efflux: it is relatively nonspecific. In vivo, with endolymph on the apical cell surface, K^+ must, in fact, carry most of the receptor current.

A number of monovalent cations were consequently tested with an in vitro microphonic preparation (Corey and Hudspeth {1979}, Biophys. J. 26: 499), in which the fluid facing the apical hair cell surface may be changed independently of that facing the basal surface. The microphonic potential is maintained when endolymph in the apical chamber is replaced with salines containing 130 mM Li^+, Na^+, K^+, Rb^+, Cs^+, or NH_4^+. The response is reduced with tetramethylammonium, choline, or carbamylcholine ion, and is small but measurable with glucosamine, triethanolamine, or tris(hydroxymethyl)aminomethane ion. If the channel is a pore, the largest of these ions would require a minimal pore diameter of ∿ 0.7 nm. In addition, the receptor current measured under voltage-clamp varies non-linearly with voltage, and the current-voltage relation can be adequately fitted by a model which posits a single energy barrier to ion permeation placed about 40% of the way through the membrane. We suggest that the transducer channel of hair cells is a large, water-filled pore with a "tight spot" near the middle.

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53 AUTORADIOGRAPHIC DEMONSTRATION OF AUDITORY AND VESTIBULAR PATHWAYS IN THE PIGEON BY MEANS OF ANTEROGRADE TRANSNEURAL TRANSPORT. M.J. Correia, A.R. Eden, K.N. Westlund, and J.D. Coulter. Depts. of Otolaryng., Physiol. & Biophys., Psychiat. & Behav. Sci., and the Marine Biomed. Inst., Univ. Tex. Med. Br., Galveston, TX 77550

An equal parts mixture of L-[^{3}H] proline and 1-[6-^{3}H] fucose (50 µCi/µl) was injected into the endolymphatic space of the left membranous labyrinth of each of two White King pigeons. A total amount of 1 µl of the above solution was injected through a micropipette glued into a transsected anterior semicircular duct over a period of 1 hr. Following injection, the cut ends of the ducts were sealed by cauterization and the animals were allowed to survive 15 days. The brain, spinal cord, and labyrinths were fixed with 10% buffered formalin delivered by bilateral transcardiac intracarotid catheterization. Serial paraffin sections (15 µm) were prepared by standard autoradiographic techniques.

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

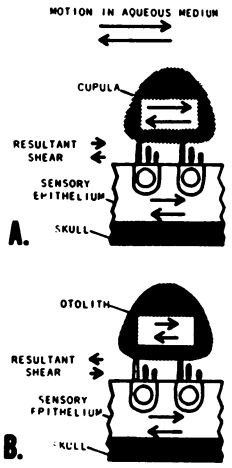
Vestibular structures, ipsilateral to the side of injection, which were heavily labeled were: the vestibular nerve, all six vestibular nuclei, and the cerebello-vestibular lateral process. Less heavily labeled ipsilateral structures included: the medial longitudinal fasciculus, terminations on motoneurons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

Different densities of labeling were found within the oculomotor nuclei. The lateral half of the dorsal part and the majority of the ventral part of the contralateral oculomotor nucleus showed heaviest labeling; whereas, the medial half of the dorsal part of the ipsilateral oculomotor nucleus was labeled the heaviest.

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

54 PARALLEL CHANNELS FOR SOUND DETECTION IN THE FISH EAR. Jeffrey T. Corwin Dept. Neurosci., Sch. Med., UCSD, La Jolla, CA 92093.

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



In the present study unit and population responses in nerve VIII have been evoked by acoustic stimulation of relatively intact sharks. The initial population response to a click is composed of a series of brief high amplitude peaks lasting 5-10 msec. Certain peaks are believed to originate at the macula neglecta, as they are reversibly altered by covering the fenestra ovalis, a sound portal important in macula neglecta stimulation. Other peaks are believed to originate at otolithic maculae, as they are unaffected by this manipulation. The response characteristics and the anatomical distribution of units within the VIIIth nerve also suggest origins at different detectors. Thus parallel channels may be active in audition by sharks, and as a result of their dissimilar modes of stimulation they may provide different types of information concerning the nature of acoustic stimuli.

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55 RESPONSES TO THE DIFFERENCE TONE IN THE ANTEROVENTRAL COCHLEAR NUCLEUS OF THE CAT. John Dickson, Robert Wickesberg* and Mary Morton Gibson, Dept. of Neurophysiology, Univ. of Wisconsin, Madison, Wisconsin, 53706.

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

With the difference tone frequency and the levels of the two primaries held constant, one might expect that the discharge rate would be independent of the primary frequencies. This, however, is not the case: the response varies with primary frequency, sometimes from a negligible response to saturation. Both enhancement and diminution of the response could be accounted for by production by the primaries of other combination tones such as $2f_1$-f_2, $3f_1$-$2f_2$, etc. Thus enhancement could result when a combination tone falls within the response area of the neuron, while reduction could result when it falls within the suppression region. It seems unlikely, though, that increases and decreases in responsiveness at higher primary frequencies could be altogether accounted for by interference with higher order distortion products. In some cases, there is an overall decrease in response with increasing stimulus frequency which may be the result of middle ear filtering. (Supported by grant NS-12732).

56 ULTRASTRUCTURE OF NEURONS AND NEUROPIL IN THE ACOUSTIC TUBERCLE OF THE RED-EARED TURTLE. A.B. Drakontides and R.H. Browner. Dept. of Anat., New York Medical College, Valhalla, NY 10595.

Audition is dependent upon the presence of a minimal core auditory pathway. Essential auditory structures, such as the cochlear duct and basilar membrane are first evident in reptiles. Since reptiles are also the stem stock for all amniotes, the study of the auditory system in this Class has proven to be of increasing significance in the understanding of the hearing mechanism.

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

The cytoplasm of "clear" cells has a diffuse Nissl substance, the rough endoplasmic reticulum appearing in single, isolated rows. Mitochondria are small (270 nm diam.) and dispersed throughout the cytoplasm; numerous lysosomes (900 nm) are usually present. The Golgi complex is present characteristically around the circumference of the nucleus. The eccentric nucleus lacks chromatin particles and has a clear vesicular appearance, while the nucleolus appears as a typical dense, spherical mass. Portions of the periphery of these "clear" cells are often encased by lamellae. This is especially evident when the cell abuts on another cell body. Axo-somatic and dendro-somatic synapses are seen, often surrounded by astrocytic processes. In addition to a dense concentration of mitochondria within dendritic terminals, mitochondrial clusters are also present within dendritic spines. The apposing synaptic membranes present an array of various specializations which have been previously described in a number of species. The majority of sampled synaptic morphology indicates a chemical means of transmission. At least 20% of axon terminals contain large dense-cored vesicles (1300Å), in addition to clear-core vesicles (500Å diam.). These dense-cored vesicles are usually described as neurosecretory. Still to be determined are the cells of origin of these terminals and their synaptic relations.

57 ADAPTATION IN A VERTEBRATE HAIR CELL: STIMULUS-INDUCED SHIFT OF THE OPERATING RANGE. R. A. Eatock*, D. P. Corey* and A. J. Hudspeth, Division of Biology, California Institute of Technology, Pasadena, CA 91125

The operating range of hair cells in the bullfrog sacculus, measured as the hair bundle displacement which brings the receptor current from its minimal to its maximal value, is only 0.3 μm, or 2° of hair bundle bend. Larger displacements might thus be expected to move the cells out of their sensitive range, rendering them unresponsive to further stimuli. Instead, we observe that the hair cell's operating range shifts in such a way as to restore sensitivity during maintained displacement, and that this form of adaptation involves calcium ion.

The operating range of hair cells was determined in vitro by giving step displacements to the otolithic membrane, which is attached to the cells' hair bundles, and measuring the resultant transepithelial currents. For small regions of sacculus that encompass hair cells of nearly uniform orientation, the operating range is about 0.4 μm. Adapting steps of various amplitudes and durations were delivered, and the shift in operating range measured by giving another set of test steps at various times during or after the adapting step. For a displacement that increases the receptor current, the adaptive shift in operating range is such as to reduce it; a step in the opposite direction, which initially eliminates the receptor current, causes a shift such that responsiveness returns. The sigmoidal shape of the operating curve is largely unaffected. For displacements in either direction, the time course of the adaptive process is approximately exponential with a time constant of 10 - 50 ms. This process thus acts as a high-pass filter with a rolloff frequency around 5 Hz. The rate of adaptation varies with Ca^{++} concentration at the apical surface of the preparation, being greater for higher Ca^{++} concentrations and small if Ca^{++} is replaced with Sr^{++}.

These observations have been extended with intracellular recordings in which the operating ranges of individual cells were determined with 10-Hz stimulation of their hair bundles. The tips of the bundles were displaced by as much as 4 μm, held until the response returned, and fixed in situ. Scanning electron microscopy confirmed that individual cells, once adapted, can respond to stimuli even with their hair bundles bent by as much as 30° in either direction.

Thus, by shifting their operating range, hair cells are able to adapt to saturating static stimuli in such a way as to maintain high sensitivity to small, superimposed stimuli.

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58 PRENATAL OTOTOXICITY OF KANAMYCIN IN THE CHICK. C. D. Fermin and G. M. Cohen. Department of Biological Sciences, Florida Institute of Technology, Melbourne, Florida 32901.

Recently several investigators showed that kanamycin exerts similar ototoxic actions during intrauterine life as in the adult. In order to compare the avian ototoxic response to the mammalian, we injected kanamycin sulfate into the yolk sac of White Leghorn embryos on the 7th day (stage 31) of the 21 day incubation period. Cytoplasmic damage, though widely varying and often extensive, occurred in all lagenar cells. By the 15th day (stage 41), tall hair cells, which morphologically resemble mammalian outer hair cells, frequently show severe damage but the short hair cells display little, if any, damage. The cytoplasmic texture, which is pocked by exploded mitochondria and empty spaces, alternates between coarse and clear areas. Organelles lose their usual arrangement and regroup. Osmiophilic granules, believed to be lysosomes, lie below the cuticular cone and are occasionally incarcerated within it; the stereocilia show no evidence of damage. The synaptic vesicles that normally form a symmetrical ringlet around the synaptic bar largely disappear. The pre- and post-synaptic membranes lose density, as does the subsynaptic web; the intersynaptic material disperses. Depending upon whether their terminals are mildly or severely damaged, afferent nerves either bloat or explode; efferent nerves, which form synaptic contacts several days later than afferent, show negligible evidence of intoxication. Throughout intoxicated supporting cells, circular zones of clear cytoplasm that are usually bounded by membranes or dense borders presage the empty holes. In the basal region, the endoplasmic reticula (ER) are irregularly arranged and do not form coiled patterns as in normal cells. In the neck, the Golgi complexes lose their concentric arrangement and disperse. Kanamycin, which apparently intoxicates along a gradient, damages tegmental cells above the inferior fibrocartilaginous plate more severely than those above the superior. Tegmental dark cells leave a mosaic of large spaces as they withdraw their tangle of snugly fitting cytoplasmic processes; in light cells the sequence precedes similarly but less conspicuously because of their limited processal number. In intoxicated cells of both types, levels of glycogen increase, though to a greater extent in dark cells. The tightly stacked columnar cells pull apart when intoxicated, leaving large intercellular spaces that occasionally contain cellular debris and blind processes filled with glycogen and fragments of rough ER. Because damaged columnar cells retain their fibrillar matrix, cytoplasmic organelles remain fixed at the sides, basal, and luminal ends. The tectorial membrane does not detach from the relatively undamaged luminal end. In necrosing columnar cells, the fibrillar matrix congeals to form dense spicules.

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59 RESPONSE PROPERTIES OF SINGLE AUDITORY UNITS IN THE OYSTER TOADFISH (OPSANUS TAU). Michael L. Fine, Robert R. Capranica, Section of Neurobiol. & Behavior, Cornell Univ., Ithaca, NY 14850.

To date, no studies have compared the response properties of auditory units with the parameters of communicatory sounds produced by any fish. Because of the large body of descriptive and experimental studies on sound production in the toadfish Opsanus tau, it is an ideal species for such a study. Single units were isolated extracellularly from saccular branches of the auditory nerve in submerged toadfish in response to underwater sounds. Tuning curves are complex and variable, and best frequencies (BFs) range from 25 to 90 Hz. There is a strong modal peak at 40 Hz and 83% of the units have BFs between 25 and 40 Hz. The thresholds of the most sensitive units are below -23 dB (re: 1 dyne/cm^2) at 40 and 90 Hz. The upper tails of tuning curves vary from ones that increase monotonically with frequency to others that exhibit plateaus or even regain sensitivity. These secondary regions of sensitivity often occur at 200-250 Hz, where most sensitive thresholds are near 0 dB. Above 250 Hz sensitivity falls off rapidly.

The response properties of a unit do not depend on its BF. Maximal spike rate and slope of spike-rate curves increase and latency to tone bursts decreases with increasing frequency regardless of a unit's BF, and dynamic range is not necessarily greatest at BF. At BF, units are tonically active and phase locked with no short-term adaptation during a 300 ms stimulus. After stimulus termination, units with high rates of spontaneous activity exhibit momentary suppression, while those with low spontaneous rates (37 spikes/sec or fewer) can remain suppressed for periods exceeding half a second.

No information exists on how the toadfish utilizes sound of 40 Hz and below. The grunt, an agonistic call produced by both sexes, has a fundamental frequency of 90 Hz and is matched to the upper end of the fish's most sensitive auditory region. The sound pressure level of the male's mating call, the boatwhistle, is 40 dB at 1 meter, and its fundamental frequency varies from under 150 Hz to over 250 Hz, depending on temperature and assumed hormonal state. Even though the toadfish has no neurons primarily tuned to the boatwhistle, behavioral and single-unit data indicate the fish is quite capable of hearing it. This mismatch may enable toadfish to respond preferentially to the relatively loud boatwhistle of nearby callers, since it is difficult to localize distant sounds underwater.

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

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

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

FIG. 1

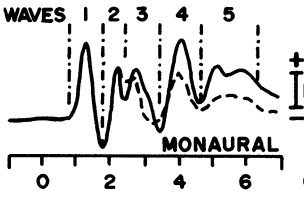
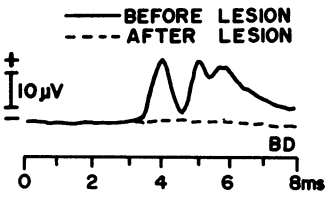


FIG. 2



61 A PRELIMINARY REPORT ON THE ORIGIN(S) OF THE BINAURAL-INTERACTION COMPONENT OF THE BSER IN THE GUINEA PIG. John N. Gardi* and Charles I. Berlin* (Spon: C. Norris). Kresge Hear. Res. Lab, Dept. Otorhinolaryngol., LSU Med. School, New Orleans, LA 70119.

An ablation technique under visual control was used to clarify the possible sites of origin of the binaural-interaction-component of the brainstem auditory evoked response in guinea pigs (BIC-BSER). The BIC is obtained by summing BSERs elicited by monaural clicks to each ear and subtracting this sum from the BSER elicited by binaural clicks. Typically, the BIC waveform is biphasic (negative - positive). At stimulus intensities of 80 dB SPL, latency values are 3.5-4.0 ms and 4.5-5.0 ms, respectively (Dobie and Berlin, Arch. Otolaryngol. in press and Clopton, personal communication).

Under urethane anesthesia (20%, I-P injections), the scalp and skull were removed. Cortex overlying the inferior and superior colliculi was aspirated to give a clear, unobstructed view of the colliculi. Scalp-recorded monaural and binaural BSERs were collected at this point and compared to pre-surgical controls. If no change in the waveforms was observed, the ablation procedure was continued. After the colliculi had been bilaterally aspirated, a series of final recordings was made.

Among the results were the following: 1. The BSER remained virtually unchanged following collicular aspiration. 2. The binaural-interaction-component, likewise, was not significantly affected or distorted by this direct ablation procedure.

These results suggest that although the inferior colliculus is a powerful integration center for the processing of binaural signals, the neural structures necessary for the maintenance of the BIC must be located caudal to the colliculi yet rostral to the cochlear nuclei. Based on a myriad of previous micro-electrode binaural-interaction studies, the nuclei of the superior olive are to be expected to be most vital to the maintenance of the scalp-recorded binaural-interaction components. Future investigations will attempt to pinpoint these sources. (This research was supported by NIH NS-11647 and NS- 07058. Laboratory facilities were provided by the Kresge Foundation.

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

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

The responses of single cells with low spontaneous firing rates were recorded extracellularly in the anteroventral cochlear nucleus of barbiturate anesthetized cats. The stimulus generally consisted of 500 msec. best-frequency tone bursts presented once per second. The sound pressure level (SPL) was varied in 1dB steps from below threshold to 10 or 20 dB above threshold with 100 presentations at each SPL.

We define threshold as that SPL at which 50% of the tone presentations evoke at least one spike. When so defined the SPL at threshold is an orderly monotonic function of the duration of the sampling time, i.e., the time following stimulus onset during which the spike is accepted as a response. Within limits, the longer the sampling time, the lower the threshold. The magnitude of the effect seems commensurate with the psychoacoustic observations.

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63 DISTRIBUTION OF ENZYMES OF ACETYLCHOLINE METABOLISM IN THE RAT COCHLEAR NUCLEUS. D.A. Godfrey and F.M. Matschinsky*. Dept. Pharmacol., Washington U. Sch. Med., St. Louis, MO 63110.

The involvement of acetylcholine as a neurotransmitter in the rat cochlear nucleus was assessed by measuring the activities of the enzymes responsible for its synthesis and degradation: choline acetyltransferase (ChAc) and acetylcholinesterase (AChE). Application of radiometric assays for samples of freeze-dried tissue weighing less than one microgram, and quantitative histochemical mapping procedures, provided maps of the distributions of the enzyme activities within the cochlear nucleus and other nearby locations. Averaged data are presented in table form as mean ± SEM (no. of rats). (DCN, PVCN, AVCN: dorsal, posteroventral, anteroventral cochlear nucleus; CbF: cerebellar flocculus.)

	Enzyme activity in amt./kg dry wt./min.	
Region	ChAc (μmoles)	AChE (mmoles)
Granular region, DCN	311 ± 91 (3)	54 ± 11 (3)
Granular region, PVCN	214 ± 42 (4)	44 ± 2 (4)
Granular region, AVCN	319 ± 35 (3)	52 ± 2 (3)
DCN molecular layer	214 ± 29 (4)	42 ± 3 (4)
DCN fusiform-cell layer	375 ± 34 (4)	62 ± 3 (4)
DCN deep region	295 ± 18 (3)	42 ± 5 (4)
PVCN	292 ± 44 (4)	25 ± 1 (4)
AVCN caudoventral part	450 (1)	28 (2)
AVCN caudodorsal part	611 (1)	39 (2)
AVCN rostral part	351 ± 41 (4)	39 ± 5 (3)
Auditory nerve root	30 (2)	2.5 (2)
Trapezoid body	125 ± 33 (4)	4.8 ± 0.4 (3)
Acoustic striae	216 ± 30 (3)	25 ± 4 (4)
CbF molecular layer	27 ± 4 (4)	5.4 ± 0.7 (3)
CbF granular layer	93 ± 25 (4)	62 (2)
CbF white matter	95 (2)	30 ± 9 (3)
Facial nucleus	4735 ± 376 (3)	169 ± 13 (3)
Facial nerve root	6619 ± 1035 (4)	46 (2)
Spinal trigeminal tract	5.1 (2)	2.2 ± 0.4 (3)

Comparison of the data for auditory nerve root, trapezoid body and acoustic striae with that for facial nerve root implies that axons in the first- and second- order auditory pathways are overwhelmingly non-cholinergic. Within the cochlear nucleus, relatively high activities of both enzymes were found in the fusiform-cell layer, but these activities are much lower than those in the facial nucleus. On a quantitative basis, the distributions of choline acetyltransferase and acetylcholinesterase activities differ considerably from those reported previously for cat cochlear nucleus. The results are consistent with a function of acetylcholine as a neurotransmitter in the rat cochlear nucleus, probably mostly at synapses of interneurons and/or feedback pathways.

64 INTRACELLULAR AND EXTRACELLULAR POTENTIALS IN THE ORGAN OF CORTI OF THE MONGOLIAN GERBIL. David A. Goodman* and Robert L. Smith (SPON: J. J. Zwislocki). Institute for Sensory Research, Syracuse University, Syracuse, NY 13210.

Glass microelectrodes were inserted through scala tympani into the organ of Corti of anesthetized Mongolian gerbils and aimed toward the inner hair cells. The resting and sound-evoked potentials encountered were consistent with those reported by Russell and Sellick (J. Physiol. 284, 261-290, 1978) in the guinea pig with respect to resting potentials and response magnitude, polarity, and frequency-selectivity. As in that study two classes of cells were encountered. Cells contacted first in a penetration, presumably supporting cells, had large resting potentials of -70 to -100 mV. In these cells the depolarizations in response to sound (summating potentials) were less than 3 mV. The second class, presumably inner hair cells, had smaller resting potentials of -15 to -45 mV and large summating potentials of 5 to 15 mV. The extracellular summating potential increased as the electrode advanced and reached up to 3 mV in the vicinity of the inner hair cells where it exhibited a frequency-selectivity comparable to that of auditory nerve fibers. With further small advances of the electrode, scala media was entered as evidenced by the positive endolymphatic potential and a polarity reversal of the summating potential. The magnitude of the summating potential measured in the extracellular space near a supporting cell was generally smaller than that measured in the cell. A possible explanation for this inequality is that the summating potential spreads passively through electrically coupled supporting cells with a space constant exceeding that of the extracellular space.

65 PHYSIOLOGY OF MNTB NEURONS RECEIVING LARGE ENDINGS John J. Guinan Jr.* (SPON: N. Y. S. Kiang). Eaton-Peabody Laboratory of Auditory Physiology, Mass. Eye and Ear Infirmary, Boston, MA. 02114.

In the medial nucleus of the trapezoid body (MNTB) each principal neuron receives one large ending (calyx of Held), and many small endings. MNTB units often have unusual waveforms, typically with a positive first component (C1) followed 0.5 ms later by a negative second component (C2). Electric shocks in the region of the axons that give rise to calyces in the contralateral trapezoid body (CTB) evoke C1+C2 responses in which the latency of C1 is between 0.1 and 0.3 ms. Because these latencies are too short for there to be an intervening synapse, C1 must represent activity in a presynaptic element, presumably a calyx of Held (the only large ending nearby). Electric shocks in the ipsilateral nucleus of the lateral superior olive (LSO), a region to which MNTB neurons project, evoke a spike with a latency of 0.1 to 0.3 ms. This "A spike" must be an antidromic activation of the same neural element as a C2 spike because they have similar waveshapes and mutually interact showing refractory effects when LSO and CTB shocks are combined. C2 almost certainly represents activity from a postsynaptic element such as the soma of the principal neuron because C2 can be evoked antidromically (in the form of an A spike) but normally follows C1 by a time appropriate for a synaptic delay. In dozens of cats under the effects of Dial-urethane and in two under ketamine, both spontaneous and sound-evoked responses in units with C1+C2 waveshapes never had C1 or C2 components alone. Occasionally CTB shocks evoked a long latency (1-3 ms) "LL spike" which, by refractoriness and waveshape, is from the same neural element as C2 and A spikes. Since LL spikes do not follow every shock and have long variable latencies, it seems likely that a synapse is interposed between the axons stimulated and the elements producing LL spikes. Thus an LL spike appears to be an orthodromic, postsynaptic spike from a neuron which receives a large ending. Since LL spikes are not preceeded by C1 spikes, LL spikes most likely are mediated through small endings. Further work is needed to determine the significance of LL responses and small synapses on principal neurons in acoustic signal processing.

66 PHYSIOLOGY AND ANATOMY OF SINGLE CELLS IN THE CAT INFERIOR COLLICULUS STUDIED WITH THE TECHNIQUE OF INTRACELLULAR RECORDING AND INJECTION OF HORSERADISH PEROXIDASE. L.B. Haberly, S. Kuwada*, T.C.T. Yin, and D.C. Mountain. Depts. of Neurophysiology and Anatomy, Univ. of Wisconsin Medical School, Madison, Wis. 53706

We report here preliminary data on the morphology and physiology of neurons in the inferior colliculus of the cat. Single cells were impaled with beveled glass micropipettes filled with a buffered HRP solution. Intracellular potentials in response to auditory stimulation were recorded followed by iontophoresis of HRP. Cats were perfused and frozen sections (80μm) processed by the $CoCl_2$-DAB procedure. A small number of neurons has thus far been studied physiologically and injected successfully with no visible traces of extracellular leakage of HRP or damage to the neuron, but with an apparently complete labeling of dendritic trees and extensive labeling of axonal arborizations.

There is a marked variation in the morphology of neurons injected. Cell somas ranged in size from approximately 12-35 μm in diameter. Dendritic trees conforming to Rockel and Jones' (JCN 147:11) fusiform, bitufted, large, and multipolar categories have been labeled. In most cases these dendritic trees appear considerably more extensive than in previous Golgi studies, presumably since serial reconstruction through many sections could be readily performed on the present material. An interesting feature of the dendrites of all neurons studied was the presence of varicosities. The extent to which these varicosities are artifactual is unknown, although pyramidal cells injected and processed with identical techniques show no evidence of dendritic beading. Many dendrites are of extremely small diameter. Dendritic shafts between varicosities (often widely spaced) can be less than 0.5μm in diameter for distances of 100μm or more. Dendritic spines are always present but their morphology and numbers vary greatly in different neurons.

Extensive axonal arbors within the colliculus were found for all neurons injected. In one case the local arborization of a large cell, whose axon entered the brachium of the inferior colliculus, gave rise to more than 2000 presumed terminal swellings within the central nucleus.

In all cases the physiological properties of the cells were characterized by their intracellular responses to sinusoidal tone bursts delivered dichotically at the cell's characteristic frequency before injection. Some cells were found to be sensitive to the interaural phase difference. The spike discharge properties of the cells could sometimes be inferred from the postsynaptic membrane potentials. However, our sample of cells in which both the physiology and morphology are well-studied is still too small to draw correlations between structure and function.

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67 ACOUSTIC ANALYSIS OF MATERNAL RETRIEVAL TO KITTEN STRESS CALL. J.B. Harrison*, J.S. Buchwald, and R.J. Norman, C. Hinman*, Dept. Physiology, Mental Retardation Research Center, Brain Research Institute, University of California, Los Angeles, California.

Maternal retrieval in response to kitten stress calls has been used as a means of determining significant features of this complex stimulus. The test subject was placed in a cylindrical chamber in which there were two wall apertures of approximately 6 x 6 cm covered by opaque curtains. Experimental sessions consisted of twelve 2 min trials separated by inter-trial intervals of 5 min with no more than 2 sessions per week per subject. During each 2 min trial, one of 5 test stimuli was continously delivered: stress calls from a vocalizing kitten within one of the wall-mounted boxes, tape-recorded stress calls from one of the two speakers, or synthesized components of the stress call. These stimuli were delivered pseudo-randomly so that presentations of the vocalizing kitten always occurred throughout the session to mitigate against habituation effects. "Retrieval" responses were scored when the test subject's head contacted the curtain of the box from which the call originated. Twenty-two adult females were tested with these stimuli during the month immediately before and after parturition. None of the animals retrieved in the absence of a call stimulus. The highest levels of retrieval occured post-natally to the vocalizing kitten. A response gradient to the kitten, the taped kitten call, and the synthesized components was consistently seen in all animals. There was no difference in retrieval levels to a sequence of taped stress calls compared to their digitized version. A single repeated call was also an effective stimulus. To determine what aspects of the call were most effective, digitized components of the call were played forward or backward, spectrally altered by high pass and low-pass filtering, or temporally blocked into FM and sustained epochs.

Taken together, our results indicated that the kitten stress call seems preeminent over visual, olfactory or thermal cues as a trigger for maternal retrival, since retrievals persisted at relatively high levels when tape recorded calls were substituted for the kitten. On the other hand, synthetic, stimuli which lacked certain aspects of harmonic structure, resonance and band width in the tape recorded calls induced little or no retrieval. (Supported by USPHS HD-05958 and HD-04612).

68 NEURONAL RESPONSES TO THE KITTEN STRESS CALL IN CAT MEDIAL GENICULATE NUCLEUS. C. Hinman*, J. Buchwald, J. Harrison*, and R. Norman, Dept. of Physiology, Mental Retardation Research Center, Brain Research Institute, University of California Medical Center, CA.

Anatomical studies in the cat have demonstrated direct connectivity between the medical geniculate body (MGB) and several cortical areas. While the ventral nucleus of the MGB projects to primary auditory cortex, the dorsal nucleus projects primarily to the insulo-temporal cortical area. Insulo-temporal cortex in the cat appears to be necessary for some kinds of complex acoustic processing which do not require primary auditory cortex (Dewson, J.H., Science 144: 555, 1964). Moreover, regions within the dorsal MGB are relatively unresponsive to clicks or pure tones. Thus, different kinds of ascending acoustic information may be transmitted by the dorsal MGB-insulo-temporal system and the ventral MGB-primary auditory cortex system. In order to investigate this problem, we have been recording MGB single unit responses in the awake, unanesthetized cat to species-specific vocalizations which are known to produce behavioral responses in the cat. A maternal retrieval assay of the kitten stress call has been used to determine which aspects of the call have behavioral significance. (See Harrison et al, this volume) Forward and reverse time segments extracted from the call were used as stimuli in addition to filtered calls (high/low, band-pass/reject) and individual FM call components. Responses to these complex stimuli were compared with responses of the same units to clicks and to pure tone bursts at several intensities. Preliminary data suggest that correlations can be made between gross MGB anatomical subdivisions and unit response selectivity for isolation call components. Examples will be presented from different classes of responsive units, and their implications for central auditory processing discussed. (This work supported by USPHS grants HD-05958 and HD-04612).

69 PATTERNS OF IPSILATERAL AND CONTRALATERAL CORTICOCORTICAL INPUT TO AI ARE RELATED TO BINAURAL AND BEST FREQUENCY MAPS. Thomas J. Imig and Richard A. Reale. Department of Neurophysiology and Waisman Center on Mental Retardation and Human Development, University of Wisconsin Medical School, Madison, WI 53706.

The primary auditory cortical field in cat receives input from a number of other cortical areas in the same and opposite hemispheres. Using the axoplasmic transport of tritiated amino acids we have studied the crossed projection from the anterior (A) auditory field and the ipsilateral projections from the anterior and posterior (P) auditory fields upon AI. In each experiment best frequency maps were obtained to serve as guides for placement of isotope injections. Labeling of each of these pathways produces dense aggregates of silver grains in several locations within AI which are separated from each other by areas of less dense labeling. These projections connect similar portions of the best frequency representations in each field. In tissue sections cut parallel to the flattened cortical surface dense aggregates of silver grains could often be seen which occupied patches of cortex elongated along the low-to-high best frequency gradient. Thus the patchy distribution of corticocortical input and the elongation of the patches appears similar to the terminal patterns formed by callosal fibers interconnecting the primary auditory fields in each hemisphere (Imig and Brugge, JCN, 182:637-60, '78).

We have studied the relation between the ipsilateral projection patterns from field A and the binaural organization of AI. In some experiments each physiologically defined suppression column corresponds with a region of dense labeling and each physiologically defined summation column corresponds with a region of sparse labeling. This is exactly opposite to the pattern formed by terminals of callosal fibers which interconnect the primary fields (ibid.). However more complex patterns of labeling were seen in other experiments in which suppression columns still appeared to be the major recipients of the projection but in addition dense projections were also seen to some summation columns.

Variation was also seen in the pattern of projection from field P upon AI. Injections into the same isofrequency strip produced dense elongated bands of label in the center of AI in some experiments while in others labeling was confined to regions near the dorsal and ventral borders. This variability in labeling suggests that neurons which differ in location along an isofrequency strip in field A or P may also differ in their pattern of projection upon field AI. (NSF 76-19893, HD-03352)

70 PLASTICITY OF BINAURAL INPUT TO NEURONS IN CAT INFERIOR COLLICULUS. D. R. F. Irvine* and D. R. Moore* (SPON: J. F. Brugge). Neuropsychology Lab., Dept. Psych., Monash Univ., Clayton, VIC 3168, Australia.

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

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

71 VISUALIZING THE RABBIT AUDITORY PATHWAY WITH ^{14}C 2-DEOXY-D-GLUCOSE. L. Sargent Jones and J.F. Disterhoft, Department of Anatomy, Northwestern Univ. Med. Sch., Chicago, Ill. 60611.

Previous work in mice has shown that the ^{14}C 2-deoxy-D-glucose (2-DG) technique can be applied to studies of the auditory pathway (Jones and Disterhoft, '79). The present work in rabbit is part of an examination of metabolic changes which may be evident during tone signalled nictitating membrane conditioning in rabbit. Male albino rabbits were restrained with their heads non-traumatically immobilized in an IAC single walled acoustic chamber. A silastic earmold fitted to the rabbit's left ear positioned the earphone, sound measuring microphone and calibrated probe tube assembly close to the eardrum. 50 msec. tone pulses with 10 msec. rise fall times were presented one per sec. at 2, 8 and 20 KHz, 30 dB above the rabbit's behavioral threshold (Martin et al., '77). 45 min. after an IV injection of 2-DG the brain was removed and sectioned as previously described for mice.

All of the auditory nuclei caudal to the medial geniculate nucleus (MGN) were labeled by the 2-DG in all three conditions. Differences between them were seen as differences in the overall pattern of 2-DG uptake rather than in the amount. Cochlear nucleus (CN), lateral superior olive (LSO), medial superior olive (MSO), medial nucleus of the trapezoid body (MNTB), nuclei of the lateral lemniscus (LL), and the inferior colliculus (IC) all demonstrated relatively high metabolic levels in the control. The dorsal CN always showed the highest uptake in the CN. The lateral curve of the LSO was more heavily labeled than the rest of the nucleus; this was less noticeable in the control. The MSO and the LL were labeled less than the other auditory brainstem nuclei under all conditions, while the MNTB was always highly labeled. The IC pattern of activation varied among conditions. In the control, metabolic activity was high in the ventromedial portion of the IC as was shown in mouse. White noise stimulation activated the whole central and external nuclei, particularly contralaterally. Although the entire central nucleus was activated, bands of greater uptake, usually three in number, ran dorsomedial to ventrolateral. They were particularly marked after pure tone stimulation when densely labeled bands with adjacent bands of reduced activity were seen. This pattern may have been the metabolic result of concentrations of excitatory and inhibitory neuronal activity caused by repeated pure tone presentations. MGN was labeled more heavily than surrounding thalamic regions but there was no striking difference among conditions. As in mouse, rabbit auditory cortex showed no substantial nor organized labeling.

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72 CYTOARCHITECTURE OF THE DORSAL NUCLEUS OF THE LATERAL LEMNISCUS (DNLL) OF CATS. Eileen S. Kane. Dept of Anat., Univ. of Mass. Med. Sch., Worcester, MA 01605.

The boundaries, nerve cell types and neuronal distributions in the dorsal nucleus of the lateral lemniscus (DNLL) were studied in several sets of Nissl-stained or Golgi-impregnated mature cat brains, cut in one of the three standard planes. Maps of DNLL neurons and their orientations in Nissl sections showed a nearly cubical nucleus with maximal dimensions of 1.1 mm (dorsoventral) X 1.1 mm (mediolateral) X 1.5 mm (rostrocaudal). DNLL neurons are clustered with individual clusters separated by thick fascicles of lemniscal axons. The width:length (W:L) ratios of single neurons provided three classes based on somatic shape: elongate (E) = W:L ratio $\leq$ 0.65, ovoid (O) = 0.65 < W:L ratio <0.80, and round (R)=W:L ratio $\geq$ 0.80. Three somatic size categories were present within each shape category such that large cells (L) were 21-30 µm X 26-45 µm, medium (M) cells were 11-20 µm X 16-25 µm, and small (S) cells were 5-10 µm X 10-15 µm. Counts of DNLL neurons within each of the resulting nine classes showed the following percent distributions: LR = 12%, LO = 24.5%, LE = 13.5%, MR = 17.5%, MO = 17%, ME = 14%, SR = 1%, SO = 0.5%, and SE = 0%. Dendritic orientations of Golgi-impregnated neurons were defined for six of the nine categories and showed a preferred horizontal orientation for LR, LO, ME and MO cells, preferred vertical orientation for most LE neurons, and a radiate orientation of neuronal dendrites in the MR class. The tendency toward horizontal vectors of both large and medium-sized neuron receptive fields suggests possible divergent inputs from lemniscal axons of several frequencies. Our findings suggest an additional horizontal organization to the DNLL that may be superimposed upon the dorsoventral tonotopic organization shown in other studies. That horizontal component may reflect known phase sensitivity or broad tuning of individual DNLL neurons.

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73 DEVELOPMENT OF CENTRAL AUDITORY FUNCTION IN THE RAT: A [^{14}C] DEOXYGLUCOSE STUDY. Jack Kelly and Leslie C. Skeen. Department of Psychology, Carleton University, Ottawa, Ontario, K1S 5B6, and Department of Psychology, University of Delaware, 19711.

The [^{14}C]-deoxyglucose method was used to examine the course of development of the auditory system in the rat. The distribution of labeled deoxyglucose in the brain was determined following auditory stimulation of infant rats at ages 8, 11, 14, 17 and 20 days. These ages were selected to cover the period of most rapid development of auditory function as estimated from physiological and behavioral studies. In addition 30 and 60 day animals were included to provide reference points for post weaning ages. Following either intraperitoneal or intravenous injections of deoxyglucose the animals were placed inside a small cage in a sound-proofed room and were stimulated for 45 minutes from an overhead loudspeaker with an 80 dB SPL noise containing frequencies from 2 to 40 kHz. Frontal sections were taken through all levels of the auditory pathway and autoradiographs were made on X-ray film. Cresyl violet stains of selected sections provided histological verification of auditory structures. For control purposes, cases with ear blocks and without auditory stimulation were also examined. The results showed no stimulus-related response in the central auditory system of 8 or 11 day animals although a distinctive pattern of activity could be seen in auditory structures even without stimulation. The earliest response to auditory stimulation was found in 14 day animals, and was most noticeable in the cochlear nucleus and inferior colliculus. The superior olivary nucleus was very active in both stimulated and non-stimulated conditions and did not provide a reliable basis for determining the onset of auditory function. Stimulus-related responses in the cochlear nucleus and midbrain auditory areas were more pronounced at older ages, but again activity in the superior olive was high without stimulation. At all ages forebrain responses were less clear than brainstem responses and little contrast was found between stimulated and non-stimulated conditions. Evoked potential studies of auditory sensitivity and behavioral studies of auditory spatial orientation were carried out to complement the anatomical studies with deoxyglucose. Generally, the results of the deoxyglucose study corresponded closely with both behavioral and physiological indices of the onset and development of hearing in the rat.

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74 THE TORUS SEMICIRCULARIS IN THE TOKAY GECKO, GEKKO GECKO. M.C. KENNEDY AND R.H. BROWNER. Dept. Biol., New York Univ., New York, NY 10003; Dept. Anat., New York Med. Coll., Valhalla, NY 10595.

The cytoarchitecture of the torus semicircularis in the Tokay gecko was examined in Nissl and fiber stained brains (Klüver-Barrera method) sectioned in the three standard planes. Tissue impregnated using a modified Golgi-Kopsch technique was embedded in Araldite and sectioned in the three standard planes at 120-160 um for use in the analysis of neuronal morphology.

From a superficial position under the cerebellum, the torus semicircularis continues rostrally, extending under the caudal half of the optic tectum and causing a distinct bulge in the floor of the tectal ventricle. Caudally, the two tori abut upon one another; as they proceed rostrally, they diverge. The torus semicircularis is composed of a central nucleus, a laminar nucleus and a superficial nucleus.

The central nucleus is an ovoid mass of pyriform, fusiform, triangular and oval neurons. These neurons exhibit dendritic arrays which ramify mainly within the central nucleus. The complexity of the dendritic branching increases at more caudal levels, as does the incidence of dendritic spines. At its center, the central nucleus includes a number of large neurons with extensive dendritic fields and numerous dendritic spines. Some of the cells of the central nucleus have dendrites which project ventrally into the tegmentum.

The laminar nucleus is a sheet, 3-5 cells thick, which bounds the central nucleus on its medial, ventral and dorsomedial aspects. The nucleus is populated mainly by fusiform neurons with dendrites oriented parallel to the boundaries of the central nucleus. These processes exhibit a paucity of dendritic spines.

The caudal superficial nucleus is present dorsal, dorsolateral and caudal to the central nucleus. It is of variable width and is widest at its lateral margins. The superficial nucleus is sparsely populated and is traversed by fibers apparently derived from the lateral lemniscus.

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75 EAR OCCLUSION CAUSES SYSTEMATIC SHIFTS IN THE RECEPTIVE FIELDS OF AUDITORY UNITS. Eric I. Knudsen and Masakazu Konishi. Div. of Biology, Calif. Inst. of Technology, Pasadena, CA 91125

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

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

We used a movable sound source to map the receptive fields of these units before and after plugging one ear. All units were affected in the same way. Plugging the left ear caused receptive fields to move down and to the left. Plugging the right ear caused fields to move up and to the right.

If the activity of a unit coded the location of a sound source in space, then an induced receptive field shift down and to the left, for example, would cause the owl to make a localization error above and to the right of the sound source - the effect that was, in fact, observed. These results support the contention that neurons in this specialized region of MLD are involved with spatial analysis of sounds.

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

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

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

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

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

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77 POSTNATAL DEVELOPMENT OF THE DORSAL COCHLEAR NUCLEUS. S.A. Larsen. Department of Neurophysiology and Waisman Center on Mental Retardation and Human Development, University of Wisconsin, Madison, Wisconsin.

Maturation of cells of the dorsal cochlear nucleus (DCN) was studied in kittens ranging in age from 2 to 81 days and adult cats using Nissl (thionin) stained serial sections. At selected developmental stages, Nissl-stained cells were drawn using a Zeiss microscope equipped with a camera lucida and a Talos cybergraphic tablet interfaced to a Harris 6024/5 computer. The cochlear nucleus doubles in size from birth to adulthood. The fusiform cell layer is 5-6 cells thick; cells are small, densely packed and poorly differentiated at birth. The central DCN is shallow and poorly developed. Cells in the central DCN are small and not well differentiated at birth. Many signs of immaturity are observed: (1) cells which appear to be migrating (2) large cells with eccentrically placed nuclei and with Nissl material congregating at the periphery of the cytoplasmic membrane (3) cells with two nucleoli and (4) many cells with heavily clumped Nissl material. Cells of the fusiform cell layer increase from 761 cells at 2 days to 1355 cells in the adult. Cells found in central DCN also increase from 715 cells/mm^3 at 2 days to 1057 cells/mm^3 in the adult. Two types of fusiform cells are recognized in Nissl-stained material as early as 2 days postpartum. These types are (1) fusiform cells with pale-staining cytoplasm and nucleus and with an adult Nissl pattern of clumped Nissl arranged along the longitudinal axis of the cell and (2) fusiform cells with dark-staining cytoplasm, lightly stained nucleus and with an adult Nissl pattern of finely dispersed Nissl material distributed evenly throughout the cytoplasm. Pale-staining fusiform cells tend to group in the ventral half of the fusiform cell layer with dark cells grouped in the dorsal half. Cell growth is a nonlinear function of postnatal age. Both types of fusiform cells are approximately 35% of adult size at 2 days and follow a similar growth rate although the pale-staining cells are larger at all ages. Pale-staining cells increase 186% and dark-staining cells increase 172% in size from 2 days postpartum to adulthood; both are approximately 90% of adult size at 81 days postpartum. Both types develop adult cytoplasmic patterns by 34 days postpartum. Cells of central DCN are 50% of adult size at 2 days increasing 70% in size by adulthood. These cells are 99% of adult size by 81 days postpartum. Nuclei of fusiform cells are 43-45% of adult size at 2 days postpartum increasing 121-131% by adulthood. The nuclei of cells of central DCN are 92% at 2 days and 98% at 81 days postpartum. Microneurons are found scattered throughout the DCN. These microneurons show little change in cytoplasmic pattern or size with age. The microneurons of DCN are larger than microneurons located in other areas of the cochlear nucleus.

78 DICHOTING LISTENING IN CHILDREN SUFFERING FROM AGENESIS OF THE CORPUS CALLOSUM. Maryse C. Lassonde, Jean Lortie* and Maurice Ptito. Lab. de Neuropsychologie expérimentale, Univ. du Québec, C.P. 500, Trois-Rivières, P.Q. Can., G9A 5H7.

Numerous studies have established beyond question the existence of functional asymetries between the two cerebral hemispheres in man. Dichotic listening proved to be a powerful tool for investigating such asymetries. This technique has often been used to study hemispheric specialization in normals and commissurotomized subjects. However, very few studies have been conducted in order to assess functional asysmetries in patients suffering from a congenital absence of the corpus callosum. The purpose of the prescut study is therefore to investigate hemispheric specialization in patients suffering from callosal agenesis using the dichotic paradigm. Two subjects were included in the experimental group; MG, age 8 and LG age 16 had a complete callosal agenesis as revealed by CT scan. The patients were matched with two control groups: a. subjects with normal IQ, b. subjects with an IQ comparable to the acallosal patients ($\simeq$75). Each was right handed and had bilaterally normal hearing at the speech frequencies. All subjects were tested in an auditory recognition task under dichoting listening and their performances (mean errors and reaction time) were compared. Verbal (common words and nonsense syllables) and non verbal (pure tones) stimuli were binaurally presented to the subjects through earphones. Results have shown that 1) in the normal and matched IQ groups, the right hemisphere is superior when confronted with the non verbal stimuli whereas the left hemisphere performed better with the verbal stimuli 2) in the acallosal patients, the right hemisphere is always superior independantly of the nature of the stimuli (verbal vs non-verbal). Moreover, the reaction times recorded from the acallosal patients are three times longer than the other groups. These results tend to show that acallosal patients acquire during their development a brain asymetry different from normal.

79 BRAINSTEM AUDITORY EVOKED POTENTIALS AND AUDITORY RADIATION ACTIVITY IN THE MONKEY. Alan D. Legatt, Joseph Arezzo*, and Herbert G. Vaughan, Jr. Dept. of Neuroscience, Albert Einstein College of Medicine, Bronx, New York 10461.

Short latency or brainstem auditory evoked potentials (BAEPs), recorded during the first few msec following a click stimulus, are believed to reflect activity in the brainstem auditory pathways. The BAEP waveform recorded in the monkey resembles that seen in man, although more components can be identified. We have examined the relationship between evoked activity in the geniculocortical radiations and the far-field BAEP in the monkey.

100 usec 95 dBSPL clicks were delivered monaurally at a rate of 2 or 10 per second to an alert monkey which had been implanted with arrays of vertically-oriented guide tubes for depth electrodes. Depth passes were made through contralateral auditory cortex and into the underlying white matter. Three-dimensional field mapping delineated primary auditory cortex on the basis of potential gradients, polarity inversions, and multiple unit activity (MUA) maxima.

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

Wave 8 at the vertex peaked at 4.9-5.3 msec. The corresponding depth-recorded potential was much larger; it could be traced upwards from auditory cortex into its far field. It did not invert in polarity beneath auditory cortex, as did the cortical AEP. MUA in the deeper layers of primary auditory cortex showed two bursts preceding the cortical MUA increase, peaking at 4.5-4.6 and 5.5-5.6 msec.

A depth pass penetrating the auditory radiations recorded a MUA burst, peaking at 5.5-6.0 msec, restricted to a distance of approximately 0.5 mm. Its gross potential correlate was a positive-negative-positive waveform with onset at 4.5 msec and peaks at 5.5, 6.0, and 6.2 msec. The initial positivity could be traced upwards, its latency gradually approaching that of wave 8. A small MUA burst with an onset at 4.3 msec and a peak at 4.5 msec was recorded nearby, but over a smaller distance.

Thus it appears that wave 8 at the surface to a large degree reflects propagating action potentials in the geniculocortical radiations. The activity reflected in the earlier MUA burst and additional positivity in the depth recordings involves only a small part of the radiations and cortical projection areas, and is not seen as an individual wave at the scalp.

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81 TERMINAL PORTIONS OF AFFERENT FIBERS TO INNER HAIR CELLS IN THE CAT AS STUDIED IN SERIAL ULTRATHIN SECTIONS. M. Charles Liberman* (SPON: T. F. Weiss). Dept. Anat., Harvard Med. School, Boston, MA 02115.

Recent electrophysiological data from single auditory-nerve fibers suggest that three unit types can be differentiated on the basis of thresholds for tones and rates of spontaneous discharges (Liberman, J. acoust. Soc. Amer. 63: 442, 1979). Anatomical data show that at least 95% of the auditory-nerve fibers comprising these physiological samples are radial fibers innervating inner hair cells (Spoendlin, Acta Oto-Laryngol. 67: 239, 1969). The purpose of the present study was to search for morphological differences among the fibers innervating inner hair cells which might be related to the observed unit types in the auditory nerve.

Cochlear tissue was taken from a young animal born and raised in a low-noise environment. Single units recorded from this animal were exceptionally sensitive to acoustic stimuli. Serial sections were cut (in a plane parallel to the endolymphatic surface of the hair cells) from the habenula perforata to the supranuclear region of the hair cells. These sections included the entire unmyelinated portions and terminations of the afferent fibers in a small cochlear region.

The complete afferent innervation of two adjacent inner hair cells (including 56 afferent fibers) was reconstructed. Particular attention was paid to the following morphological features: 1) branching patterns, 2) fiber diameters, 3) sizes and shapes of the synaptic complexes, 4) axoplasmic contents, and 5) number, type and position of synaptic contacts with efferent fibers. All the afferent fibers were unbranched with a single synaptic complex situated less than a micron from the fiber terminus. Several types of synaptic complexes could be differentiated among the fibers contacting each hair cell. Differences in the synaptic morphology could be correlated with neuron size and with the position and number of synaptic interactions between efferent fibers and these afferents. Possible classification schemes will be discussed.

80 DIRECT EVIDENCE FOR AN AUDITORY PLACE MECHANISM IN THE FROG AMPHIBIAN PAPILLA. Edwin R. Lewis and Ellen L. Leverenz*. Biophysics Group, Univ. of California, Berkeley, CA 94720.

A place mechanism whereby low-frequency sensitivity is confined to the anterior region and high-frequency sensitivity is confined to the posterior region has been postulated for a single auditory organ, the amphibian papilla, in the ear of the frog (E.R. Lewis, Scan. Electr. Microsc./1977, Chicago:IITRI, 429-436). This hypothesis was based on comparative physiological results of Capranica and his colleagues (Frog Neurobiol., Berlin: Springer-Verlag, 551-575, 1976) considered in the light of comparative morphological studies carried out in our laboratory on the same species. More recently, on the basis of click response latencies, Yano et al. (J. acoust. Soc. Am. 64: s85, 1978) proposed the converse- high-frequency sensitivity in the anterior region, low-frequency sensitivity in the posterior region.

In order to obtain direct evidence on this matter, intracellular recordings were made from primary afferents of the bullfrog amphibian papilla. These afferents were penetrated in the posterior branch of the VIIIth cranial nerve medial to its emergence from the intact otic capsule with intact circulation. After characterization of the frequency response properties of each unit, we attempted to fill the axon by iontophoretic injection of Lucifer Yellow (W.W. Stewart, Cell 14: 741-759, 1978) and trace it to its origins on the papillar sensory surface. Those units that have been filled so far corroborate our version of the place mechanism hypothesis. Low-frequency units originate in the anterior region of the papilla; high-frequency units originate in the posterior region. It is expected that this line of research soon will yield a complete tonotopic map of the amphibian papilla.

In addition to tonotopic organization in the amphibian papilla, this method has confirmed the observations by Capranica and others that some high-frequency units originate at the basilar papilla. Furthermore, the method reveals innervation patterns at the sensory surface. Afferent axons from the basilar papilla and those from the high-frequency region of the amphibian papilla exhibit very confined patterns of innervation, contacting at most four or five neighboring hair cells. Afferent axons from the low-frequency region of the amphibian papilla have been found with branches innervating separate groups of hair cells quite remote from one another. It is expected that this line of research soon will yield a more complete picture of afferent branching patterns and their possible relationship to function.

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82 PHYSIOLOGICAL ANALYSIS OF EIGHTH NERVE INPUT TO CAT DORSAL COCHLEAR NUCLEUS. Paul B. Manis* and William E. Brownell (SPON: D. C. Teas). Depts. of Neuroscience and Surgery (ENT) University of Florida College of Medicine, Gainesville, Florida 32610.

The dorsal cochlear nucleus (DCN) is divisible into a molecular layer (ML), fusiform cell layer, and deep or polymorphic layer (DL). The primary efferent cells of the DCN, the fusiform cells, have their apical dendrites in ML, and their basal dendrites in the superficial region of DL. The lamination of the DCN has permitted us to electrophysiologically assess the distribution of eighth nerve terminals on fusiform cells. Field potentials in response to eighth nerve shock were collected from unanesthetized, decerebrate cats at successive 50 micrometer depth increments. The current source density (CSD) at each point along the electrode track was then calculated from the field potentials.

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

Excitability cycle studies of the field potential in upper DL revealed no significant effects of conditioning stimuli on the test response at intervals between 10 and 200 msec, suggesting that a monosynaptic or very secure disynaptic pathway is responsible for the evoked potential. One possible disynaptic pathway originates in the anterior ventral cochlear nucleus (AVCN). In one cat, the DCN source-sink distribution and time course in response to eighth nerve shock were not modified by transection of the dorsal intranuclear association fibers from the AVCN.

Anatomical studies have shown that the eighth nerve terminates on the basal dendrites of fusiform cells. If we assume that the eighth nerve is excitatory, then the current sink in the region of the fusiform cell basal dendrites reflects activation of eighth nerve terminals. The current source in the ML would then correspond to the expected passive current flow through the apical dendrites of the fusiform cells. The observed distribution of current sources and sinks is in full accord with the known anatomical distribution of eighth nerve synapses on fusiform cells.

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83 BINAURAL SUMMATION OF LOUDNESS: WIDE-BAND AND NARROW-BAND NOISE. Lawrence E. Marks. John B. Pierce Fndn. Lab. and Yale Univ., New Haven, CT 06519.

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

84 MAPPING OF PRIMARY AUDITORY PROJECTIONS FOLLOWING INTRACOCHLEAR INJECTIONS OF HRP. Glen K. Martin*, Brenda L. Lonsbury-Martin, Ben M. Clopton*, and Renee P. Wise*. Depts. Otolaryngol. and Physiol. & Biophys., Sch. Med., U. of Washington, Seattle, WA 98195.

The axonal projections of spiral ganglion neurons which provide the primary afferent innervation to the ipsilateral cochlear nucleus were investigated by the technique of orthograde axonal transport of HRP to obtain information about its organization for experimental studies. Young adult guinea pigs received intracochlear injections of 50% chromatographically-purified HRP (Worthington) dissolved in 5 ul of physiological saline. Following post-injection times of 24 hrs, the animals were perfused with a 2% glutaraldehyde in phosphate-buffered fixative and the brainstems post-fixed for one day in a 2% glutaraldehyde in 15% sucrose solution and for an additional day in a 30% sucrose solution. After fixation, the brain was prepared for frozen sectioning and cut at 40 um. The unmounted tissues were then reacted with tetramethylbenzidine (TMB, Sigma) following a procedure similar to that described by Mesulam (J. Histochem. Cytochem., 26:106, 1978). Sections were subsequently mounted and stained with neutral red.

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

85 THE EFFECTS OF APOMORPHINE ON ACOUSTIC STARTLE IN MICE. Michael D. McGinn* and Kenneth R. Henry. (SPON: L. F. Chapman). Dept. of Psych. University of California, Davis, CA 95616.

Dopamine agonists induce stereotyped motor responses and hypothermia in rodents. However, these agents may not have the same effects in different rodent species in terms of auditory responses; acoustic startle in rats is enhanced by apomorphine (APO), yet it protects mice from audiogenic seizures (AGS). Latencies of auditory brainstem evoked responses (BSER's) are increased by hypothermia, which offers a possible explanation for seizure protection. We looked for direct effects of APO on auditory afferents by obtaining BSER's while body temperature was maintained. C57BL/6 mice were anesthetized with pentobarbital and core temperature was maintained at 37.5 - 37.8°C. BSER's were recorded before and 15 min. after an injection of 3.0 mg/kg APO. [Dose and post-injection time are those which affect acoustic startle and AGS.] Latencies and amplitudes were virtually the same before and after the injection. In order to assess the relative motor and hypothermic contributions to the APO effect, we tested acoustic startle in C57BL/6 mice with and without body temperature maintained and compared these groups with saline injected controls. Startle was evaluated by averaging latencies and amplitudes of responses to a 112 dB click stimulus. Due to rapid habituation of startle to repeated stimuli, we used only 5 stimulus presentations before and another 5 after injection. When compared to saline injected controls, APO mice which were hypothermic had startle response latencies increased by 5.4 msec ($p < .01$) and response amplitudes reduced by 84% ($p < .01$). The APO injected mice whose temperature was maintained had latencies increased by 1.9 msec ($p < .01$) with a 49% amplitude reduction ($p < .01$). The two APO groups also differed from each other, with the hypothermic subjects having longer latencies ($p < .01$) and smaller amplitudes ($p < .01$). These data are in contrast to those obtained in the rat. However, the APO induced depression of acoustic startle in mice does correlate with the protection from AGS's that APO affords.

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86 RABBIT AUDITORY CORTEX: ELECTROPHYSIOLOGICAL EVIDENCE FOR TONOTOPIC ORGANIZATION. N.T. McMullen and E.M. Glaser, Dept. of Physiology, Univ. of Maryland School of Med., Baltimore, MD. 21201

The lissencephalic cortex of the rabbit provides opportunities for the correlative study of its functional and architectonic features, opportunities that are not available when studying gyrate brains. Using multiunit and evoked potential activity, we have studied the auditory cortex of urethane anesthetized Dutch Belted and New Zealand rabbits. Glass-coated tungsten microelectrodes were advanced tangentially through the auditory cortex in a direction perpendicular to the horizontal plane. With this technique, several parallel tracks can explore a large portion of the auditory cortex. The electrode tracks were placed so as to pass as much as possible through lamina III and IV. As much as an 8 mm extent of temporal cortex has been found to be responsive to auditory stimulation along a single track. Both amplitude discriminated multiunit activity and averaged evoked potentials (AEPs) were recorded in response to tone bursts, white noise and clicks monaurally delivered to the contralateral ear. Sound pressure levels were monitored. Electrolytic lesions were placed at selected sites and the electrode tracks were histologically verified from Nissl stained sections. Some of the brains have been processed by the Golgi-Cox method to permit subsequent analysis of the spatial orientation of the dendrite systems (Glaser et. al, Exp. Brain Res., 1979, in press). The AEPs have been found to be useful "signposts" of the electrode's location. The AEPs were often observed well before responsive unit activity was encountered. In the regions where the AEP was observed earliest, it exhibited an initially positive component. The electrode's initial invasion of the region yielding acoustically-responsive unit activity was accompanied by AEPs that were multiphasic and initially positive. At the electrode sites within this active zone, large amplitude negative AEPs predominated. Once within the active zone, the electrode was advanced in 250 µm steps and the best frequencies of neuron clusters were determined at lowest thresholds. Neurons within the active zone were found to be responsive to tones ranging from 0.4 to 35 KHz. Although some neurons were found which had no apparent best frequency, our results indicate that higher frequencies (15-35 KHz) are represented dorsally and lower frequencies (0.4-10 KHz) ventrally in the temporal cortex. These findings provide evidence for the tonotopic organization of rabbit auditory cortex, and are in agreement with the organization of the auditory fields observed by others in squirrel and guinea pig. The orientation of the isofrequency contours and the possible existence of several auditory fields are presently under investigation. Supported by NSF Grant BNS 78-05502.

87 OTOTOXIC ACTIONS OF AMINOGLYCOSIDES IN COCHLEAR PERFUSIONS CORRELATE WITH IN VITRO EFFECTS ON POLYPHOSPHOINOSITIDES. Iris N. Mechigian*, Shahid Lodhi*, Norman D. Weiner*, and Jochen Schacht. Kresge Hearing Research Institute and College of Pharmacy, Univ. of Michigan, Ann Arbor, MI 48109.

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

Aminoglycoside antibiotics have specific toxicity against the inner ear and the kidney. The ototoxicity of seven aminoglycoside antibiotics and fragments was measured quantitatively by cochlear perfusion: artificial perilymph was perfused for one half hour allowing a stable CM to be achieved, followed by 10 mM drug in artificial perilymph for one hour. Continuous measurements of CM were made throughout the perfusions. Kanamycin B and neomycin caused the most rapid decline of CM followed by gentamicin C_{1a}, ribostamicin and kanamycin A. Neamine and methylneobiosamine did not show significant ototoxicity.

We have previously shown that aminoglycoside antibiotics inhibit the turnover of polyphosphoinositides in the inner ear and the kidney in vivo as well as in vitro. Furthermore, these drugs were shown to interact directly and specifically with polyphosphoinositides in monomolecular films of these lipids, implicating polyphosphoinositides as possible receptors for the ototoxic drugs. To test further this hypothesis, we measured the interactions of the above seven antibiotics with monomolecular films of polyphosphoinositides. Neomycin and kanamycin B induced the largest increases in surface pressure, followed by the other drugs in the same order as seen for ototoxicity. The correlation between the in vivo and in vitro action of the drugs was r=0.91. These results support the hypothesis that binding to polyphosphoinositides is an important part in the mechanism of aminoglycoside ototoxicity. Furthermore, an in vitro assay system seems possible for the assessment of this drug toxicity.
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88 COMPARATIVE STUDIES OF SHORT-TERM AUDITORY ADAPTATION AND UNIT RESPONSE PATTERNS IN THE EIGHTH NERVE OF ANURANS. Andrea L. Megela and Robert R. Capranica, Neurobiology and Behavior, Cornell University, Ithaca, N.Y. 14853.

Previous studies have indicated that the two peripheral auditory organs of anurans, the amphibian and basilar papillae, differ in their frequency sensitivities and in mechanisms of frequency analysis. The amphibian papilla likely operates by a place mechanism and is sensitive to low (100 to 500 Hz) and mid (500 to 1200 Hz) frequencies. The basilar papilla appears to be a simply tuned resonant organ which is maximally sensitive to higher frequencies. The mid- and high-frequency ranges are species-specific while the low-frequency range is similar in all species. We now report that fibers from the amphibian and basilar papillae differ in their sensitivities to the effects of short-term auditory adaptation and in their firing patterns as determined from PST histograms.

Over 200 single fibers from the eighth nerve of the leopard frog, Rana pipiens, and the green treefrog, Hyla cinerea, were studied. In both species, the number of spikes to short probe tones was decreased by prior exposure to longer adapting tones (with both tones at a fiber's best excitatory frequency). The magnitude of response reduction and the time course of recovery varied with the interval between the adapting and probe tones. Basilar papilla units exhibited less response reduction at all interstimulus intervals than did amphibian papilla units. Within the amphibian papilla, low-frequency-sensitive fibers showed less response reduction than mid-frequency-sensitive fibers.

Firing patterns of units from the amphibian papilla formed a continuum from very flat or "tonic" to very peaked or "phasic" onset-type responses. Units from the basilar papilla tended to show more homogeneous firing patterns. The effects of short-term adaptation were more pronounced in units with more peaked PST histograms. Shapes of the histograms were related to each fiber's best excitatory frequency and to spontaneous activity, but not to threshold or to sharpness of tuning.

Different adaptation rates and unit response patterns were found in R. pipiens and H. cinerea that were consistent with the differences in vocal signals produced by these two species. These results suggest that adaptation may be important in the communicative behavior of these animals.

Supported by NSF and NIH.

89 A COMPARISON OF THE SUMMATING POTENTIALS TO RESISTANCE CHANGES MEASURED IN SCALA MEDIA OF THE GUINEA PIG COCHLEA. D. C. Mountain* and C. D. Geisler (SPON: L. Roth). Dept. of Neurophysiology, Univ. of Wisconsin, Madison, WI 53706.

The summating potential (SP) and the input resistance of scala media were measured simultaneously during the presentation of tone bursts. A resistance decrease was observed during the tone burst in response to a wide range of frequencies and intensities. The most effective acoustic frequency was the same for both the negative SP and the resistance change. Little or no resistance change was observed for stimuli that elecited a positive SP. The frequency tuning of the resistance change was broader than that of the negative SP but narrower than that of the cochlear microphonic (CM). The data indicate that the positive and negative summating potentials originate from different sources, one of which has a resistance change associated with it (the negative SP). This same pattern of two sources has been observed in the harmonic distortion of the CM (Mountain, Geisler, and Hubbard, J. Acoust. Soc. Am., 63:s43, 1978 and Hubbard, Mountain, and Geisler, J. Acoust. Soc. Am., in press). The negative SP may be due to an assymetrical response at or before the transduction stage and the positive SP may be due to a voltage dependent channel in the hair cell membrane which rectifies the cochlear microphonic. (work supported by NIH)

90 COCHLEAR NUCLEUS PROJECTIONS TO THE INFERIOR COLLICULUS OF THE CAT STUDIED WITH LIGHT AND ELECTRON MICROSCOPIC AUTORADIOGRAPHY. Douglas L. Oliver and D. Kent Morest, Department of Anatomy, Univ. Conn. Health Center, Farmington, CT 06032.

In this study we have begun to identify the axons of cochlear nucleus neurons which terminate in the inferior colliculus. Despite the fact that many of these neurons have been recognized with retrograde tracing techniques, many details of their axonal terminations remain unresolved.

Using highly concentrated tritiated leucine and/or proline solutions (50-200 μCi/μl), we made injections in the dorsal cochlear nucleus. After a survival time of 2-3 days, the experimental animals were perfused for electron microscopy. The midbrains were then tissue-chopped or Vibratome-sectioned and alternate sections were prepared for electron microscopy. The remaining sections were prepared for light autoradiography. In selected cases, EM autoradiographs were prepared according to the method of Kopriwa ('73). Most injections included the entire dorsal cochlear nucleus as well as parts of the underlying posteroventral cochlear nucleus and sometimes the small cell cap. In the light microscope, intensely labeled axons were found to ascend to the contralateral inferior colliculus primarily via the dorsal acoustic stria, medial to the lateral lemniscus. Within the inferior colliculus they terminate chiefly in the pars medialis and pars centralis of the central nucleus. Significant numbers of labeled axons were also located in the ventrolateral nucleus and in layer IV of the dorsal cortex, in continuity with the labeled axons of the central nucleus. Little or no labeling of axons was found in pars lateralis. The lateral portion of pars centralis also had little label, particularly in the rostral half of the colliculus. In these autoradiographs bands of labeled axons, which parallel the dendritic laminae of the central nucleus, were often seen. These bands are wider than the dendritic laminae and are separated by less intensely labeled regions of similar width. In the ipsilateral colliculus only very sparse label was found in the homotopic regions.

Our initial results of the electron microscopic autoradiography came from pars medialis of the central nucleus. After 8-12 weeks exposure, the majority of grains appeared to lie over myelinated axons or terminals. Most profiles of the labeled terminals were 1.5-3 μm in average diameter and contained medium-sized, round synaptic vesicles. These terminals formed asymmetric synaptic contacts usually on small-to-medium-sized dendrites. Similar terminals, forming axosomatic contacts and terminals with pleomorphic or flat vesicles, did not tend to be labeled.

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91 THE STRUCTURE AND CORTICAL PROJECTIONS OF THE MEDIAL GENICULATE BODY OF THE RAT. Hugh A. Patterson* and W. Bruce Warr. Department of Anatomy, School of Medicine, UCSF, San Francisco, CA., and Human Communication Laboratories, Boys Town Institute for Communication Disorders in Children, Omaha, NE.

The structure of the rat medial geniculate body (MGB) was studied in photographic reconstructions from normal material stained with Nissl, myelin and reduced silver methods. Largely consistent with previous work in various species, the rat MGB is best described as consisting of two longitudinally adjacent principal cell groups, a medial (magnocellular) and a larger laterally adjacent ventral division, the latter of which is enveloped by a shell-like surface cap of lesser cell groups. These groups include a large dorsal division, a caudal division, a laterally placed marginal zone and a ventrolateral nucleus. In addition, a small-celled group occupying a niche between the ventral, medial and dorsal divisions, here termed the supragenículate nucleus, was recognized.

The cortical projections of these nuclei were studied by both the HRP and Fink-Heimer methods. All parts of the MGB have cortical projections and these projections complement the structural parcellation just described. The ventral division projects topographically to a core area of koniocortex, area 41 of Krieg. Terminal degeneration was localized primarily in layers IV and III. The medial division projects diffusely to both area 41 and to extensive areas dorsally and anteriorly contiguous to it. This projection appeared to involve all layers of cortex, but with heaviest concentrations in layer VI. The shell-like cap of nuclei, which partially surrounds the ventral division, projects topographically to a C-shaped belt of cortex centered caudoventral to Area 41, comprized by areas 36 and 20. This projection terminates primarily in layers IV and III. The projections of the supragenículate nucleus to belt cortex were demonstrable by the HRP technique, but its laminar distribution remains to be determined.

Taken together, these findings support the notion that at least three distinct projection systems originate from comparably distinct structural regions of the rat MGB, and that the possibility of convergent interaction of two of these in area 41 must be considered.

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92 RESPONSE PLASTICITY OF AUDITORY CORTEX NEURONS DURING TONE - SIGNALLED CONDITIONING. N. Kraus Perkins and J.F. Disterhoft. Dept. Anat., Med. Sch., Northwestern Univ., Chicago, Ill. 60611

The activity of single neurons in auditory cortex of rabbit was monitored continuously throughout the acquisition of a classically conditioned, nictitating-membrane response to an auditory stimulus at the characteristic frequency of each unit. Stimulation and recording techniques were previously described (Perkins and Disterhoft,'79). Stimulus-evoked and spontaneous activity recorded during initial conditioning trials, in which the animal was behaviorally naive, was compared with that occurring during the later trials in which the conditioned response was established. Pseudoconditioning - random, non-paired presentations of the CS and UCS - was used as a control for general arousal, sensory habituation and random fluctuation of unit activity over time. In addition, the cell's response to selected, repetitive acoustic stimuli was assessed before and after behavioral manipulations.

Alterations in unit response to both the CS and UCS were prevalent in conditioned animals. Response plasticity was most frequently expressed as an increase in unit activity, although decreases also occurred. Subcomponents of a response pattern could change independently of each other. New responses, absent prior to conditioning, emerged after training. Shifts in spontaneous rate did not necessarily occur in the same direction as changes in stimulus-evoked activity. In pseudoconditioned animals, response patterns remained predominantly unchanged, or became attenuated over time. Some of the changes described above also occurred, but to a lesser extent, in this group.

Both conditioned and control animals showed response alterations to auditory stimuli presented prior to and following the behavioral trial series. This is consistent with previous reports of labile response properties of auditory cortex neurons to repetitive sound stimuli (Miller et al., '72). However, some features of these changes could be attributed specifically to auditory conditioning.

This study supports previous work in which multiple unit activity was shown to vary systematically with the acquisition of a behavioral response to an auditory stimulus (Buchwald et al., '66, Oleson et al., '75, Disterhoft and Stuart,'76). The fact that changes did not occur in all of the units studied, suggests that these changes are specific to a sub-population of neurons in auditory cortex. The variety of response changes observed reflects the heterogeneous response properties characteristic of single neurons in auditory cortex.

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93 COCHLEAR MICROPHONIC POTENTIALS FROM INNER AND OUTER HAIR CELLS? Martha G. Pierson* and Aage Møller* (SPON: Maryanna Henkart). Neurobiology Department, Armed Forces Radiobiology Research Institute, Bethesda MD 20014; Dept. of Otolaryngology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213

Generally it is not considered feasible to distinguish the cochlear microphonic (CM) originating from outer hair cells from that originating from inner hair cells in extracellular recordings. The large number of contributing hair cells, their stimulated phase distribution, and the finding that there is a 40-dB difference in their sensitivities has promoted this notion. In the present study it is reported that within a narrow window defined by intensity and frequency, two types of CM can be discerned simultaneously. Specifically, within a quarter octave band, 1/5 to 1/2 octave above the best frequency of a differential electrode pair, the CM intensity function displays a feature suggestive of a dual origin: it has two maxima separated by a cancellation notch at about 70 dB SPL.

The stimuli used in the present study consisted of 120-msec pure-tone pulses with linear rates of rise and fall, obtained by using analogue multipliers and trapezoidal control signals. The rise-fall times were generally 40 msec, the on time was less than 120 msec, and the period was 1 sec. The CM was band passed with a 1/3 octave-band filter tuned according to the stimulating frequency. The amplitude measurements were obtained by the full wave rectification of the CM, followed by a low pass filtering (470 Hz) and subsequent averaging. In experiments testing the influence of basilar membrane position, CM responses were processed as above except that the stimuli consisted of continuous pure tones. Basilar membrane position was modulated sinusoidally by presenting a 100-Hz signal together with the continuous high-frequency probe signal. Cycle histograms of the relative amplitude of the CM were then generated when the averager was triggered by the 100-Hz signal.

On the basis of these experiments it is proposed that the CM originating in outer hair cells has a lower threshold, a smaller dynamic range, is more subject to acoustic fatigue, and is more labile due to metabolic impairment (hypoxia). The magnitude of this type of CM is enhanced by a movement of the basilar membrane toward the scala tympani. On the other hand, the CM presumed to arise from inner hair cells is insensitive, has a vast linear operating range, and is almost unassailable by intense acoustic exposure or by hypoxia. This latter type of CM is enhanced by movement of the basilar membrane toward the scala vestibuli.

94 DISPROPORTIONATE FREQUENCY REPRESENTATION IN THE INFERIOR COLLICULUS OF HORSESHOE BATS:EVIDENCE FOR AN ACOUSTIC FOVEA. George Pollak and Gerd Schuller*. Dept. Zoology, Frankfurt Univ., FRG.

The horseshoe bat, Rhinolophus ferrumequinum, identifies and tracks its prey by emitting loud orientation cries and listening to the returning echoes. Each echolocation sound is composed of a constant frequency (CF) component of about 83 kHz lasting for 10-100 msec followed by a brief terminal frequency modulated part. While hunting, the CF component of the echo will have a higher frequency than the emitted CF due to a Doppler-shifting of the echo caused by the bat's approach towards its target. In response to the increase in echo frequency horseshoe bats lower the emitted CF by an amount almost equal to the Doppler-shift in the echo. As a result of the Doppler-compensation, subsequent echoes are stabilized and actively held at a frequency called the reference frequency. The reference frequencies of the bats studied by us were between 83.6-84.1 kHz.

By recording the best frequencies (BF) of several hundred neurons it was found that the inferior colliculus is functionally divided into two regions: one dorsal and one ventral. In the dorsal region, extending to a depth of about 800 microns, the BFs increase systematically with depth from 9 kHz to about 77 kHz. In marked contrast, the BFs in the ventral regionare nearly constant along a particular dorsoventral axis with neurons having similar BFs being arranged in cortical-like columns. The range of BFs in the ventral region extends from about 78-88 kHz along the anterioposterior axis with columns tuned to 78 kHz located most anteriorly and columns tuned to 88 kHz being situated most posteriorly. Thus, the neurons in the ventral region are tuned to the full range of frequencies that the emitted and echo CF components can assume during echolocation.

There also exists a pronounced disproportionate representation of frequencies within the ventral region where 33% of the neuronal population have BFs between 83-84.5 kHz. This small frequency band corresponds closely to the frequencies at which these bats stabilize the echo CF components during echolocation. This suggests that the over-represented frequency region of the brain is analogous to the foveal regions in the visual system.

The auditory system of horseshoe bats appears to be similar to the visual system is several respects. Instead of eye movements, these bats manipulate their voices (compensate for Doppler-shifts) in order to stabilize echoes within a frequency region to which a disproportionately large number of neurons are tuned and in this way enhance the resolving power for fine target features.

95 INNER EAR AUDITORY RECEPTORS IN OSTEOGLOSSOMORPH FISHES. Arthur N. Popper, Department of Anatomy, Georgetown University, Sch. Med.& Dent., Washington, D.C. 20007

Scanning electron micrographic studies of the ears in four species of osteoglossomorph (Division II) fishes show considerable diversity in a number of features which may be associated with differences in auditory mechanisms or capabilities. Variation is particularly great in the hair cell orientation patterns on the sensory maculae. Species studied were the butterfly fish (Pantodon bucholzi), a mormyrid (Gnathonemus sp.), an African knifefish (Notopterus chitala), and the arawana (Osteoglossum bicirrosum).

Typically, the caudal part of the saccular macula in nonostariophysan teleosts has a group of dorsally oriented hair cells (kinocilium on the dorsal side of the cell) above a group of ventrally oriented cells. The rostral macula region has posteriorly oriented cells above a group of anteriorly oriented cells. Variants on this basic pattern are found in each osteoglossid except Osteoglossum. Pantodon has alternating anteriorly and posteriorly oriented cells on the rostral macula region. Gnathonemus is unique in having only vertically oriented cells on the saccular macula. This pattern has heretofore only been known for the saccular maculae in Ostariophysi and terrestrial vertebrates. The saccular pattern in Notopterus also differs from that seen in previous studies of fishes. The saccular macula is essentially tripartate, and thin 'bridges' connect each of the three regions. The most anterior macular region contains a group of anteriorly oriented cells at the rostral tip, followed by a continuation of the anteriorly oriented cells ventral to a posteriorly oriented cell group. The middle part of the macula contains a group of ventrally oriented cells rostral to a group of dorsally oriented cells while the third part contains dorsally oriented cells above ventrally oriented cells.

The lagenar macula in Osteoglossum, Pantodon, and Notopterus is typical of most nonostariophysans in having a dorsally oriented group of hair cells located anterior to a group of ventrally oriented cells. However, the macula in Gnathonemus more nearly resembles the pattern in Ostariophysi and has hair cells oriented in a wide range of directions.

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

96 RATE-INTENSITY FUNCTIONS OF SINGLE NEURONS LOCATED WITHIN BINAURAL SUPPRESSION COLUMNS OF CAT PRIMARY AUDITORY CORTEX. Richard A. Reale, Thomas J. Imig and Donal G. Sinex*. Dept. of Neurophys., Waisman Cntr. on Mental Retard. and Human Dev., Univ. of Wisconsin Medical School, Madison, WI 53706.

In addition to the tonotopic organization, the primary auditory field (AI) of the cat also contains a binaural representation in which neurons with similar binaural properties form functional columns within the high-frequency representation of the field (Imig, T.J. and Adrian, H.O., Brain Res. 138:241-257, 1977). We have mapped the best frequencies and binaural sensitivities of neuron clusters within AI to define binaural columns. The response of single neurons to tone-burst stimuli were then quantified during microelectrode penetrations directed into mapped suppression columns. Neuron clusters displaying suppression are excited by stimulation to the contralateral ear and inhibited by simultaneous stimulation to the ipsilateral ear. Monaural and binaural rate-intensity functions were determined for each isolated neuron at its best frequency. Neurons were driven by contralateral monaural stimulation, whereas stimulation of the ipsilateral ear alone was ineffective in eliciting discharges regardless of stimulus intensity. Binaural rate functions were obtained by holding the intensity to the contralateral ear constant while varying the intensity to the ipsilateral ear. With few exceptions, the average discharge rate produced by a contralateral stimulus at any chosen intensity could be significantly reduced when paired with a higher intensity tone delivered to the ipsilateral ear. In some experiments, several suppression columns were examined within a selected isofrequency strip. The majority neurons isolated within suppression columns located on the most ventral portion of an isofrequency strip exhibited non-monotonic rate-intensity functions for monaural stimulation. Plots of discharge rate versus stimulus intensity produce a Λ-shaped curve in which near zero discharge rates occur at both the low and high ends of the effective intensity range. The maximum rates elicited from these neurons were usually obtained within 30 dB of threshold. By comparison, high contralateral intensity rarely reduced the monaural discharge rates of neurons located within suppression columns nearest the dorsal end of an isofrequency strip by more than 50% relative to their maximum rates. Additionally, the discharge rates of several neurons increased monotonically over a 30-to-80 dB range. Taken together these data suggest that many neurons in a binaural column exhibit similar rate-intensity functions and that spatially separate groups of neurons with similar best frequencies and binaural properties possess dissimilar intensity sensitivities to monaural stimulation. (NS-05459, NSF76-19893, HD-03352)

97 SINGLE UNIT ANALYSIS OF THE POSTEROVENTIAL COCHLEAR NUCLEUS OF THE DECEREBRATE CAT. Louis A. Ritz and William E. Brownell. Depts. of Neuroscience and Surgery (ENT), Univ. of Florida, Gainesville, Florida, 32610.

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

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

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

Within the OCA of the unanesthetized cat, the spontaneous rates range from 0 to 135, with a mean of 34 spikes/sec. Approximately 80% of the responses recorded from the OCA are of an onset type. One response consists of an onset, followed by a profound inhibition for the remainder of the tone burst. A second response type consists of an onset, followed by a low level of excitation for the remainder of the tone burst. These onset units have tuning curves that are generally broader than those from eighth nerve fibers. These data will be compared to other findings from the auditory system of decerebrate cats.
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98 CHANGES IN AUDITORY THRESHOLD SENSITIVITY AND FREQUENCY SELECTIVITY AFTER NOISE EXPOSURE IN NEONATAL C57BL/6J MICE. James C. Saunders and Linda L. Restifo*. Dept. Otorhinolaryngol. and Human Communication, and Human Genetics, University of Pennsylvania, Philadelphia, Pa. 19104.

Eighteen day old mice were exposed to noise for 90-seconds at 110 dB SPL (bandwidth, 8.7-13.3 kHz). At 23 days a bipolar concentric electrode was placed in the cochlear nucleus (CN) of anesthetized mice and two measures of auditory function were sought in animals exposed to the noise and in non-noise exposed control subjects. First, tone-burst evoked response threshold sensitivity was measured at 8 test frequencies between 1.0 and 39.0 kHz. Next , a simultaneous two-tone masking procedure was introduced to define evoked-response tuning curves at each of 6 center frequencies. The results showed a loss in threshold sensitivity in the noise exposed animals that was between 12 and 20 dB for test frequencies from 10.0 to 26.0 kHz. At other frequencies no threshold loss was noted. The frequency selectivity (i.e. the sharpness) of tuning curves in the region of maximum threshold loss was the same in both control and noise exposed animals. However, the sensitivity of the tuning curve, in noise exposed mice, showed a reduction that was equal to the threshold loss. These results are similar to data reported by other investigators that show a relation between cochlear trauma and changes in threshold sensitivity and frequency selectivity, and suggest that the effects of noise in the present experiment may be damage confined to the outer hair cells of the organ of Corti. The C57BL/6J mouse strain exhibits a critical period of heightened susceptibility to acoustic trauma at 18 days of age and the present data contribute to our understanding of this phenomena.

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99 DIFFERENTIAL DISTRIBUTION OF GLUTAMIC ACID, ASPARTIC ACID AND GLYCINE UPTAKE IN THE COCHLEAR NUCLEUS. Ilsa R. Schwartz. Department of Surgery/Head & Neck, School of Medicine, UCLA, Los Angeles, CA 90024.

Recent biochemical and histochemical studies of the cochlear nuclei (CN) in normal, genetically deaf and surgically deafened guinea pigs have shown a correlation between levels of glutamic and aspartic acid and the presence of normal primary auditory endings (Wenthold & Gulley, Br. Res. 138:111-123, 1977; Wenthold, Br. Res. 143:544-548, 1978). This study sought to demonstrate the presence and localization of high affinity uptake systems for these substances morphologically.

Cats and C57BL/6 mice were perfused with cold saline-nitrite solutions. The brain stems were rapidly removed, sliced transversely at 235μ and the CN dissected out in the cold. Tissue was preincubated at room temperature in oxygenated Ringer's bicarbonate solution (RBS) for 10 minutes, transferred to oxygenated RBS containing approximately 10^{-8}M tritiated amino acids (100μCi/ml) for 20 minutes, rinsed in RBS, fixed with 1% paraformaldehyde and 1.25% glutaraldehyde in 0.12M phosphate buffer, pH 7.2, and processed for autoradiography with standard methods (Schwartz & Bok, J. Neurocytol. 8:53-66, 1979).

Preliminary results indicate that following incubation with aspartic acid, glutamic acid or glycine, intense labelling is present over many small structures in the granule cell and molecular layers. Synaptic terminals appear to be the major structures labeled, and the pattern differs with the amino acid. Most neurons throughout the CN are contacted by a few terminals which label with glycine. With glutamic acid fewer perisomatic and peridendritic terminals and small structures (presumably terminals) are labeled. Concentrations of label are observed in the ventral CN and the intermediate acoustic stria, with less intense labelling in the dorsal CN. Aspartic acid produced intense labelling of terminals in the mouse ventral CN which had a similar distribution to that of primary auditory terminals. Electron microscopic autoradiography is underway to identify the labeled structures.

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100 QUANTITATIVE [^{3}H] AND [^{14}C]-2-DEOXYGLUCOSE MAPPING OF THE AUDITORY CENTRAL NERVOUS SYSTEM IN XENOPUS LAEVIS. T. J. Sejnowski, D. B. Kelley, J. A. Paton and M. L. Yodlowski. Princeton University, Princeton, N. J., 08544 and The Rockefeller University, New York.

The 2-deoxyglucose (2DG) technique was used to study CNS activity of male South African clawed frogs during acoustic stimulation. Five μCi [^{14}C]-2DG or 50 μCi [^{3}H]-2DG was injected into the dorsal lymph sacs of 20 males; frogs were then presented with 2 hours of taped conspecific vocalizations. Half of the frogs were transcardially perfused with saline followed by 10% formalin. The [^{14}C]-2DG brains were processed as described in Sokoloff et al., J. Neurochem. 28: 897, 1977. The [^{3}H]-2DG brains were sectioned at -22°C on a cryostat in a darkroom. Sections were picked up on slides precoated with NTB-3 emulsion and dried at room temperature. Slides were developed after 4 weeks and some were counterstained with cresyl violet to identify the labelled areas. Optical densities of 125μ spots were measured with a photodensitometer. The measurements were normalized against a standard region of the dorsal optic tectum in each animal.

The following regions were consistently labelled: dorsal auditory medulla (MED), superior olive, anterior and posterior thalamus, caudal torus semicircularis (CTOR) and laminar nucleus of the torus (LTOR). A ^{14}C autoradiogram of a section through the torus and dorsal medulla is shown below. Unilateral removal of the middle ear bones decreased uptake in contralateral LTOR but not in CTOR. The 2DG labelling in the torus was used to quantitatively compare perfused with nonperfused tissue and [^{14}C]- with [^{3}H]-2DG autoradiograms. The table below gives the mean and standard deviation of the maximum optical density for [^{14}C] perfused and nonperfused autoradiograms. Perfusion did not reduce the label densities in LTOR or CTOR. The pattern of labelling using [^{3}H]-2DG was the same as that seen with [^{14}C]-2DG; optical densities were, however, more variable.

These results indicate that the 2DG method can be used to functionally map the auditory system in an anuran amphibian, that autoradiograms can be successfully prepared using perfused tissue and that [^{3}H]-2DG is an alternative but less reliable method for obtaining 2DG autoradiograms.

	LTOR	CTOR
Perfused	2.9±.2	1.9±.3
Unperfused	2.6±.5	2.2±.5

N=4 in each group

101 A NEW BIODETECTOR OF AUDITORY NERVE ACTIVATING SUBSTANCE (ANAS). William F. Sewell, and Paul S. Guth. Dept. of Pharmacology, Tulane University School of Medicine, New Orleans, La. 70112.

Auditory nerve activating substance (ANAS) (which may be the primary afferent transmitter of audition) is found in the perilymph of the frog or the guinea pig following appropriate stimulation with sound. ANAS was originally detected by the increase in firing rate of single afferent units serving the amphibian papilla of the bull frog. This means of biodetection although originally useful proved extremely clumsy and therefore a major impediment to the isolation and characterization of ANAS, the ultimate goal of this research. The major drawbacks of the original biodetection preparation were the seasonal variations and the small number of samples assayable per preparation. We therefore attempted to discover and develop new and better means of detecting ANAS. To this end we tried: single units of the toad lateral line afferents, the goldfish saccule-saccular nerve preparation, guinea pig auditory nerve single unit and bull frog saccule-saccular nerve preparation. Only the last mentioned preparation responds regularly to the presence of ANAS. Bull frogs of either sex are double-pithed and the saccule and saccular nerve exposed by drilling through the otic ridge. The perilymphatic sac is opened and a pipette used to deliver substances to be assayed is placed over the saccule. Glass micropipette electrodes are used to record single unit activity in the saccular nerve. This preparation responds not only to small volumes of ANAS but to glutamate (5 μl of 3 mM glutamate) in a reliable manner and allows the assay of many samples per preparation. (This work supported by grants from the U.S.P.H.S., NS 11647 to the Kresge Hearing Research Lab. of the South and from the Veteran's Administration.)

102 DEOXYGLUCOSE DEMONSTRATION OF A NEW AUDITORY PROJECTION FIELD WITHIN THE INFERIOR COLLICULUS. Martin S. Silverman* (SPON: Russell L. Snyder). Dept. Phys., Univ. of Calif., San Francisco, Ca. 94143.

A new auditory projection field with unique functional characteristics has been located within the inferior colliculus using the ^{14}C-2-Deoxyglucose metabolic mapping technique, (Science, 187: 850, '75). This field consists of a band of highly active elements located at the ventromedial extremity of the central nucleus of the inferior colliculus. In barbiturate anesthetized preparations receiving no acoustic stimulation, this projection area (VM) is seen autoradiographically as a dense band of about 200μ in width that rings the ventromedial extent of the central nucleus of the inferior colliculi, bilaterally. In similar preparations that received pure tone stimulation to one ear, this ventromedial projection area is seen in the colliculus contralateral to the stimulated ear, but is substantially diminished in density, or altogether absent in the colliculus ipsilateral to the intact ear. A variety of tones spanning at least 3 octaves located in the center of the rat's auditory range suppressed activity in the VM ipsilateral to the stimulated ear, indicating that ipsilateral suppression of activity in the VM is broadly tuned. To further investigate the effects of unequal auditory activity on the labeling of the VM band, some animals were unilaterally labyrinthectomized with the intact ear left acoustically unstimulated. Again, dense labeling was seen in the VM contralateral to the intact ear with no such labeling seen ipsilaterally. This result indicates that even the unequal binaural activity condition achieved by the elimination of the spontaneous 8th nerve activity on one side can eliminate VM labeling ipsilateral to the intact ear. The above results demonstrate that the VM can be strongly inhibited ipsilaterally by unequal sound-evoked, or spontaneous activity eminating from the 8th nerve. These cases by themselves alternatively do not show that activity within the auditory system is necessary for VM activity, (i.e., the VM could in itself be spontaneously active, and/or receive a facilatory input from extra-auditory sources). That the VM area requires auditory input for its substantial activity was demonstrated in preparations in which the spontaneous activity in the 8th nerves was eliminated by bilateral cochlear destruction. In these cases the labeling in the VM area was bilaterally eliminated, indicating that cochlear activity is necessary for VM activity. In summary, these experiments show the existance of a new and discrete collicular projection field having unique functional characteristics that are different from auditory projection areas so far reported.

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103 RESPONSE OF SECOND AND THIRD ORDER AVIAN AUDITORY NEURONS TO DEAFFERENTATION. Anthony N. van den Pol, Michel Kliot*, Richard Kuritzkes*. Sect. Neurosurg., Yale Sch. Med., New Haven, Ct. 06510.

Nucleus magnocellularis (NM) and nucleus laminaris (NL) second and third order avian auditory nuclei, respectively, were examined in 43 six to ten day hatchling chickens at several time intervals after unilateral cochlea removal combined with ganglion cell aspiration, and in controls. NM receives afferent input from the ipsilateral cochlea, and NL dorsally from the ipsilateral NM and ventrally from the contralateral NM. Two days after cochlea removal, degeneration is found with EM and LM in primary afferents and NM somata ipsilaterally. Furthermore, early signs of axonal degeneration are found dorsal to the ipsilateral NL, and ventral to the contralateral NL; both axon swelling and collapse are more evident ($p<.05$) here than in the normally innervated NL. As a recent report (Parks & Rubel, JCN 180, 439, 1978) found no direct projections from cochlea to NL in chicks, and since we find degeneration of axons in NL occurring simultaneous with cell death in NM, the degenerating axons in NL two days after cochlea removal are probably secondary, and from NM. Similar results were obtained with a three day post-surgery survival. Seven days after unilateral cochlea removal, many collapsed osmiophilic axons were found in the ipsilateral NM, dorsal ipsilateral NL, and contralateral ventral NL; only a few swollen axons were seen at this time. Eight hours after cochlea removal we find no qualitative signs of axonal degeneration in NM or NL. In a parallel series of experiments with ^{14}C 2 deoxyglucose (2DG) autoradiography in unanesthetized chicks, relative to the surrounding neuropil, 2DG uptake appeared high in the synaptic region of NL, and also in the non-synaptic region of myelinated fibers and glial cells ventral and dorsal to NL. Unilateral cochlea removal caused a statistically significant ($p<.05$) decrease in 2DG uptake, as measured with microdensitometry, in the ipsilateral NM, ipsilateral dorsal NL, and contralateral ventral NL 5, 24, and 48 hours after surgery. This was true at low and high sound levels (45-92dB). In a third set of experiments, uptake of tritiated amino acid was examined in NM after unilateral cochlea removal in four chicks. Three days after surgery, ^{3}H-lysine uptake 45 minutes after intraperitoneal injection was decreased in NM on the side from which the cochlea was removed by 45% compared to the contralateral side. Since dying cells can be found in the ipsilateral NM after unilateral cochlea removal at this time interval, grains were counted only over normal appearing neurons. The use of converging techniques to analyze structure, synthesis (amino acid incorporation), and energy demand (2DG) in the auditory system may lead to a better understanding of the relationships between morphological and functional response to deafferentation.

104 RESPONSES OF SINGLE COCHLEAR NUCLEUS NEURONS OF HORSESHOE BATS TO SINUSOIDALLY FREQUENCY AND AMPLITUDE MODULATED SIGNALS. Marianne Vater* (SPON: W.J. THOMPSON). Dept. of Biology, Frankfurt Universitat, FRG.

The greater horseshoe bat, Rhinolophus ferrumequinum, emits a long constant frequency component (CF) of about 83 kHz during echolocation. The echoes are Doppler-shifted upward in frequency due to the relative movement between the bat and target. The bat compensates for these deviations in the echo frequency by lowering the frequency of its emitted calls so that the echo frequency remains constant within a narrow frequency band. The wing beats of prey insects result in periodic frequency and amplitude modulations of this carrier frequency which can be used by the bat as clues for detection and probably for the identification of moving prey objects. In order to examine the neuronal encoding characterisitcs of these complex time varying singals within the ascending auditory pathway of horseshoe bats, recordings were made from single cochlear nucleus (CN) neurons. The sample comprises neurons from all subdivisions of the CN-complex. Variable parameters of the sinusoidal frequency and amplitude modulated (SFM and SAM) stimuli were presented. The discharge patterns of most tonic units faithfully reproduced the time course of the periodic modulations. It can therefore be expected that signals with more complex temporal patterns, as for example generated by the wing beats of insects, are also processed with only little distortion and are relayed as input information to higher centers. Phasic neurons did not preserve the stimulus time structure: they reponded with a transient discharge activity to distinct portions of the modulation cycle. Synchronization was present over a wide range of signal intensities and modulation depths, important parameters for evaluations of the target distance and size. The sharply tuned CN "filter" neurons, tuned to 78-88 kHz, are able to encode modulation depths as small as +50 Hz. Neurons of the different subdivisions of the CN differ in the response properties to SFM signals. This becomes clear in the responses to different modulation rates. Primary-like neurons in the anteroventral CN are able to synchronize to rates as high as 900 Hz. The stimulus coding in the time domain therefore far exceeds the frequencies of prey insect wing beat frequencies (i. e., between 30 and 100 Hz). Build up neurons in the dorsal CN were unable to follow the periodic modulations in the frequency range tested (minimum modulation rate of 20 Hz). Phasic neurons in the dorsal CN typically lock only at modulation rates below 300 Hz, a behavior also reported for inferior collicular neurons, but a range largely covering the biologically important range of insect wing beat frequencies.

105 EVIDENCE OF INHIBITORY INTERACTIONS BETWEEN NEURONS IN DORSAL COCHLEAR NUCLEUS. Herbert F. Voigt* and Eric D. Young, Dept. of Biomedical Engineering, Johns Hopkins School of Medicine, Baltimore, MD. 21205.

Neurons in the dorsal cochlear nucleus (DCN) of unanesthetized, decerebrate cats can be classified as Type II/III (excited at all levels by best-frequency (BF) tones) or Type IV (inhibited by moderate and high level BF tones). The properties of Type II/III neurons are all consistent with the hypothesis that they are interneurons which inhibit the activity of Type IV cells. We have obtained further evidence of such a connection by studying the functional relationships between pairs of neurons in the DCN. Recordings from pairs of neurons that were physically close together were obtained by using a single microelectrode. The spike trains of the two neurons were separated on-line using a template matching technique. Each neuron was classified as Type II/III or Type IV and cross correlograms of spontaneous or acoustically-driven activity were computed. Where recording time allowed, complete frequency-intensity response maps were also obtained. For 12 of 17 Type II/III-Type IV pairs, the cross correlograms showed statistically significant decreases in the Type IV's discharge rate following spikes in the Type II/III unit. In these cases, the excitatory region of the Type II/III unit's response map was within the inhibitory region of the Type IV unit's response map. The remaining five pairs showed no signs of inhibitory interaction in their cross correlograms. In four of these cases, there was extensive overlap of the excitatory regions of the cells' response maps. A portion of the inhibitory region of the response map of one Type IV neuron was converted to an excitatory area after injury discharge and disappearence of an accompanying Type II/III unit whose discharge had shown an inhibitory correlation with that of the Type IV unit. The region in which the change occurred was centered on the BF of the Type II/III neuron. Cross correlograms of the spontaneous activity of Type IV-Type IV pairs were featureless in 10 of 20 cases, displayed signs of shared input in 7 cases, and were complex in the remaining 3 cases. There was extensive overlap of the response maps in all Type IV-Type IV pairs. (Supported by NIH grant NS-12524).

106 SPIRAL GANGLION NEURONS FOLLOWING DRUG-INDUCED ORGAN OF CORTI LOSS. Molly Webster* and Douglas B. Webster. Kresge Hear. Res. Lab., Dept. Otorhinolaryngol., L.S.U. Med. Ctr., New Orleans, LA 70119.

The guinea pig organ of Corti was destroyed by a single treatment of the ototoxic combination of kanamycin followed by ethacrynic acid (West et al., Arch. Otolaryngol., 98:32-37, 1973). The resulting loss of spiral ganglion neurons was quantitatively studied in 10-µm serial sections of 23 ears from 15 animals, including 2 normals and 13 with post-treatment survivals from 2 weeks to 15 months. For each ear, mid-modiolar sections were selected for the first half-turn (above the hook), designated as turn 0.5, and for half-turns 1.0, 1.5, 2.0, 2.5, and turn 3-4. For each turn or half-turn, all neurons were counted in the selected section and in the second sections before and after, and the three counts averaged. The cross-sectional areas of Rosenthal's canal were measured in the same sections and averaged, and the number of neurons per 10,000 μm^2 was calculated to correct for differences in orientation of the ears. By 2 months the organ of Corti has degenerated and the spiral ganglion population is greatly depleted. Neurons continue to disappear between 4 and 8 months. A small and apparently stable population of neurons is reached by 8 months.

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107 DUAL POPULATIONS OF EFFERENT AND AFFERENT FIBERS OF THE BASILAR PAPILLA IN THE CHICKEN. Mark Whitehead and D. Kent Morest. Department of Anatomy, University of Connecticut Health Center, Farmington, Connecticut 06032.

Neurons with cochlear efferent fibers have been localized in adult chickens by the axonal transport of horseradish peroxidase. Injections of peroxidase into the inner ear resulted in diffuse filling of cells associated with the cochlear nerve.

Labelled efferent (olivocochlear) neurons were scattered bilaterally in the reticular formation, lateral to the abducens nerve root, and medial and ventromedial to the superior olive. Ventrally situated cells were located among the trapezoid fibers. Some cells were characterized by stellate dendrites, while others were fusiform. Axons of contralateral efferent neurons extend dorsally adjacent to 6th nerve fibers, cross beneath the ventricle, course ventrolaterally mingled with fibers of the facial genu, and exit with the ipsilateral axons through the vestibular nerve root as thick and thin fibers. In plastic-embedded preparations of the inner ear, thin, beaded, labelled fibers, resembling Golgi-impregnated efferent axons (Whitehead & Morest, Soc. Neuroscience Abstracts 4: 397, 1978), were traced to endings on hair cells of the basilar papilla. These labelled axons form large, cup-shaped endings on short hair cells, while smaller terminals contact the tall hair cells, which thus resemble outer and inner mammalian hair cells, respectively.

Afferent components of the cochlear nerve also exhibited Golgi-like filling. Primary afferents bifurcate into lateral and medial branches. The lateral branch gives rise to thin, wavy terminals in nucleus angularis. The medial branch contains mostly thick fibers forming end-bulbs in nucleus magnocellularis, where, in addition, there is a thin, heavily varicose type of primary afferent which is branched and tortuous. These latter axons may represent a class of ganglion cells different from that forming end-bulbs; or, these fibers could be collaterals of the thick primary afferents. Similar delicate, beaded fibers have been seen in Golgi impregnations. In the ear, peripheral processes of ganglion cells appeared in one case to be filled with granules of reaction product, ending on hair cells of all heights. In another case, in which no efferent neurons were diffusely labelled, a few diffusely filled, presumably afferent fibers were seen to make large, foot-shaped contacts with tall hair cells.

In conclusion, as in mammals, the avian olivocochlear neurons form at least two populations; likewise there may be two types of sensory neurons.

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108 CORTICAL ABLATION AND PITCH GENERALIZATION OF COMPLEX TONES. I. C. Whitfield. Neurocommunications Research Unit, Univ. Birmingham, Birmingham 15 U.K.

A combination of overtones e.g. 1600, 2000, 2400 Hz (a) has an apparent pitch equivalent to a simple tone of 400 Hz (missing fundamental). The combinations 1600, 1942, 2284 Hz (b) and 1716, 2058, 2400 Hz (c) have lower pitches than (a), while 1600, 2058, 2516 Hz (d) and 1484, 1942, 2400 Hz (e) have higher pitches (about a minor third) in each case.

Cats were first trained to withhold a lick response when a falling pair of tones, 400-342 Hz (safe), was replaced by a rising pair, 400-458 Hz (warning). The animals transferred readily to the complex tone sequences ab (safe) and ad (warning). Animals were overtrained on this paradigm and the absolute frequencies randomly varied 10% to ensure that the response was to direction rather than spot frequency. The cats were then tested on a combination of ab, ad, ac, and ae. They treated ac like ab (safe) and ae like ad (warning) indicating that it was the direction of the fundamental pitch change that was significant, and not that of the harmonics (Heffner & Whitfield, J. acoust. Soc. Amer. 59, 915-919, 1976). The cats were then given a bilateral cortical ablation involving AI, AII, Ep. After recovery the initial training was found to have been lost. However response to the simple tones was rapidly relearned, and the response to the complex pair ab/ad somewhat less rapidly. After retraining on ab/ad, the test programme ab, ad, ac, ae was presented. Unlike the unoperated cats, the operated animals now failed to distinguish between ac and ae. Furthermore one cat, which was presented with the complex ad, but with frequencies all changed by 200 Hz, failed to treat it as warning. It is concluded that while bilaterally decorticate cats can be trained to discriminate specific complex tones; pitch generalization between different complexes of the same pitch is lost.

109 WIENER KERNEL ANALYSIS OF THE RESPONSES OF CAT ANTEROVENTRAL COCHLEAR NUCLEUS NEURONS.
Robert Wickesberg*, John Dickson, and Mary Morton Gibson, (SPON: C. Daniel Geisler) Dept. of Neurophysiology, Univ. of Wisconsin, Madison, Wisconsin 53706.

In order to determine the usefulness of Wiener kernel analysis in the estimation of second-order distortion products encoded in the peripheral auditory system, we have compared second-order Wiener kernels with neural responses to pairs of clicks. In addition, we have compared the Fourier transforms of the second kernels with neural responses to a specific distortion product, the f2-f1 difference tone (f2>f1).

Responses to pseudo-random Gaussian white noise were recorded extracellularly from anteroventral cochlear nucleus neurons in barbiturate anesthetized cats. A 8192-point noise sequence with 40 μsec. resolution and the same sequence with polarities reversed were presented alternately. For each noise sequence a peri-stimulus time (PST) histogram was made from the recorded spike times and normalized for the number of presentations and the binwidth. To calculate the first-order kernel, the PST histogram made for the noise sequence with polarities reversed was subtracted from the histogram made for the other noise sequence. To calculate the second kernel, the two PST histograms were added together. This method of kernel calculation reduces errors due to the finite length of the noise sequence and the approximation to a Gaussian distribution. The subtraction for the first kernel calculation eliminates errors contributed by even-order terms, and the addition for the second kernel calculation eliminates errors from the odd-order terms.

Preliminary results indicate that this analysis might provide insights into processes involved in the neural encoding of second-order distortion products. (Supported by NIH grant NS-12732).

110 PROPRANOLOL REDUCES AUDITORY NERVE AND BRAIN STEM RESPONSES TO CLICK STIMULI. Michael L. Wiederhold and Helen E. Savaki*. Neurobiology Department, Armed Forces Radiobiology Research Institute and Laboratory of Cerebral Metabolism, National Institute of Mental Health, Bethesda, MD 20014.

Intravenous administration of beta-adrenergic-blocking agents has been shown to decrease glucose metabolism in nuclei throughout the auditory CNS (Savaki et al., Nature 276: 521-523, 1978). In an attempt to determine where this effect might be exerted, we have recorded click-evoked electrical responses generated in the cochlea (cochlear microphonic, CM), auditory nerve (N_1), and brain stem (BSER recording). All potentials were recorded simultaneously from the external ear canal of barbiturate-anesthetized cats before, during, and after intravenous infusion of propranolol for 1 hour. Dose rates from 0.01 to 1.0 mg/kg/min were used. The amplitudes of both N_1 and BSER in response to clicks approximately 40 dB above threshold were reduced by up to 70% in a dose-dependent manner by propranolol. A half-maximal effect was obtained at approximately 0.3 mg/kg/min. Maximal effects were generally seen about 10 minutes after infusion was stopped. Recovery began immediately thereafter but was never complete within 2 hours. No consistent dose-dependent effects on CM were seen. Since comparable reductions of N_1 and BSER responses were obtained at all dose rates, a major portion of the effects of propranolol in these experiments must be mediated within the cochlea. Although most of the effect on brain stem responses can be accounted for by a reduction in auditory nerve input to the CNS, the data do indicate some additional response reduction within the brain stem.

111 EXTERNAL NUCLEUS OF THE INFERIOR COLLICULUS: A SITE OF OVERLAP FOR ASCENDING AUDITORY AND SOMATOSENSORY PROJECTIONS IN THE MOUSE. F.H. Willard* and D.K. Ryugo. Depts. Anatomy, Univ. Vermont, Burlington, VT 05401 and Harvard Med. Sch., Boston, MA 02115; and Eaton Peabody Lab., Mass. Eye and Ear Infirmary, Boston, MA 02114.

We have examined second-order auditory and somatosensory pathways which project to the external nucleus(EN) of the inferior colliculus(IC). The cells of origin that project to EN have been identified consequent to horseradish peroxidase(HRP) injections into EN. In the contralateral dorsal cochlear nucleus(DCN), HRP-labelled fusiform cells of the fusiform cell layer, and elongate, horizontal and pyramidal cells of the polymorphic layer could be distinguished. In addition, HRP-labelled fusiform cells were concentrated around the margins of the contralateral dorsal column nuclei(NG&C); large, round HRP-labelled cells were also occasionally observed within these nuclei. Finally, HRP-labelled fusiform cells were localized along the lateral border of pars interpolaris in the contralateral spinal trigeminal nucleus.

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

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

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112 COMPARISON OF INFERIOR COLLICULUS NEURONAL RESPONSE PROPERTIES IN C57BL/6 AND DBA/2 INBRED MOUSE STRAINS. James F. Willott and Gregory P. Urban*. Dept. Psychol. Northern Illinois Univ., DeKalb, IL 60115.

Response properties of neurons in the inferior colliculus (IC) were studied in mice of two inbred strains--DBA/2 mice, which are innately susceptible to audiogenic seizures and nonsusceptible C57BL/6 mice. The IC was chosen for study since it appears from lesion studies to play a critical role in audiogenic seizures. Using tranquilized mice, extracellular records were obtained throughout units' response areas with intensities of up to 80 dB SPL. More than 100 neurons from mice of each strain were thoroughly studied.

For most neurons, comparison of various response parameters (e.g., frequency range, intensity functions, discharge patterns, response latencies, response to 10 sec. duration tones) showed surprisingly little difference between the two strains. There was generally poorer representation of high frequencies and slightly reduced overall threshold sensitivity in IC neurons of DBA/2 mice. However, a small proportion of neurons in the latter strain displayed dramatically abnormal response properties. These neurons showed sustained discharges with prolonged afterdischarges when stimulated with 200 ms tones of relatively high intensities. In some cases, increased levels of neural activity lasting for up to several seconds were associated with exposure to tones of 80 dB SPL. Most neurons with abnormal responses were located deep in the ventrolateral nucleus of the IC and were usually most effectively driven by tones of 15-20 kHz. Since similar abnormal responses have been reported in IC neurons of C57BL/6 mice made susceptible to audiogenic seizures by acoustic priming (Urban and Willott, EXPER NEUROL 63: 229-243, 1979), it seems likely that such responses are correlated with susceptibility to audiogenic seizures. This suggests that a relatively small population of neurons having hyperexcitable responses to high intensity sound may be responsible for audiogenic seizure susceptibility.

113 LAMINAR CONNECTIONS IN THE CAT'S AUDITORY CORTEX (AI). D. Wong* and J.P. Kelly*. (SPON.: D. Toran-Allerand). Department of Anatomy, P&S, Columbia University, N.Y., New York 10032.

Neurons in all areas of the cerebral cortex are organized in layers and this laminar pattern is related directly to the connections established by a given area. To examine the connections of layers in the cat's primary auditory cortex (AI), we have used horseradish peroxidase (HRP) for retrograde mapping and autoradiography for anterograde mapping of neuronal connections. Micropipettes filled with a solution of HRP (30%) or ^{3}H-proline (.25mCi/ul) were used to record the responses of neurons in anesthetized cats to auditory stimuli presented in the free field of a sound isolation booth. After the responses of several single units were documented, thereby localizing the injection site physiologically, the tracer was injected with pressure. Tetramethyl benzidine (TMB) was used to demonstrate HRP. Autoradiographs were prepared from frozen sections of the brain after injections of ^{3}H-proline. The results from the autoradiographic experiments indicate that the vast majority of axons from the medial geniculate body (MGB) terminate within AI in a broad band (500-675 um thick) that encompasses layers III and IV. Callosal axons, originating in the contralateral AI, terminate in irregular "patches" ranging in width from 400-800 um. These patches are found principally in layers I, II and III, but it is also possible that some callosal terminals are found in layers V and VI among the fibers of passage ascending to higher layers. HRP experiments show that these callosal axons arise from pyramidal cells in layer III, in the inner rim of layer V, and in layer VI of the contralateral AI. The cells with callosal axons in layer III are grouped in clusters, each spanning about 1100 um along the horizontal axis of the layer. The dense cellular clusters are separated by smaller spaces, $\sim$ 500 um wide, that contain only a few or no callosal neurons. Interestingly, the callosal neurons in layers V and VI are not found in distinct clusters, but rather have a homogeneous distribution along the horizontal axes of these layers. HRP experiments also show that there is a separation in the projections from layers V and VI of AI to the midbrain and the thalamus. After injection of HRP into the inferior colliculus, labeled cells in AI are found exclusively in the outer rim of layer V. These cells are the largest pyramidal neurons of the layer. After HRP injection into the MGB labeled cells are found principally in layer VI, but some are also found in the outer rim of layer V. It remains to be determined whether separate populations of neurons generate the differential projections that may arise from an individual layer in the auditory cortex. (Supported by NIH Grant NS13403 and by the Sloan Foundation).

114 BINAURAL INTERACTION IN THE CAT INFERIOR COLLICULUS STUDIED WITH INTERAURAL DELAYS AND BINAURAL BEATS. T.C.T. Yin, S. Kuwada*, and R.E. Wickesberg. Dept. of Neurophysiology, Univ. of Wisconsin Medical School, Madison, Wis. 53706.

Most cells in the inferior colliculus receive binaural input and many of the low frequency cells (<3000 Hz) have been shown to be sensitive to differences in the time of arrival of the sound to the two ears, thereby suggesting that they are involved in sound localization. Most studies of these cells have employed dichotically presented sine wave signals with variable delays in the onset of the tone to one ear with respect to the other. The repetitive cycling nature of the interaural delay curves suggests that the neurons are sensitive to the interaural phase difference. An orderly and continuously changing phase difference can also be generated by delivering sinusoids of slightly different frequencies to the two ears, i.e. a stimulus which evokes the well-known sensation of binaural beats. We have used this "binaural beat stimulus" and the traditional interaural delay paradigm to study the phase sensitivity of neurons in the cat inferior colliculus.

Platinum-gold plated indium microelectrodes were used to record single cell activity extracellularly in cats anesthetized with sodium pentobarbital. We have recorded the responses of low frequency cells that receive binaural input to the interaural delay and binaural beat stimuli. Over 90% of the cells that show phase sensitivity will respond reliably to both stimuli. With the binaural beat stimulus the neurons responded cyclically at a period corresponding to the beat frequency and maximally at a particular interaural phase difference. There is a high correlation between the values of the optimal interaural phase found with the two stimuli. In addition there was usually qualitative agreement between the shapes of the curves obtained at low beat frequencies. Thus, the binaural beat provides a means for gathering the same information as the interaural delay paradigm. The binaural beat stimulus has the additional benefit that it simulates the phase relationship obtained from a sound source moving in space. By changing the beat frequency or by interchanging the frequencies delivered to the two ears, we could also study the sensitivity of the neurons to speed and direction of movement, respectively. We have found cells that will follow up to beat frequencies of at least 80 Hz. We have also observed a few cells that show marked sensitivity to movement in a particular direction or to different speeds. Thus, the binaural beat stimulus is a very effective and efficient means for gathering information concerning binaural interaction that is not available with the traditional interaural delay stimulus.

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115 REGENERATION OF THE AUDITORY NERVE IN ANURANS: AN ANATOMICAL AND PHYSIOLOGICAL STUDY. H. Zakon and R.R. Capranica, Section of Neurobiology & Behavior, Cornell University, Ithaca, NY 14853.

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

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

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

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116 CONNECTIONS OF THE NUCLEI OF THE LATERAL LEMNISCUS IN THE MUSTACHE BAT, PTERONOTUS PARNELLII PARNELLII. J. M. Zook and J. H. Casseday, Depts. Psychology and Surgery (Otolaryngology), Duke University, Durham, NC 27710.

In the mustache bat the nuclei of the lateral lemniscus stand out as three large and cytoarchitecturally distinct areas, called here dorsal (DNLL), intermediate (INLL), and ventral (VNLL) nuclei of the lateral lemniscus. The cytoarchitectural appearance of DNLL and VNLL suggests that these nuclei have homologues in other mammals, but INLL appears so cytoarchitecturally unique as to question whether it is really part of the auditory pathway. We studied the connections of these nuclei by placing electrophoretic deposits of horseradish perixidase (HRP) or [^{3}H]-leucine in these nuclei or in subdivisions of the cochlear nucleus, superior olivary complex or inferior colliculus. Evidence from anterograde transport shows that the anteroventral cochlear nucleus supplies the main ascending projection to both VNLL and INLL, but there are little or no projections from the cochlear nucleus to DNLL. Deposit of HRP within the borders of VNLL reveal large numbers of labeled cells in the anteroventral cochlear nucleus; in the posteroventral cochlear nucleus only octopus cells were labeled. Deposits of HRP within INLL also revealed labeled cells throughout the anteroventral cochlear nucleus; in the posteroventral cochlear nucleus, octopus cells were not labeled, but other types of cells were. Very few labeled cells were found in the dorsal cochlear nucleus after deposit of HRP in any part of the lateral lemniscus.

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

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

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AUTONOMIC FUNCTION

117 SYMPATHETIC NERVE RHYTHM OF BRAIN STEM ORIGIN. Susan M. Barman and Gerard L. Gebber. Dept. Pharmacol. & Toxicol., Michigan State University, East Lansing, MI 48824.

A study was made to determine the origin of the 2-6 c/s rhythm in preganglionic splanchnic and postganglionic renal sympathetic nerve discharge (SND) in the chloralose anesthetized, baroreceptor denervated cat. Specifically, it was of interest to learn whether the rhythm is inherent to the brain stem network responsible for basal SND or whether, as suggested by the work of Camerer et al. (Pflügers Arch. 370: 221-225, 1977), it is dependent upon the integrity of interconnections between the forebrain and brainstem. For this purpose, the frequency characteristics of SND and cortical activity (parietal-frontal EEG) were compared using crosscorrelation and power spectral analyses before and after baroreceptor denervation and after midcollicular decerebration. Crosscorrelation analysis failed to reveal a relationship between SND and cortical activity before baroreceptor denervation. At this time, the predominant frequency in power spectra of SND was that of the heart rate, while the frequency of EEG activity varied between 2-6 c/s. Following bilateral section of the carotid sinus, aortic depressor and vagus nerves, crosscorrelation analysis revealed a weak relationship between SND and EEG. SND lagged cortical activity by an average of 30 ms. Power spectral analysis showed complex rhythms in the 2-6 c/s range for both SND and EEG (i.e., the frequency of slow wave occurrence varied between 2-6 c/s). Although no longer related after decerebration, complex rhythms in the 2-6 c/s range persisted in both SND and EEG. The complex rhythm in SND of the baroreceptor denervated and decerebrate cat was transformed into a simple rhythm (i.e., constant interburst intervals) which approached the upper limit of the 2-6 c/s band during short periods (15-30 s) of asphyxia. Furthermore, the rhythm was synchronized to single shocks applied to cardiovascular reactive sites in the medulla and to afferents in the sciatic nerve. These data indicate that although normally entrained to each other, the 2-6 c/s rhythms in SND and EEG are independently generated. That is, the 2-6 c/s rhythm in SND is not dependent upon the integrity of interconnections between the forebrain and brain stem. Rather, the results, in conjunction with an earlier report (McCall and Gebber, Brain Research 89: 139-143, 1975) which eliminated the spinal cord as the site of origin of the 2-6 c/s rhythm in SND, indicate that the rhythm is generated in the brain stem. (Supported by PHS Grant HL-13187.)

118 PARTICIPATION OF AUTONOMIC EFFECTOR PATHWAYS IN THE CARDIOVASCULAR EFFECTS OF ELECTRICAL STIMULATION OF THE CANINE AREA POSTREMA. Karen L. Barnes and Carlos M. Ferrario. Research Division, Cleveland Clinic Foundation, Cleveland, Ohio 44106.

We have shown that electrical stimulation of the dog's area postrema (AP) mimics the pressor response produced by intravertebral infusion of low-dose angiotensin II, producing an increase in mean arterial pressure associated with onset tachycardia and increased peripheral resistance (Barnes et al. Circ. Res., in press). In order to determine the effector pathways mediating the increases in mean arterial pressure and heart rate during AP stimulation, this structure was stimulated in 12 dogs anesthetized with morphine-chloralose before and after selective blockade of parasympathetic and cardiac or peripheral sympathetic nervous systems. The maximal increases in mean arterial pressure (+30 ± 5 mm Hg) and heart rate (+22 ± 9 bpm) were not significantly changed after bilateral cervical vagotomy (+32 ± 3 mm Hg; +21 ± 8 bpm). Beta-adrenergic blockade with propranolol (10 mg iv) eliminated the initial tachycardia and slowed the rise in arterial blood pressure without reducing significantly the final magnitude of the pressor response (+23 ± 3 mm Hg). Sympathetic ganglionic blockade with either pentolinium (3 mg/kg im) or hexamethonium (10 mg/kg iv) completely abolished the pressor effects of AP stimulation.

These data reveal that the sympathetic rather than the parasympathetic division of the autonomic nervous system was responsible for the cardiovascular effects of electrical stimulation of the canine AP, since vagotomy did not affect the response. Because the sympathetically mediated onset tachycardia was not necessary for the occurrence of the rise in mean arterial pressure, we conclude that central activation of peripheral sympathetic vasoconstriction is the essential component of the AP pressor response. These results provide additional support for our view that the area postrema participates in the central adrenergic control of arterial blood pressure.

These studies were supported in part by grants from NHLBI #HL-6835 and American Heart - HANEO.

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

As early as 1920, Billigheimer proposed that epinephrine has a modulatory effect on the sympathetic-cholinergic electrodermal system in humans (Billigheimer, Naunyn-Schmiedebergs Arch. exp. Path. Pharmak., 1920, 88, 172). Previous studies in our laboratory have indicated that systemic administration of epinephrine depresses the amplitude of postganglionically evoked electrodermal responses in cats. This effect was antagonized by 10 mg/kg phentolamine (Koss, Davison and Bernthal, Psychopharmacology, 1976, 50, 149). The present studies were undertaken to determine if systemic administration of epinephrine would inhibit reflexly evoked electrodermal responses in a similar fashion, and if this inhibition could also be produced by endogenous release of catecholamines. Cats were anesthetized with pentobarbital (36 mg/kg, ip) or a combination of α-chloralose (30 mg/kg, ip) and urethane (300-500 mg/kg,ip). Reflex electrodermal responses were evoked by stimulating the proximal portion of a ligated peroneal nerve once every 60 sec with a 10-30V, 6-12 Hz train of 1 sec duration. Graded doses of epinephrine (0.3-10.0 ug/kg, iv) depressed the amplitude of electrodermal reflexes in a manner that was quantitatively equivalent to that observed with the peripherally evoked response. Asphyxia (5 min) also resulted in an inhibition of both the reflexly and peripherally evoked responses which was antagonized by bilateral adrenalectomy. In order to further elucidate the mechanism of this effect, epinephrine was administered to cats before and after phenoxybenzamine or yohimbine. Yohimbine (0.5 mg/kg, iv), an α-adrenergic blocker which is postulated to act preferentially at pre-synaptic sites, effectively antagonized the inhibitory action of epinephrine on peripherally evoked electrodermal responses. Phenoxybenzamine (2.5 mg/kg, iv), an α-adrenergic blocker considered to act mainly at post-synaptic sites, did not antagonize epinephrine's inhibition. As expected, the pupillary effects of epinephrine, which have been shown to be post-synaptic in nature, were strongly antagonized by phenoxybenzamine, but not by yohimbine. The results of these studies demonstrate that systemically administered epinephrine, as well as endogenously released catecholamines, act to depress the amplitude of both reflexly and peripherally evoked electrodermal responses. The relative degree of antagonism of this effect by yohimbine and phenoxybenzamine suggests that catecholaminergic modulation of this sympathetic-cholinergic sudomotor system may occur at pre-synaptic sites.

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120 SYNAPTIC HYPERFUNCTION IN A SYMPATHETIC GANGLION AND ITS POTENTIAL SIGNIFICANCE IN ESSENTIAL HYPERTENSION. R.I. Birks, Physiology Department, McGill University, Montreal, Canada H3G 1Y6.

Patterning of the preganglionic neural input to the cat superior cervical ganglion into brief high frequency trains causes a progressive and maintained increase in ganglionic acetylcholine (ACh) stores of up to 170% over those of the contralateral control ganglion. It was proposed that the effect might be related to accelerated sodium pump activity resulting from the rise in internal nerve ending sodium during the trains (Birks, R.I., J. Physiol. 280, 559-572, 1978).

It has now been found that the ACh stores increase to the same extent as with patterned stimulation during perfusion with normal K^+ following sodium-loading of the ganglia by removal of extracellular K^+. Since no increase in ACh occurred during sodium loading when the pump was inhibited, but only during accelerated pumping in the recovery period, it is concluded that the effect is related to accelerated sodium pumping, rather than to a direct effect of the raised intracellular Na^+ at the nerve terminals.

ACh output was found to increase 2.5-3.0 fold during 60 min activation by patterned stimulation, or by equally spaced low frequency pulses during recovery in normal K^+ following pump inhibition. These effects on ACh output are greater than can be accounted for by the increases in ACh stores (Birks, R.I., J. Physiol. 271, 847-862, 1977). Because ACh release is known to be increased by raised $[Na]_i$ (Birks, R.I. and Cohen, M.W. Proc. Roy. Soc. B. 170, 401-421, 1968; Charlton, M.P. and Atwood, H.L. Br. Res. 134, 367-371, 1977) it is proposed that the extra effect of patterned stimulation on ACh output is related to the increase in $[Na]_i$ and perhaps also to the hyperpolarization which accompanies accelerated sodium pumping. The general conclusion that arises from this work is that the internal sodium load at synapsing preganglionic nerve endings modulates ACh output and thus effector activity.

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

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121 A NONINVASIVE LASER-DOPPLER AND RADIOMETRIC MONITOR OF CUTANEOUS AND SKELETAL MUSCLE MICROCIRCULATION. R.F. Bonner*, P.D. Bowen*, R.L. Bowman*, A.J. Tahmoush, and W. King Engel (SPON: R. Curtis Graeber). NIH, Bethesda, MD 20205

A laser-Doppler, blood flow monitor and an LED-phototransistor reflection radiometer have been simultaneously used to measure the dynamics of the microvasculature in skin of human finger and forearm and exposed rat muscle. These noninvasive techniques measure changes in blood flow, blood volume, and red blood cell velocity within small (mm^3), superficial regions of the microvasculature. The Doppler-broadening of laser light scattered by moving red blood cells is analyzed in real-time by an analogue processor which has been shown to be linearly correlated with flow (Am. J. Physiol. 232:H441-8, 1977). The radiometric technique employs a calibrated near-infrared light source and phototransistor to detect light backscattered from underlying tissue (Techniques in Psychophysiology,1979). The radiometric signal is inversely related to tissue blood volume.

In six male subjects during basal condition, the results (1-4) were obtained. 1) Maximum laser-Doppler flow values were 2.7 ± 1 with a fractional pulsatile component of 11 ± 3% from the fingertip and .05 ± .01 with a pulsatile component of 20 ± 7% from the forearm skin. The corresponding radiometric values were 15 ± 5 μ W with a fractional pulsatile componentof 0.4 ± .1% from the fingertip and 28 ± 1 μ W with 0.04 ± .03% pulsations from the forearm skin. 2) During the Valsalva maneuver, the laser-Doppler blood flow value from the fingertip was transiently reduced to less than 10% of the resting value with an associated 5-7% increase in radiometric signal suggesting a decrease in local blood volume. 3) During graded, externally-applied pressure increases, the laser flow values decreased in a sigmoidal manner with a corresponding increase in pulsatile flow. The radiometer showed a corresponding increase in average blood volume but a decrease in pulsatile changes. 4) Following total occulsion, a 6-fold increase in laser flow values was observed in the forearm skin. The magnitude of this observed hyperemic response increased with occlusion duration from 15 to 120 sec. In the rat muscle, autoregulation of blood flow was observed with partial aortic compression and hyperemic responses were measured following total occlusion.

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

122 THE RATIO OF PREGANGLIONIC AXONS TO POSTGANGLIONIC CELLS IN THE SYMPATHETIC NERVOUS SYSTEM. Rebecca Brooks-Fournier* and Richard E. Coggeshall. (SPON: A.E. Applebaum). Departments of Anatomy and of Physiology and Biophysics and the Marine Biomedical Institute, The University of Texas Medical Branch, Galveston, Texas 77550.

It is widely believed that the postganglionic cells outnumber the preganglionic axons in the sympathetic division of the autonomic nervous system by at least 11 to 1. This figure is usually contrasted to the parasympathetic innervation where the ratio is felt to be approximately 2 to 1. These figures give rise to the idea that the sympathetic system has a "diffuse" innervation as compared to the "precise" parasympathetic innervation. There are two major difficulties with the studies that established these ratios, however, First, the preganglionic axons were counted using the light microscope which does not allow an adequate assessment of the numbers of unmyelinated axons. Second, the axons were counted in the sympathetic chain just caudal to the superior cervical ganglion and there was no attempt made to prove that all the axons in this location were preganglionic in nature; it was assumed that there were no sensory or postganglionic axons present to contaminate the counts. To take account of these difficulties, the number of axons in the sympathetic chain was reassessed by using the electron microscope and found that the ratio of preganglionic axons to postganglionic neurons was 1 to 1.9, which is far higher than reported in the classic literature. The second part of the study, to check for the presence of sensory and/or postganglionic fibers in the chain is reported here. Ventral roots C8 - T4 were transected in rats. This operation would remove the preganglionic fibers. The remaining fibers were counted and in three rats, the average number of surviving axons was approximately 600 out of a total population of approximately 4500. If further work confirms these preliminary observations, then two conclusions are possible; 1) that the ratio of preganglionic to postganglionic axons in the sympathetic system is approximately 1 to 2.5, which is much higher than previously reported and 2) that there are a significant number of postganglionic and/or sensory fibers in the cervical sympathetic trunk. This work is partially supported by NIH grants NS 07377 and NS 10161 and a teaching and research assistantship of the University of Texas Medical Branch.

123 CENTRAL AND PERIPHERAL CATECHOLAMINERGIC ACTIVITY DURING SODIUM DEPLETION. K. Bridget Brosnihan, Julianna E. Szilagyi* and Carlos M. Ferrario. Research Division, Cleveland Clinic Foundation, Cleveland, Ohio 44106.

Reduction of sodium intake affects both the renin-angiotensin and sympathetic nervous systems, but the effects on the latter are less well understood. During sodium depletion it has been demonstrated histochemically that the adrenergic nerve terminals are depleted of neural transmitter (Ljundqvist et al. Acta path. microbiol. scand. 83:661,1975). Others (Rocchini et al. Am. J. Physiol. 233: 196, 1977) have shown the presence of depressed sympatnetic reflexes which they attributed to a decreased ability to release norepinephrine from peripheral sympathetic nerve endings.

In order to assess whether these alterations in sympathetic activity could originate centrally, samples of cerebrospinal fluid (CSF) from the cisterna magna and plasma were simultaneously taken to assess differences in the peripheral and central levels of catecholaminergic activity in mongrel dogs placed first on a diet of normal followed by a diet of low sodium content (40 mEq sodium/day vs. 4 mEq sodium/day respectively). Animals on the low sodium diet received furosemide (40 mg/day i.m.) during the last three days of the 21-day period of dietary sodium depletion. Samples were taken under pentobrabital anesthesia. Catecholamines [norepinephrine (NE), epinephrine (EPI), and dopamine] were measured by the radioenzymatic method.

After sodium depletion plasma NE rose from 127 ± 19 to 223 ± 28 pg/ml ($p< 0.01$) but EPI and dopamine did not change (111 ± 3 vs. 193 ± 43 pg/ml and 90 ± 43 vs 50 ± 6 pg/ml respectively). In CSF, NE rose from 87 ± 10 to 121 ± 14 pg/ml ($p< 0.05$) while EPI and dopamine levels remained unchanged (9 ± 3 vs 6 ± 1 pg/ml and 53 ± 10 vs. 63 ± 27 pg/ml respectively). Plasma sodium decreased from 151.3 ± 2.4 to 143.8 ± 1.1 mEq/l ($p< 0.05$) and similarly CSF sodium decreased from 154.3 ± 4.0 to 146.8 ± 2.3 mEq/l ($p< 0.05$). No correlation was found in plasma between sodium and NE levels, but in CSF the levels of sodium appears to be inversely correlated to the levels of NE.

Our findings of an inverse correlation between CSF sodium and NE suggests the possibility of a sodium-mediated effect influencing central noradrenergic activity. Such augmented central noradrenergic activity indicates that the previously reported alterations of the sympathetic nervous system are not confined to the peripheral site but may originate centrally. The presence of elevated plasma NE levels may result secondarily from the elevated plasma renin activity or depleted plasma volume, factors which are both known to be associated with sodium depletion.

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

Blood-borne angiotensin (AII) may rapidly increase arterial pressure not only by its direct vasoconstrictor action on vascular smooth muscle but also by activation of central nervous system mediated pressor mechanisms. Although some evidence also implicates more rostral brain structures, AII sensitive brainstem sites (area postrema, subnucleus medialis) have been identified in dog, cat, and rabbit which initiate the centrally mediated component of the pressor effect of circulating AII. In the rat however, intracarotid but not intravertebral artery infusion of AII produces an exaggerated pressor response suggesting that in this species, AII sensitive brain sites mediating the central component of the pressor effect are located in the forebrain region rather than brainstem. Consistent with this view is the finding of no change in pressor response to circulating or intraventricular AII after area postrema ablation in rat. Previous stimulation and ablation studies have demonstrated the importance of preoptic-hypothalamic periventricular tissue surrounding the anteroventral third ventricle (AV3V) for centrally mediated pressor, dipsogenic, and antidiuretic effects of AII in the rat. An adjacent circumventricular structure, the subfornical organ (SFO), is also exceptionally sensitive for pressor and dipsogenic responses to AII. The supraoptic nucleus, site of cell bodies synthesizing antidiuretic hormone, has been reported to receive projections from both AV3V and SFO. This study assessed the effect of SFO ablation on drinking and pressor responses elicited by intracarotid (IC) or intraabdominal aortic (IA) infusions of AII in conscious rats with chronic indwelling catheters. SFO ablation did not affect body weight, basal blood pressure or heart rate but compared to rats with control lesions, SFO lesioned rats showed a reduced drinking response to IC AII infusion. SFO lesioned rats also had reduced pressor responses to IC but not IA infusion of 25, 50, or 100 ng AII/min. The reduced pressor response to IC AII infusion is not an exclusive property of SFO ablation since the same effect has been reported after AV3V ablation. The finding that SFO ablation abolished the increased pressor sensitivity to IC infusion of AII is consistent with the hypothesis that both SFO and AV3V are part of an AII sensitive periventricular system in rat forebrain which mediates the central component of the pressor action of blood-borne AII. (This work was supported by a grant in aid from the American Heart Association and with funds contributed in part by the SC Heart Association.)

125 THE INVOLVEMENT OF MEDIAL MEDULLARY RETICULAR FORMATION IN CARDIOVASCULAR EFFECTS OF CLONIDINE IN EXPERIMENTALLY-INDUCED HYPERTENSIVE CATS. Yih Huey Chen and Samuel H.H. Chan. Dept. Life Sci., Indiana State Univ., Terre Haute, IN 47809.

Clonidine, a potent antihypertensive agent, has been demonstrated in our laboratory to exert its hypotensive and bradycardiac actions via the activation of α-adrenoceptors in the medial medullary reticular formation (MMRF) (Chen and Chan, Neuroscience Abst. 4:18, 1978) in normotensive cats, actions that require the presence of vagus nerve. In addition, the MMRF has been identified in the rat to be at least one of the central sites of clonidine induced actions (Chan and Koo, Neuropharmacol. 17:367, 1978). The present study was undertaken to further investigate the cardiovascular effects of clonidine in experimentally-induced hypertensive cats and the involvement of MMRF in these actions.

Cats that were lightly anesthetized with pentobarbital sodium (35 mg/Kg, i.p.) or precollicularly decerebrated were used. Cannulations of left carotid artery and left femoral vein were performed for measurement of arterial blood pressure (ABP) and injection of drug. Heart rate (HR) was determined by conventional EKG. Experimental hypertension was induced at the beginning of the recording session by common carotid occlusion, which elicited a maintained elevation of ABP without a significant change in HR.

Systemic injection of clonidine (10 μg/Kg, i.v.) in the experimentally-induced hypertensive cat produced an initial, transient increase in ABP (mean, systolic, and diastolic), followed by a significant decrease of the same parameters and a prolonged suppression of HR for 60 min. Apart from the transient hypertension, which represents a peripheral vascular effect of clonidine, depression of ABP and HR by this imidazoline compound was eliminated after bilateral vagotomy, indicating that these circulatory events were mediated by the vagus nerve. Likewise, bilateral focal electrolytic lesions of MMRF (three 1 mm apart lesions on each side) resulted only in the transient hypertension without the subsequent hypotension and bradycardia after systemic injection of clonidine (10 μg/Kg). Preliminary results indicated that unilateral microinjection of clonidine (1 μl in vol.) directly into three 1 mm apart loci in MMRF at an ineffective dose (0.2 μg/Kg) when administered systemically produced a significant decrease of ABP and HR without the preceding hypertension, while control microinjections of saline (vehicle) did not produce any cardiovascular changes.

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

126 EFFERENT PROJECTIONS OF THE CANINE AREA POSTREMA. C.L. Chernicky,** K.L. Barnes, J.P. Conomy and C.M. Ferrario. Division of Research and Dept. of Neurology, Cleveland Clinic Foundation, Cleveland, Ohio 44106.

The canine area postrema (AP) has been shown by Barnes et al. (Circ. Res., in press) to be the site of a physiological pressor pathway. We have previously described the morphological composition of this structure (Chernicky et al., Neurosci. Abstr. 4: 13, 1978). In order to demonstrate the anatomic pathway from the canine AP, efferent projections were studied by the Fink-Heimer and Wiitanen modifications of the Nauta-Gygax silver degeneration technique. Discrete electrocoagulation lesions were placed in the AP under direct vision with the aid of an operating microscope. Following survival times of 2-5 days, animals were perfused with isotonic saline followed by 10% buffered formalin. After 4-8 weeks storage in 10% formalin, the brainstems were serially sectioned at 25 μm in the sagittal, horizontal, or transverse plane. Every fifth section was stained with luxol fast blue and/or cresyl violet for both anatomic orientation and establishment of the extent of the lesion. Adjacent sections were silver stained for degenerating axons and terminals.

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

The observation of terminal degeneration in the AP-NTS junctional zone confirms the finding in Golgi studies of AP neurons with short axons projecting into this region. The present study also demonstrates an efferent projection from the AP to the medial NTS. The degenerating fibers seen crossing the midline in the nucleus commissuralis of Cajal provide evidence for bilateral interconnectedness of the two halves of the AP. This study suggests that the AP projects only to the closely adjacent medullary structures, and implies that the pathways which mediate its facilitative effects on the sympathetic vasomotor outflow are multisynaptic.

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127 ADRENERGIC AND CARDIOVASCULAR RESPONSIVENESS OF 28-MONTH OLD FISHER-344 RATS FOLLOWING STRESS. C.C. Chiueh and S.I. Rapoport. Lab. Neuro Sci., NIA, NIH, Balto City Hosp. Balto.,MD 21224.

The blood pressure and heart rate of freely moving or restrained male Fisher-344 rats at 3 months (young), 12 months (adult) and 28 months (old) of age were monitored through a chronic indwelling catheter which was implanted in the ventral tail artery at least 20 hrs before the experiment (Chiueh and Kopin, Amer. J. Physiol. 234:H690-H695, 1978). The sympathetic activities of the animal were determined by radioenzymatically measuring the catecholamines in 50 μl of plasma. Conscious and unrestrained Fisher-344 rats undergo a decrease in heart rate and an increase in diastolic blood pressure with increasing age. The heart rate of old rats, 369±12 bpm, was significantly lower than those of young (396±6 bpm) or adult (385±5 bpm) rats. The systolic and diastolic blood pressure of unrestrained young, adult and old rats were 124±5/71±4, 141±3/78±2 and 120±4/92±4 mm Hg, respectively.A 3 min or 30 min forced immobilization was applied to the animals and used as physical and emotional stressors in order to investigate the effect of the aging process on the amplitude and rate of responsiveness in the sympathetic and cardiovascular systems. Three minutes of stress failed to increase the blood pressure and heart rate of old rats, but increased cardiovascular responses in young and adult rats. Following 30 min of stress, the heart rate of aged rats increased slightly (Δ29±9 bpm), approximately 1/3 to 1/2 of the increase seen in young rats. The immobilized rats had slightly lower blood pressures than unrestrained controls because of their massive release of epinephrine following the 30 minute stress. The resting plasma levels of norepinephrine in unrestrained Fisher-344 rats ranged from 0.4 to 1.1 ng/ml and increased slightly with increasing age. Aged rats showed no decrease in the maximal adrenergic release due to stress, but had a prominent delay in the cathecholamine release. The respective increments of plasma norepinephrine after 3 min and 30 min stresses were 1.8±0.5 and 3.5±0.4 ng/ml. The delay of norephinephrine release may be responsible for the lack of blood pressure change after 3 min stress. A decrease in the activity of adrenergic receptors, especially β receptors, in the target organs was evident because the 30 min stress induced a greater increase in plasma epinephrine in old rats (Δ7.3±1.1 ng/ml) than in adult rats (Δ4.3±0.6 ng/ml) while producing a decrease in β-adrenergic responses; i.e., heart rate (old = Δ29± 9 vs adult = Δ88±6 bpm) and blood sugar (old = 141±3 vs adult = 217±5 mg%). The present results indicate that the release mechanism of the sympathoadrenal medullary system of aged Fisher-344 rats can respond maximally but not efficiently to stress due to a decline in the rates of catecholamine release and a decrease in the activities of adrenergic receptors or effectors in the target organs.

128 DIRECT PROJECTIONS FROM THE NUCLEUS TRACTUS SOLITARIUS TO THE HYPOTHALAMUS IN THE CAT. J. Ciriello and F. R. Calaresu, Dept. of Physiology, Univ. of Western Ontario, London, Canada N6A 5C1.

Although it is known that the function of the hypothalamus can be affected by cardiovascular afferent information in the buffer nerves the course and distribution of these afferent fibers from the first relay in the n. tractus solitarius (NTS) to the hypothalamus is not known. The traditional view is that the NTS relays afferent information to the hypothalamus via numerous connections in the brain stem reticular formation, but recent evidence obtained in the rat suggests a direct connection. Two series of experiments were done in cats in an attempt to obtain anatomical and electrophysiological evidence concerning this issue. In the first series, small injections (20-40 μl) of a mixture of tritiated amino acids were made in the caudal NTS of 11 cats and after 1-15 days' survival the brains were processed for autoradiography. Fibers from the NTS appeared to project rostrally, through the lateral periaqueductal gray matter and medial lemniscus bilaterally, to several hypothalamic regions: the periventricular hypothalamic area, the paraventricular hypothalamic n. (PAH), the dorsomedial hypothalamic area (DMH), and the subcommissural part of the bed n. of the stria terminalis (BST). In three animals in which the injection missed the NTS and was mainly confined to dorsal column nn., no label was seen in the hypothalamus although the contralateral medial lemniscus was heavily labeled. In the second series, the electrical activity of 84 single units widely distributed in the hypothalamus was monitored in chloralosed cats for changes in firing frequency during stimulation of the carotid sinus nerve (CSN) and NTS. CSN stimulation excited 46 of the 84 units, 23 in the PAH (mean latency, 27.1 ± 3.0 ms), 14 in the DMH (mean latency, 19.2 ± 3.2 ms), and 9 in the BST (mean latency, 14.9 ± 1.0 ms). These units were also excited by NTS stimulation with significantly shorter latencies (mean latencies, 13.1 ± 2.1 ms, 9.5 ± 2.0 ms, 6.9 ± 1.2 ms, respectively). These results demonstrate the existence of a fiber tract originating in the NTS conducting at 2.3 to 4.4 m/s and carrying cardiovascular afferent information directly to the hypothalamus.

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

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

A typical classic migraine attack is characterized by a biphasic pattern of vasomotor behavior. The prodrome stage involves the reduction of intracranial blood flow followed by a reactive dilatation of the intracranial and extracranial arteries. It has been hypothesized that hand-warming through biofeedback training may result in a decrease in sympathetic outflow, thereby, interrupting the vasomotor pattern of change in a migraine headache.

Normal subjects were 12 right handed women and they were compared to 12 right handed female migraineurs. Age ranges for the normal group were 18 to 35 and for the migraine group 27-52. Migraineurs were selected on the basis of the following characteristics: manifesting either classic or common migraine and having a minimum of two migraine attacks per month. Each volunteer was randomly assigned to either a hand-warming or a hand-cooling temperature biofeedback group and given extensive training in skin temperature self-regulation in a series of 12-15 sessions.

Each subject was subsequently given 2 measures of regional cerebral blood flow (rCBF) utilizing the non-invasive ^{133}Xe inhilation technique. One rCBF run was given during a relaxation steady-state condition and a second while subjects were attempting to manipulate their skin temperature in the trained direction.

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

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

130 THE EFFECT OF LEFT CARDIAC SYMPATHECTOMY ON CARDIAC CHANGES DURING BEHAVIORAL STRESS IN DOGS. Lewis K. Clarke* and Richard A. Galosy. Dept. of Cell Biology, Univ. of Texas Hea. Sci. Ctr. at Dallas, TX 75235.

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

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

The neurons of origin of preganglionic motor axons that travel in the subdiaphragmatic segment of the vagus nerve classically have been ascribed to the dorsal motor nucleus of the vagus (DMX) in the medulla. To re-examine the central cell groups that give rise to such axons in rats, the ventral or dorsal branch of the subdiaphragmatic vagus was transected near the stomach and incubated in crystalline horseradish peroxidase (HRP) for 6 to 8 hours. After 48 hours post-operative survival, the animals were killed and their brains processed according to standard HRP histochemical technique, using tetramethyl benzidine as the chromagen. After counterstaining with neutral red, the tissue sections were examined under bright- and dark-field illumination for the presence of HRP-positive somata. As expected, numerous retrogradely labeled cells were present in DMX, distributed throughout the rostro-caudal extent of the nucleus. HRP-positive cells were also observed bilaterally in the rostral portion of the nucleus ambiguus. This finding is in contrast to previous accounts in which motor fibers of the nucleus ambiguus were considered to project only to the larynx, pharynx and cervical esophagus (Lawn, J. Comp. Neurol. 127, 1966, p. 293), but support functional evidence that suggests DMX is not the exclusive source of subdiaphragmatic vagal motor fibers (Kerr, J. Physiol., 202, 1969, p. 755; Lawn, J. Physiol., 174, 1964, p. 232.) Different patterns of cell-labeling were observed in DMX which could be correlated with one or the other of the two vagal branches. Incubation of the ventral branch resulted in labeled cells in only the left DMX, while incubation of the dorsal branch labeled cells on both sides, although more extensively on the right. The extent of the retrograde labeling in DMX indicates that its rostro-caudal limits extend somewhat further than is usually assumed on the basis of cytoarchitecture alone. The incubation procedure also resulted in anterograde transport of HRP in vagal afferent axons which could be traced to a circumscribed portion of the nucleus tractus solitarius immediately ventral and rostrolateral to the area postrema.

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132 EFFECT OF BILATERAL CERVICAL VAGOTOMY IN SQUIRREL MONKEYS. K.C. Corley, H.P. Mauck* and F.O'M. Shiel*. Dept. of Physiol., Pediat and Path. Med. Coll. of Virginia, VCU, Richmond, VA 23298.

Stress-induced cardiac arrest and myocardial pathology observed in squirrel monkeys has been suggested to be mediated by the autonomic nervous system (Psychophysiol. 14: 322, 1977). The involvement of parasympathetic cardiac input was studied by bilateral cervical vagotomy. This procedure in 27 monkeys was accomplished in one operation. While 9 monkeys succumbed suddenly within 24-48 hrs, no additional deaths over 35±13 days (M±SE) occurred. Lethargy and apnea were noted only during the first week. A weight loss also occurred, but body weight stabilized at a lower level by the second week. Mortality, lethargy and apnea were almost totally eliminated by vagotomy in two operations with weight stabilization used to indicate readiness for second operation. Since the only mortalities were two monkeys that had a left followed by right section, the sequence of vagi section was important. Because the right vagal input is greater than the left, the effect of a right-left denervation was more evenly distributed between the two operations than a left-right denervation. This observation suggested autonomic imbalance may be a factor in vagotomy deaths. Seventeen monkeys after the two stage vagotomy were studied for 40±8 days before stress with no other mortalities. While resting heart rate (335±4 bpm; N=28) was greater than that of intact monkeys (300±8 bpm; N=28; $p > .01$), blood pressure did not differ (vagotomy: 151/120mm Hg; N=3 and intact: 149/108mm Hg; N=6). Vagotomy monkeys subjected to shock stress (1 sec.; 4mA; 60Hz) showed the same heart rate or blood pressure changes as intact monkeys. Tachycardia was followed by bradycardia with cardiac arrest which had an incidence comparable to that of intact monkeys stressed for similar periods of time. While acute myocardial pathology was like that of intact monkeys, myocardial fibrosis was also observed in 5 vagotomy monkeys sacrificed after a single 24-hr. stress session. Since no fibrosis occurred in intact monkeys after a 24-hr. stress, cardiomyopathy was attributed to vagotomy per se and enhanced sympathetic activity after the abrupt removal of vagal restraint. Thus, squirrel monkeys survived vagotomy, and reaction to stress was comparable to that of intact monkeys. The immediate effects of vagotomy indicated autonomic involvement, but failure to affect stress-induced bradycardia and cardiac arrest suggested that parasympathetic input was not necessary for these cardiovascular changes (Supported by HE-13454).

133 PROJECTION OF SPLANCHNIC, PNEUMOGASTRIC AND SINUS NERVE AT SUPRAMEDULLARY LEVELS.

Bernard Delbarre, Alain Chatelus*, Danielle Senon* - Laboratoire de Chirurgie Expérimentale, Faculté de Médecine, 37032 TOURS FRANCE.

Projection of sinus nerve afferents are also located at supramedullary levels in structures in which autonomous and emotional events are connected. CHATELUS and al., 7 Int. Congress Pharmac. Abstracts, 1978, 2921. We have investigated the projection of splanchnic and pneumogastric nerve by using the technique of averaged evoked potential (EP) in chloralozed cats.

The projection of carotid sinus nerve, pneumogastric and splanchnic nerve are located at the same place in anterior and posterior hypothalamus, amygdala, cortex SI SII and ansate sulcus. Stimulation of these areas induces modifications of blood pressure and heart rate.

Destruction of nucleus tractus solitarii induces an increase of blood pressure, decrease of systemic hypertension evoked by norepinephrine and suppression of EP sinus nerve at supramedullary levels. Amplitude of evoked potentials of splanchnic and pneumogastric nerve are diminished.

These results suggest that a supramedullary level in some areas, there is projections of sinus nerve, sympathetic and parasympathetic nerve at the same place.

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134 REPEATED RESTRAINT STRESS AND PLASMA CATECHOLAMINES IN RATS. Kathryn H. DeTurck* and Wolfgang H. Vogel, Dept. Pharmacol., Thomas Jefferson Univ., Philadelphia, PA 19107.

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

Animals differed behaviorally; excitement ranged from mild to severe during the first stress period and this behavior declined noticeably during the next two restraint periods. Levels of NE and E were similar in all animals at the beginning of the experiments, 186.6±40 and 101±29 pg/ml, respectively; baseline values at 0 time did not change for the next two experiments. Restraint stress markedly increased NE and E levels during the first exposure with peak levels of NE (1264±333 pg/ml; 576% as compared to 0 min) and E (1230±500 pg/ml; 1118%) occurring at 5 min during the first restraint period. Levels of NE (355±58 pg/ml) and E (288±137 pg/ml) were still significantly elevated 30 min after the stressor had been removed. Increases in E (about 1000%) were greater than those seen with NE (about 500%). The second restraint period showed a decrease in peak NE (250%; $p < 0.1$) and E (300% ; $p < 0.05$) levels. The third experiment demonstrated further adaptation since peak levels of NE (260%; $p < 0.05$) and E (235%; $p < 0.05$) continued to decline slightly. Studies of the effects of various drugs on the stress response are planned.

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

Colony-bred beagles were selected for long-term studies of arterial blood pressure (AP) both prior and subsequent to bilateral lesions in the rostral fastigial nucleus (FN) as an effort to examine the autonomic role of the nucleus in the conscious animal. Previous studies with dogs in my laboratory have described the hypertensive response to electrical stimulation of the FN in anesthetized and conscious dogs. The objective of the study was to verify the absence of mean AP changes following ablation of rostral FN. Four beagles (2 male, 2 female) were implanted under sterile procedure with solid state pressure transducers in descending aortae and AP and heart rate (HR) recorded on a monthly basis, for 24 hour sessions, up to 9 months following surgery. Animals were acclimated (1 month) to the laboratory environment and recording apparatus prior to chest surgery. The 24 hour studies were performed 2-3 months prior to lesioning and 1-4 months post-lesion. The 24 hr mean ± S.D. of all the mean AP's for 4 dogs was 102.6 ± 4.7 mmHg and HR was 69 ± 10.5 beats/min (ten-24 hr records). Next, the dogs were anesthetized with alpha-chloralose (110 mg/kg i.v.) and the regions of the rostral FN were stereotaxically implanted under sterile surgery with concentric bipolar electrodes. When the maximal pressor response was obtained, the site was then lesioned with RF current until the response could no longer be elicited. The 24 hr mean ± S.D. for 3 dogs beginning 1 month following surgery was 122 ± 5.1 mm Hg and heart rate was 81 ± 11.5 beats/min. All 4 dogs showed elevated mean AP and the fourth dog post-lesioning exhibited waking mean AP of 110 to 160 mm Hg, a pulse pressure of 175/110 and 100 mm Hg mean AP during sleep but the 24 hr mean was not yet available. These results were totally unexpected, however, a plausible explanation for the induced hypertension is that a chronic FN inhibitory tone to the reticular formation is removed which is only observable over a long time period. Nevertheless, these results are in conflict with the pressor response obtained by electrical stimulation in cats and dogs. This work was supported by NIH grant HL22747.

136 SOMATOSTATIN AND SOMATOSTATIN ANALOGS INHIBIT SYMPATHETIC NERVOUS SYSTEM ACTIVITY. David Fisher*, Wylie Vale and Marvin Brown (SPON: Jon Lindstrom). Peptide Biology Laboratory, The Salk Institute, La Jolla, CA 92037.

Somatostatin (SS) and SS analogs, e.g. des-AA^{1,2,4,5,12,13}-[D-Trp^{8}]-SS (ODT8-SS) placed intracisternally (ic) or intracerebroventricularly (icv) have been demonstrated to prevent the hyperglycemia, hyperglucagonemia, and hypoinsulinemia induced by bombesin given ic or icv. SS and SS analogs inhibit these humoral changes by preventing bombesin induced adrenomedullary epinephrine secretion. These experiments suggest that SS and SS analogs may act within the brain to decrease sympathetic nervous system (SNS) activity. Therefore, we have carried out studies to determine whether SS and SS analogs prevent activation of the SNS by other neural stimuli. Plasma epinephrine (E) and norepinephrine (NE) were measured using a radioenzymatic assay, i.e. enzymatic conversion of the catecholamines to their respective 3-methoxy derivatives in the presence of [^{3}H-methyl]S-adenosyl methionine. Treatments were administered icv to rats via chronic indwelling lateral ventricular cannulas, and blood samples taken from venous jugular catheters. Human β-endorphin (20μg) and carbachol (10μg) given icv, intravenous insulin (1.5U), and handling stress induce a significant rise in plasma glucose, glucagon and epinephrine. These humoral changes were completely prevented by simultaneous administration of ODT8-SS (1μg) icv. OTD8-SS (1μg) alone did not significantly lower basal catecholamines; however, a rise of E and NE occurs 60 minutes following treatment suggesting a post-inhibitory rebound. Further evidence that ODT8-SS acts to decrease SNS activity is based on the observation that it prevents the rise in NE following icv carbachol (10μg). Recent studies have suggested that spontaneously hypertensive rats (SHR) may have an overactive SNS. Administration of ODT8-SS (1μg) ic to SHR reduces systolic blood pressure from ca. 170 mmHg to ca. 100 mmHg for at least 60 minutes. These data provide strong pharmacologic evidence that SS and SS analogs, particularaly ODT8-SS, act within the CNS to modulate SNS activity. SS's role in normal and abnormal physiology of the SNS remains to be determined.

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

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

138 BASIS FOR COMPLEX RHYTHM IN SYMPATHETIC NERVE DISCHARGE (SND). Gerard L. Gebber and Susan M. Barman. Dept. Pharmacol. & Toxicol., Michigan State Univeristy, East Lansing, MI 48824.

The cardiac-related rhythm in SND is transformed into a complex rhythm of brain stem origin (i.e., frequency of sympathetic nerve slow wave occurrence varies between 2-6 c/s) after baroreceptor denervation. The present study was designed to determine the basis for the complex rhythm. For this purpose, discharges recorded simultaneously from different postganglionic sympathetic nerves were compared before and after baroreceptor denervation (i.e., bilateral section of carotid sinus, aortic depressor and vagus nerves). The phase relations between slow waves in postganglionic nerves (e.g., internal and external carotid) which exit from the same sympathetic ganglion (i.e., intraganglionic recording) were fixed before and after baroreceptor denervation. As a consequence, crosscorrelation functions approached a value of one. Crosscorrelation analysis revealed a weaker relationship between slow wave activity recorded from postganglionic nerves (e.g., external carotid and renal) which exit from different sympathetic ganglia (i.e., interganglionic recording). The weaker relationship in the experiments with interganglionic recording was explained by marked shifts in the phase relations between slow wave activity from cycle to cycle of SND. Regarding this point, the distribution of intervals between the peaks of slow waves recorded from the external carotid and renal postganglionic nerves in 100 consecutive cycles of SND was 80±7 ms before baroreceptor denervation and 132±6 ms after baroreceptor denervation (6 experiments). Importantly, the shifts in phase relations between slow wave activity in the experiments with interganglionic recording far exceeded the range of conduction times in pathways from the brain stem to the external carotid and renal nerves (as determined from the range of onset latencies of sympathetic nerve potentials evoked by electrical stimulation of medullary pressor sites). These observations have led us to propose that the brain stem sympathetic network is comprised of a number of coupled oscillators, each of which is inherently capable of producing a 2-6 c/s rhythm in a separate group of postganglionic sympathetic neurons. Variations in the phase relations between slow wave activity in the interganglionic recording experiments further suggest that the complex form of the 2-6 c/s rhythm in SND results as the consequence of changes in the sequence of activation of coupled brain stem oscillators. That is, the leading brain stem focus is believed to shift from cycle to cycle of SND. (Supported by PHS Grant HL-13187.)

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

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

Occipital craniotomies were performed in anesthetized cats and stimulating electrodes were inserted into the DMN or NA on either side of the neuraxis. A strain gauge arch was sutured to the right ventricle and catheters were inserted into the left and right ventricular chambers for monitoring left and right ventricular pressures, respectively. The strain gauge output and the rate of rise of the left ventricular pressure curves (dP/dt) served as indices of ventricular contractility (VC). Pacing electrodes were placed in the right ventricle. The DMN and NA were stimulated with and without cardiac pacing; before and after ipsilateral vagotomy. Electrode tip locations were histologically verified.

DMN stimulation produced decreases in mean arterial blood pressure (MABP), dP/dt and strain gauge output; no change in heart rate (HR) and increases in left and right ventricular end-diastolic pressures (LVEDP, RVEDP). NA stimulation produced decreases in HR and MABP and increases in dP/dt, strain gauge output, LVEDP and RVEDP. The responses to NA stimulation were abolished by ventricular pacing. The responses to stimulation of either nucleus were abolished by ipsilateral vagotomy.

VC changes could be secondary to HR, LVEDP or RVEDP alterations. Yet, LVEDP and RVEDP increases could not produce the VC decreases observed with DMN stimulation. Since the VC changes during NA stimulation were abolished by cardiac pacing, the responses were secondary to bradycardia.

The data suggest cardiac vagal preganglionic somata are organized according to physiological function. Cell bodies of the DMN control VC while NA somata are involved in HR regulation. (Supported by NIH Grant HL08682.)

140 EVIDENCE FOR INVOLVEMENT OF A CNS GABAergic MECHANISM IN REFLEX-INDUCED VAGAL BRADYCARDIA IN THE CAT. Richard A. Gillis* and Daniel J. Williford* (SPON: R. McGee). Dept. of Pharmacol., Schs. Med. & Dent., Georgetown Univ., Washington, D.C. 20007.

Previous studies have demonstrated that blockade of GABA receptors in nucleus ambiguus with microinjections of bicuculline in the cat increases central vagal tone to the heart (DiMicco et al.: Proceed International Congress of Pharmacol., p. 755, 1978). This effect was reversed by the GABA receptor agonist muscimol. These data suggested the existence of a GABAergic synaptic mechanism for the regulation of central vagal tone. To determine the physiological importance of this GABAergic system, the effects of drugs that modify CNS GABAergic synaptic transmission were tested on reflex-induced vagal bradycardia produced by activation of baroreceptors with i.v. bolus injections of phenylephrine. Chloralose-anesthetized cats were used and all drugs were administered into the fourth cerebroventricle. Administration of the GABA receptor agonist muscimol (2.5 to 12.5 ug) to six cats prevented approximately 85% of the reflex-induced vagal bradycardia produced by phenylephrine. This blockade was not due to antagonism of the pressor response of phenylephrine. Once blockade was present, administration of the GABA receptor antagonist bicuculline (25-30 ug) restored the reflex vagal bradycardia response to near normal. This occurred without alteration in the phenylephrine-induced pressor response. Administration of bicuculline alone (1-5 ug) enhanced reflex-induced vagal bradycardia. These results suggest that a CNS GABAergic synapse comprises part of the reflex vagal pathway in the cat.

141 DISCHARGE PROPERTIES OF VAGAL CARDIAC NEURONS DURING CONDITIONED HEART RATE CHANGE. Michael R. Gold and David H. Cohen. Dept. Physiol., Univ. of Virginia, Charlottesville, VA 22908.

Classically conditioned heart rate change in the pigeon has both vagal and sympathetic components. As part of a comprehensive effort to describe the discharge characteristics of the motoneurons mediating this response, the activity of vagal cardiac neurons was recorded in trained animals. As previously reported (Fed. Proc., 38, 1200), these neurons show a sustained decrease in discharge during the conditioned stimulus. This report now describes the discharge properties of such neurons during acquisition of the conditioned heart rate change.

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

Regarding neuronal activity, the groups did not differ with respect to maintained discharge, and such discharge did not change significantly over training. The initial light presentation elicited a decrease in discharge that was most prominent during the initial 500 msec (phasic period) but persisted throughout the remaining 5500 msec of the light period (tonic period). In the conditioning group (10 cells) the phasic reduction in discharge increased in magnitude from 33% to 68% over training. The tonic decrease in discharge increased from 27% to 46%. In contrast, in the sensitization group (8 cells) the phasic response was attenuated from 35% to 18% and the tonic response from 33% to 8%. Thus, the groups changed differentially ($p<.001$). Further analysis indicated that the latency of the decrease in discharge was 120-160 msec for both groups early in training. However, in the conditioning group this latency shortened to less than 100 msec, while in the sensitization group it increased to almost 200 msec.

These results clearly indicate vagal involvement in conditioned cardioacceleration. Moreover, the vagal contribution is synergistic with that of the sympathetic cardiac innervation which shows phasic and tonic increases in discharge during conditioned stimulus presentation. (Supported by NSF grant BNS 75-20537 and NIH grants P01 NS14620 and T32 HL07284)

142 MODIFICATION BY LIGHT HALOTHANE ANESTHESIA OF THE CARDIOVASCULAR CHANGES ASSOCIATED WITH REM SLEEP IN THE CAT. Richard E. Hall, Eduardo H. Rubinstein and Richard W. Patterson.* Depts. of Physiology and Anesthesiology, Sch. Med., UCLA, Los Angeles, CA 90024.

Anesthetic sleep induced by the inhalation agent halothane is manifest by a persistent high voltage EEG pattern superimposed on a background of 10-14/sec low voltage activity. We have observed in chronic instrumented cats that light stages of halothane anesthesia were associated with the EEG desynchronization and rapid eye movement bursts characteristic of REM sleep. These changes occurred together with a marked drop in arterial pressure and heart rate that persisted for the duration of the REM episode. The cardiovascular responses differed from those observed during natural REM episodes, especially the extent and direction of the heart rate changes. Commonly, the transition to the REM stage is marked by an initial bradycardia followed by transient episodes of cardioacceleration that are associated with the REM bursts. The variability of the heart rate changes during natural REM sleep may depend on the superimposition of a tonic inhibition of cardiac sympathetic activity with a phasic inhibition of vagal activity. This assumption was based on work by others and on the effects of pharmacological agents on heart rate changes during natural REM sleep. Typically, propranolol, a beta blocking agent (0.2 mg/kg) decreased resting heart rate, prevented the REM associated bradycardia but did not block the phasic cardioacceleration associated with the REM bursts. Conversely, atropine (0.06 mg/kg), at a dose sufficient to block cardiac cholinergic receptors without altering the EEG, increased resting heart rate but did not affect the bradycardia associated with the onset of the REM stage and prevented the display of the transient episodes of tachycardia. The effect of light halothane anesthesia on heart control during REM sleep resembled that of atropine, in that the phasic tachycardia was abolished with preservation of the tonic decrease in heart rate. This apparently selective effect of halothane blocking phasic vagal control may be exerted on the CNS area that couples the phasic somatic changes displayed during REM sleep (REM bursts, tachypnea) with the vagal control. The present observations indicate that light halothane anesthesia may have differential effects on central autonomic control and on sleep mechanisms and that the detection of EEG desynchronization during clinical light halothane anesthesia may correspond to REM sleep and not to an arousal response.

This work was supported by grants from NIH GMS 22974 and AHA-GLA 437IG.

143 RESPIRATORY DYSFUNCTION IN IDIOPATHIC ORTHOSTATIC HYPOTENSION, Robert W. Hamill, Carol K. Petito* and Ira B. Black. Depts. of Neurol. and Pathol., Cornell Univ. Med. Col., New York, N.Y. 10021.

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

Laryngeal stridor presented a variable clinical picture in our patients. The first patient developed acute stridor, vocal cord paralysis and esophageal dysfunction early in the course of his disease and required intubation and tracheostomy. The second patient developed acute stridor with irregular respiration late during the course of his disease. The third patient developed intermittent stridor and irregular respirations.

To define the histological substrate of these respiratory abnormalities, lower cranial nerve nuclei and brain stem respiratory areas were examined in autopsy material obtained from these patients. Similar brainstem areas were examined in autopsies of 30 age-matched control patients. In the first patient, microscopic examination showed gliosis in the nucleus ambiguus (NA), tractus solitarius (TS), and nucleus of tractus solitarius (NTS). Pathological examination of the second patient revealed gliosis in the NA, and gliosis, dystrophic axons and one area of phagocytosis in the TS and NTS. In the third patient, microscopic studies showed focal neuronal phagocytosis with a glial nodule in the NA, and diffuse gliosis in the medullary tegmentum.

Control patients, aged 40-80, exhibited mild gliosis in the medullary tegmentum with only one patient exhibiting increased gliosis in the NA and NTS. These observations suggest that laryngeal stridor is associated with degenerative changes in the NA, and possibly NTS, in the Shy-Drager variant of IOH.

(This work was supported by the NIH and the Dysautonomia Fdn. Inc. R.W.H. is the recipient of a Teacher Investigator Development Award NS 00383. I.B.B. is the recipient of the Irma T. Hirschl Career Scientist Award.)

144 GABA CONTENT AND SPECIFIC BINDING IN A NUCLEUS ASSOCIATED WITH CENTRAL VAGAL OUTFLOW IN THE CAT. Betty L. Hamilton, Sandra C. Brown*, Wesley P. Norman*, Janette Dias Souza*, Karen Gale*, & Richard A. Gillis*. Depts. Pharmacol. & Anat., Schs. Med. & Dent., Georgetown Univ., Washington, D.C. 20007.

In previous studies (DiMicco et al.: Proceed. 7th International Congress Pharmacol., p. 755, 1978), blockade of GABA receptor function by direct microinjection of bicuculline into the nucleus ambiguus (NA) of cats produced a marked dose-related depression of heart rate which was mediated by the vagus. This effect was not obtained in other regions of the brainstem and was reversed by the GABA receptor agonist muscimol. These data suggested that the NA may be the site of a GABA receptor mediated inhibition of vagal outflow. The purpose of the present study was to determine (1) whether GABA is present in the NA, and (2) whether there are receptors for GABA in the NA. GABA content and specific binding for tritiated GABA was measured in NA as well as in several other CNS regions. To determine GABA levels, an enzymatic spectrophotofluorometric method was used. [^{3}H]-GABA binding was done on frozen and Triton X-100 treated membrane preparations. GABA content of NA was 24.4 nmol/mg protein. This level was similar to that found in caudate nucleus (28.4 nmol/mg protein), and was approximately 2.5-fold higher than the level found in surrounding reticular nuclei. Specific [^{3}H]-GABA binding in NA (120 femtomoles/mg protein measured at a concentration of 30 nmol [^{3}H]-GABA) was slightly lower than that in substantia nigra (180 femtomoles/mg protein), and was approximately 3-fold higher than the specific [^{3}H]-GABA binding obtained in tissue from surrounding reticular nuclei. This demonstration of the presence of GABA and specific binding sites for GABA in NA provides further evidence for GABAergic synaptic regulation of neural activity in nucleus ambiguus.

145 CHANGES IN CENTRAL CHOLINERGIC NEURONS IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR). Cinda J. Helke, Eric A. Muth* and David M. Jacobowitz. Lab. Clin. Sci., NIMH, Bethesda, Md. 20205.

Recent pharmacological studies demonstrate that CNS cholinergic mechanisms which may be involved in blood pressure control (Europ. J. Pharm. 29:262, 1974; Clin. & Exptl. Hypertension 1: 219, 1978) are altered in SHR (JPET 206: 644, 1978). In order to investigate whether biochemical differences in cholinergic neurons of discrete brain nuclei exist between 4, 8 and 12 wk old SHR and aged matched Wistar Kyoto (WKY) control rats, the activity of choline acetyltransferase (ChAT) was measured in numerous microdissected brain nuclei. ChAT activity is a specific marker for cholinergic nerves and changes in its activity have been correlated with changes in impulse flow in cholinergic neurons (Experientia 34: 1247, 1978). The acetylcholine (ACh) concentration was also measured in hindbrain nuclei of 12 wk old SHR and WKY.

Changes in ChAT activity of several nuclei were observed in SHR of all three age groups. The greatest alteration (79% increase) was found in the ChAT activity of the locus coeruleus of 12 wk old SHR rats. A 31% increase in ACh content was also detected. No differences were found in this nucleus in 4 or 8 wk old SHR. Other nuclei known to be involved in blood pressure control which showed differences in ChAT activity in SHR compared to WKY rats were the paraventricular nucleus (36% decrease in 4 wk SHR), dorsomedial nucleus of the hypothalamus (19% and 23% decrease in 8 and 12 wk SHR, respectively), posterior hypothalamus (25% decrease in 12 wk SHR) and the nucleus reticularis gigantocellularis (28% increase and 18% decrease in 4 and 8 wk SHR, respectively). A significant increase in the ChAT activity of the anterior ventral thalamus was detected in 4 and 8 wk SHR.

The data from this study demonstrate that cholinergic nerves in certain rat brain areas, several of which play a role in cardiovascular control, are altered in SHR. Of particular interest is the change in the cholinergic input to the locus coeruleus of 12 wk but not 4 or 8 wk old SHR. The fact that the 4 wk SHR are not hypertensive and the 8 wk SHR are still in the developmental stages of BP elevation compared to the 12 wk SHR suggests secondary changes in the nucleus in response to the hypertension. Kawamura et al. (Brain Res. 140: 137, 1978) suggest on the basis of their findings of alterated BP response to electrical stimulation of the locus coeruleus in SHR that the locus coeruleus may sensitize the descending depressor systems in an attempt to combat the hypertension. In view of the wide distribution of noradrenergic nerves of the locus coeruleus in the brain and spinal cord, further exploration of this cholinergic noradrenergic connection is warranted.

146 SMALL, INTENSELY FLUORESCENT CELLS OF SUPERIOR SYMPATHETIC GANGLION CONTRASTED IN GUINEA PIG AND RABBIT. Jean Jew. Dept. Anat., Coll. Med., Univ. of Iowa, Iowa City, IA 52242.

Dopamine, released from the small, intensely fluorescent (SIF) cell, is believed to elicit the slow inhibitory postsynaptic potential (s-IPSP) identified in the rabbit principal ganglionic neuron (PGN). The schema postulated involves an interneuron, possibly the type I SIF cell (Williams et al., in SIF Cells, Fogarty International Center Symposium, O. Eränkö, ed., pp. 143-162, 1977) which is solitary and has long processes directed among the PGNs.

The guinea pig superior sympathetic ganglion differs from the rabbit in being almost devoid of type I SIF cells. SIF cells in the guinea pig ganglion are much more numerous and, in addition, norepinephrine instead of dopamine has been identified as the transmitter present in the SIF cells. The guinea pig also lacks a dopamine receptor-adenylate cyclase complex, which is present in the rabbit. Since no s-IPSP has been found in the guinea pig, it was hypothesized that the interneuron (assumed to mediate this hyperpolarizing membrane change in the rabbit) would be absent.

5-OH-DA (100mg/kg dissolved in saline with 0.1% ascorbic acid) was administered i.p. to guinea pig and rabbit to mark catecholamine-containing vesicles for electronmicroscopy. After perfusion with a solution of 2.5% glutaraldehyde/1% paraformaldehyde in 0.1M Sorensen's phosphate buffer (pH 7.3), the superior cervical ganglia were removed and processed for electronmicroscopy. After localization of SIF cells using semithin sections, thin sections were stained with uranyl acetate and lead citrate and surveyed in a Philips 201 electronmicroscope.

The ultrastructural characteristics of the SIF cells and their connections are summarized as follows: Rabbit: Clustered and solitary SIF cells were distinguished by their conspicuous and large electron-dense vesicles. Presumptive SIF cell processes containing large and small dense vesicles could seldom be traced directly from the cell of origin (leaving the possibility that some were recurrent collaterals of PGNs). Terminals of cholinergic type synapsed on the SIF cells. SIF cell processes synapsed on presumed PGN dendrites. Guinea pig: Intraganglionic SIF cells received cholinergic inputs. However, their processes made synaptic contact with cell bodies and processes of other SIF cells. Thus there are associative synaptic connections between SIF cells in the guinea pig ganglion. Some of the smallest processes receiving SIF cell efferents were not identifiable. (Supported by HL 21914.)

147 Chemically Sensitive Vagal Endings in the Airways: Their Stimulation by Bradykinin. M.P. Kaufman, H.M. Coleridge*, J.C.G. Coleridge* & D.G. Baker*. Cardiovas. Res. Inst., UCSF, San Francisco, CA 94143.

Bradykinin (BK) is a potent algesic substance released in the lung in asthma and pulmonary anaphylaxis. Inhalation of BK aerosol causes cough and retrosternal irritation in normal subjects, and reflex bronchoconstriction in asthmatics and in some normal subjects also; it has little or no direct bronchoconstrictor effect in humans or dogs. We recorded activity in afferent vagal fibers in anesthetized dogs, in order to determine which lung receptors were stimulated by BK. We injected 20 ug BK into the right or left atrium, and examined its effects on pulmonary C fiber endings (J receptors), rapidly adapting receptors with myelinated fibers (irritant receptors) and bronchial C fiber endings. We also injected 1.5 ug BK directly into the bronchial circulation, and examined its effects on rapidly adapting receptors and bronchial C fiber endings, since both are in airways supplied by the bronchial arteries. Right atrial injection of BK had little effect on pulmonary C fibers, although their endings are directly accessible from the pulmonary circulation. Left atrial or bronchial arterial injection of BK stimulated 13/23 rapidly adapting receptors; the maximum increase in activity was from 3 to 6 impulses/sec; effects were usually brief, and if more prolonged, receptors fired with each cardiac cycle, suggesting that stimulation was secondary to the vasodilator properties of BK. (Rapidly adapting receptors in dogs fire up to 40-50 impulses/sec in response to bronchoconstrictor agents or to hyperinflation of the lung.) BK stimulated 13/14 bronchial C fibers. In 3 dogs (7 fibers) activity increased from less than 1 to 7-15 impulses/sec, and remained elevated for 1 min or more; in 4 dogs (6/7 fibers) the response consisted of brief bursts of spikes with several seconds of silence between. We conclude that there may be individual differences in afferent susceptibility to BK in dogs, as in humans, but that stimulation of bronchial C fibers probably accounts for its irritant effects on the respiratory tract. (Supported by NIH grants HL-06285 and HL-07192.)

148 PHASE SHIFTING OF THE RESPIRATORY CYCLE PRODUCED BY ELECTRICAL STIMULATION OF THE MEDIAL PONTINE RETICULAR FORMATION. C.K. Knox, Laboratory of Neurophysiology, Department of Physiology, University of Minnesota Medical School, Minneapolis, MN 55455

The pathways and mechanisms underlying the effects of reticular stimulation on respiration are not well understood. It is possible that such stimulation affects respiration directly at the brainstem level, or, as Anderson and Sears (J. Physiol., London 209) have suggested, only indirectly at the spinal level via reticulospinal pathways separate from those conveying respiratory rhythm. To test the first possibility, the medial pontine reticular formation of decerebrate spontaneously breathing cats was stimulated with short bursts of rectangular pulses (100μsec, 10 pulses at 100 pps), while noting the stimulus strength required to cause inspiratory to expiratory (I→E) or expiratory to inspiratory (E→I) phase switching of the phrenic discharge. Within medial pons, two regions could be identified from which I→E or E→I switching could be obtained: An I inhibitory region dorsally situated in the gigantocellular tegmental field, and an I facilatory region in the ventral magnocellular tegmental field. Depth/switching threshold profiles were defined for stimuli delivered halfway into inspiration or expiration. Throughout the medial pons, two depths were found at which thresholds for either I→E or E→I switching were at a minimum, with the thresholds increasing in the dorsal and ventral directions from these positions. When delivered to minimum threshold I→E switching sites at various times in inspiration, stimuli of greater strength were required for switching early as compared to late in the phase. Bivagotomy lengthened the time course of this threshold curve but did not alter its magnitude. Stimuli delivered to minimum threshold E→I switching sites at different times in expiration, revealed a similar time-dependent decrease in stimulus strength for phase switching. Stimulus bursts which caused I→E switching resulted in a decrease of the ensueing expiratory time; whereas, stimuli which caused E→I switching brought on a normal phrenic discharge. From these results it is concluded that medial pontine reticular structures can affect the respiratory phase switching mechanisms directly at the brainstem level. (NIH Grant #HL16430)

149 MECHANISM OF REGIONAL VASCULAR RESISTANCE CHANGES PRODUCED BY ACTIVATION OF A PERIVENTRICULAR-PERIAQUEDUCTAL DESCENDING PATHWAY. Mark M. Knuepfer*, A.K. Johnson*, and M.J. Brody* (SPON: R.K. Bhatnagar) Depts. Pharmacology and Psychology and Cardiovascular Ctr., The University of Iowa, Iowa City, IA 52242.

Our laboratory has reported that the integrity of the anteroventral third ventricle (AV3V) region is necessary for the manifestation of many forms of experimental hypertension. We have also reported that descending neuronal tracts from this region pass through the central gray since a lesion in this area will significantly attenuate the hemodynamic changes seen with AV3V stimulation. This study was designed to examine the effects of adrenalectomy and peripheral adrenergic blockade on regional vascular responses to central stimulation of the pathway. Bipolar electrodes were placed in the AV3V region and the rostral central gray (CG) a minimum of two days before experimentation. Rats were anesthetized and cannulated for arterial pressure determinations and for intravenous administration of drugs. Regional blood flows were determined using miniaturized pulsed Doppler flow probes placed on the renal and mesenteric arteries and the lower abdominal aorta. Electrical stimulation of the AV3V and the CG was done using 14V, 0.5 msec pulses at varying frequencies. Stimulation of the AV3V or the CG region elicited a frequency-dependent decrease in hindlimb vascular resistance and mesenteric and renal vasoconstriction. After bilateral adrenalectomy, the mesenteric and renal responses to both AV3V and CG stimulation were significantly attenuated. The hindlimb vasodilator response was attenuated by adrenalectomy for CG stimulation but not for AV3V stimulation. Intravenous infusion of epinephrine (1-2 μg/kg/min) resulted in vascular resistance changes similar to those produced by central stimulation of these two sites. After adrenalectomy, blockade (using phentolamine or guanethidine) further reduced vascular responses to central stimulation. In summary, catecholamines released from the adrenal medulla appear to play a role in vasoconstrictor responses elicited by AV3V and CG stimulation. In addition, adrenal catecholamines appear to be a significant factor in CG-induced hindlimb vasodilation. We conclude that electrical activation of the descending pathway between AV3V and CG produces complex integrated regional vascular responses mediated by both efferent sympathetic innervation and circulating catecholamines released by central activation of the adrenal medulla. (Supported in part by USPHS Grants HLP14388, GM07069, and 1-K02-MH00064.)

150 CNS EFFECTS OF α-METHYLDOPA ON TWO NONCARDIOVASCULAR AUTONOMIC SYSTEMS. Michael C. Koss. Departments of Pharmacology and of Ophthalmology and McGee Eye Institute, Univ. of Okla. Health Sciences Center, Okla. City, OK 73190.

In addition to well established CNS sympatho-inhibitory effects on the cardiovascular system, clonidine has also been shown to exert actions on other autonomic systems. For example, clonidine inhibits centrally evoked electrodermal responses (Europ. J. Pharmacol. 37: 71, 1976) and produces pupillary dilation in cats primarily by means of inhibition of central parasympathetic outflow to the iris (Invest. Ophthal. 15:566, 1976). Both of these noncardiovascular effects of clonidine are antagonized by CNS α-adrenergic antagonists such as yohimbine. α-Methyldopa (α-MD) has also been shown to exert a therapeutically effective hypotension and, like clonidine, it is suggested that this agent also acts (indirectly) to stimulate CNS α-adrenergic receptors. The present study was undertaken to determine if α-MD also causes clonidine-like effects on the sympathetic-cholinergic electrodermal system and pupil.

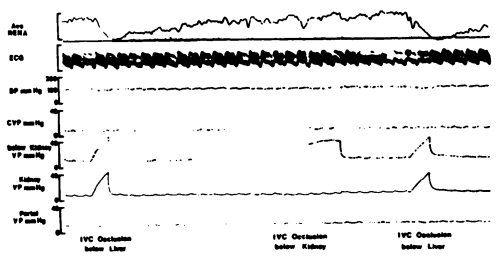
Experiments were performed on cats anesthetized with α-chloralose (60 mg/kg, i.p.) or pentobarbital (36 mg/kg, i.p.). Intravenous administration of α-MD (30 and 100 mg/kg, i.v.) caused a dose related decrease in the amplitude of electrodermal responses evoked by stimulation of the posterior hypothalamus. The peak effects were observed approximately 3 hr after injection. Administration of yohimbine hydrochloride (0.5 mg/kg, i.v.) partially reversed this α-MD sympatho-inhibition. With respect to the pupil, α-MD also caused a dose related mydriasis which was antagonized by yohimbine. Again the peak effects were not observed for 2.5-3 hr after administration. Yohimbine pretreatment abolished the α-MD-induced pupillary dilation but did not prevent mydriasis in response to epinephrine. In contrast, phenoxybenzamine pretreatment (2 mg/kg, i.v.) blocked the epinephrine but not the α-MD pupillary actions. These results were confirmed in experiments utilizing direct ciliary nerve recordings.

The present observations suggest that α-MD acts in a manner similar to clonidine on these two noncardiovascular systems and that an α-adrenergic inhibitory mechanism may be involved. The long time course to reach the maximal effects suggests that α-MD is converted to an active metabolite (i.e. α-methyldopamine or α-methylnorepinephrine).

(Supported by USPHS Grant NS 14039)

151 RENAL VENOUS CONGESTION RECEPTORS THAT CAN REFLEXLY ALTER RENAL AND CARDIAC SYMPATHETIC EFFERENT NERVE ACTIVITY. David R. Kostreva, Angel Castaner*, and John P. Kampine*. Depts. of Anesthesiology and Physiology, Medical College of Wisconsin and Wood VA Medical Center, Milwaukee, WI 53193.

Occlusion of either the left or right renal vein in open chested dogs resulted in a short latency inhibition of renal (RSENA) and cardiopulmonary (CPSENA) sympathetic efferent nerve activity before and after bilateral vagotomy. Mongrel dogs, 20-30 kg, were anesthetized using sodium pentobarbital, 35 mg/kg i.v. The animals were intubated and placed on positive pressure ventilation. The chest was split transversely to allow access to both stellate ganglia and the ansae subclaviae, CPSENA was recorded from the cut central end of either the left or right ansae. RSENA was recorded from the cut central end of a renal nerve. The SENA was amplified, filtered and time averaged using a half-wave rectifier. Central venous pressure, and renal venous pressure were monitored from catheters inserted into the femoral veins. Systemic blood pressure and left ventricular pressure were monitored from catheters inserted into the femoral arteries. The electrocardiogram (ECG) was monitored from leads placed in a lead II configuration. Heart rate was measured using a tachograph. The averaged RSENA, CPSENA, ECG, heart rate, and pressures were recorded using a polygraph. Occlusion of either the left or right renal vein, or as shown in the figure below, occlusion of the inferior vena cava (IVC) below the liver but just above the renal veins resulted in a marked increase in renal venous pressure and a reflex inhibition of RSENA and CPSENA. Heart rate, left ventricular pressure



and systemic blood pressure all decreased during occlusion of one renal vein or the IVC above the kidney. IVC occlusion below the renal veins did not alter RSENA or CPSENA. (Supported by NIH HLBI Young Investigator Research Award HL 21042 and Grant 16511, and the Med. Research Service of the VA).

152 DO SYMPATHETIC NERVES CONTROL CARDIAC GROWTH? C. Lau*, R. Morgan*, D.L. Bareis* and T.A. Slotkin. Dept. Pharmacol., Duke Univ. Med. Ctr., Durham, N.C. 27710

Development of the rat heart involves two growth phases: (1) in the first 2-3 weeks of postnatal development rapid replication of myocardial cells occurs, associated with high rates of DNA synthesis, (2) thereafter, DNA synthesis and cell replication terminate and further growth occurs by cardiac muscle hypertrophy involving RNA and protein synthesis. Previous studies have implicated involvement of sympathetic tone in both growth phases. Exposure of the neonatal rat heart to sympathetic neurotransmitters elicits a decline in DNA synthesis and an elevation in protein synthesis (Claycomb, J. Biol. Chem. 251, 6082, 1976) and in adults, sympathomimetics have long been known to cause cardiac hypertrophy. These data, together with the progressive developmental increase in endogenous stores and uptake of norepinephrine (NE) prompted the proposal that adrenergic innervation might control cardiac muscle differentiation and growth. In the present study, this hypothesis was tested by giving the neurotoxic adrenergic neuron blocking agent, guanethidine (50 mg/kg s.c.) daily to rats for 21 consecutive days to produce long-term peripheral sympathectomy in both neonatal and mature rats. Heart growth of sympathectomized animals was monitored by developmental increases in organ weight, RNA and protein synthesis. Ontogeny of the sympathetic nerve terminal was measured by the ability of synaptic vesicle preparations to take up radiolabeled NE. A 3-fold developmental increase in vesicular uptake was obtained in control rats from 2 to 40 days of age. In contrast, guanethidine treated rats did not show nerve development beyond the level reached at 2 days of age. Physiological response (heart rate change) to tyramine, which acts by displacement of NE from the sympathetic terminal, was deficient in guanethidine-treated rats, and postsynaptic supersensitivity of cardiac β-receptors (isoproterenol-induced tachycardia) was present throughout development. These biochemical and physiological indices show that sympathetic nerve destruction by guanethidine was taking place and that a sufficient lesion was obtained to interfere with basal sympathetic function. Despite the clearcut effectiveness of guanethidine to prevent formation of functional adrenergic innervation of the heart, no significant alterations in heart growth were observed in the developing rats. Results similar to those seen in the developing neonate were obtained in mature rats after chronic guanethidine treatment, i.e. no effect of sympathectomy on heart growth. These results suggest that the presence of sympathetic innervation is not obligatory for normal growth of the heart to occur. (Supported by USPHS HD-09713 and DA-00006).

153 FOUR CENTRAL NERVOUS SYSTEM SITES PROJECT TO THE PANCREAS. W. Laughton* and T.L. Powley* (SPON: Lynda Uphouse). Dept. Psychol., Yale University, New Haven, CT 06520.

Central nervous system sites projecting to the rat pancreas were identified and mapped using retrograde axonal transport of horseradish peroxidase (HRP). A total of 50ul of 30% (w/v) HRP (Sigma, Type VI) was injected into the pancreas of each animal (n=12). After 48 hours, the animals were sacrificed, and their brains and spinal cords were processed for HRP with the tetramethylbenzidine procedure (Mesulam, J. Histochem. & Cytochem. 26: 106-117, 1978). Serial 42u sections of the tissue from the sacral cord to the caudal pons were saved and systematically analyzed for HRP reaction product.

Four well organized cell groups--two in the medulla and two in the spinal cord--were found to project to the pancreas. The largest number of cells with HRP reaction product formed a coherent group occupying the medial two-thirds of the dorsal motor nucleus (DMV) bilaterally at the rostrocaudal level of the area postrema. Another tightly clustered, smaller population of cells was identified in the rostral pole of the nucleus ambiguus (NA). Thus, consistent with some degree of topographic organization, the labeled neurons were confined to specific regions of both the DMV and the NA. Labeled cells in the spinal cord were organized in two columns. One column occurred bilaterally in the cervical cord (C2 to C3), and the other bilaterally in the lower thoracic region (approximately T5 to L1); both spinal cell groups were located in the ventral horn, not the classic autonomic nucleus of the lateral horn. With the exception of a few isolated cells that were found along the intra medullary course of the vagal fibers (and that appeared to be "displaced" DMV neurons), no other CNS cell populations projecting to the pancreas were identified. In additional animals, HRP treatment of the proximal cut end of the cervical vagus high in the neck labeled the two pancreas cell groups in the medulla as well as the remainder of the neurons in the DMV and NA (virtually all ipsilateral to the nerve soak), but not the two columns in the spinal cord.

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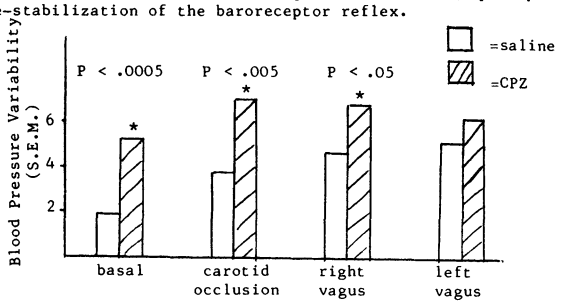
154 EFFECTS OF CHLORPROMAZINE ON BLOOD PRESSURE AND THE BARORECEPTOR RELFEX. A. L. Loeb* and R.J. Anderson. Dept. Pharmacol., Geo. Washington U., Washington, D.C. 20037.

The baroreceptor reflex in α-chloralose anesthetized cats was stimulated by (1) complete carotid occlusion and electrical stimulation of the (2) right and (3) left vagus nerves. Chlorpromazine (CPZ) was administered in cumulative doses of 0.0156, 0.0313, 0.0625, 0.125, 0.25, 0.50, 1.0, 2.0, and 4.0 mg/kg over an approximately 4-hour time period. CPZ produced no significant change in basal heart rate or blood pressure over time. The mean blood pressure response to each of the 3 test stimuli was not affected in a dose related fashion. Also, the latency between carotid occlusion and maximum increase in blood pressure was not significantly affected by CPZ.

However, in nearly all animals CPZ (n=8) produced a more variable basal blood pressure ($p < .0005$) than in control animals (n=8). In some cases CPZ caused a repetitive oscillation in blood presure (frequency=.7/min) whose duration was dose-dependent. CPZ also resulted in a more variable blood pressure response to carotid occlusion ($p < .005$) and to right vagal stimulation ($p < .05$). The response to left vagal stimulation was not significantly affected by CPZ. These effects are shown below.

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

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



155 ANATOMICAL EVIDENCE THAT THE A5 CATECHOLAMINE CELL GROUP IS A VASOMOTOR CENTER. A.D. Loewy, S. McKellar, and C.B. Saper. Dept. Anat. and Neurobiol., Wash. Univ. Sch. Med., St. Louis, MO 63110.

The A5 catecholamine cell group (Dahlström and Fuxe, '64) lies dorsal and lateral to the superior olive and medial to the root of the VIIth cranial nerve.

Two lines of anatomical evidence indicate that the A5 cell group projects to medullary and spinal vasomotor centers. First, stereotaxic injections of ^3H amino acids were made in the A5 cell group in rats. As demonstrated by autoradiography, a spinal projection from this area descends via the dorsolateral funiculus to the intermediolateral and intercalated sympathetic cell groups of the thoracic and upper lumbar spinal cord. There are also descending projections to the medial and paramedian reticular formation, the medial and parvocellular regions of the solitary complex, and the dorsal motor nucleus of the vagus. After similar injections in animals pretreated with 6-hydroxydopamine (2x250 µg, intraventricularly), there was no evidence of a spinal projection. Second, we used the combined histofluorescence-retrograde cell labeling technique of Blessing et al. ('78) to show that the A5 catecholamine cells are retrogradely labeled after injections of HRP into the T1-T2 spinal cord. Control injections of HRP into the femoral vein or subarachnoid space at the T1 levels failed to label the A5 neurons. To provide further evidence, we studied the retrograde labeling pattern in the A5 cell group of rats that had received 6-hydroxydopamine injections in the lateral ventricle and this treatment abolished virtually all retrograde cell labeling in this area from HRP injections in the spinal cord.

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

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

156 SPECTRAL ANALYSIS OF HEART RATE DURING DEPRESSOR NERVE STIMULATION: THE VALIDATION OF A NON-INVASIVE ESTIMATE OF VAGAL TONE. Philip M. McCabe*, Stephen W. Porges*, and Brandon G. Yongue* (SPON: C. S. Carter-Porges). Neural and Behavioral Biol. Prog. and Dept. Psych., Univ. Illinois, Urbana, IL, 61801.

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

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

β-adrenergic blockade (propranolol, i.v., 2 mg/kg) did not decrease the change in the spectral estimate of vagal tone during stimulation. Cholinergic blockade (atropine methyl nitrate, i.v., 2 mg/kg) decreased the spectral estimate of vagal tone and eliminated the bradycardia response to aortic nerve stimulation. During the β-adrenergic blockade there was an apparent current intensity-vagal tone relationship. This implies that differing amounts of vagal stimulation will be manifested in a monotonic fashion in the spectral estimate of vagal tone. Subsequent research will investigate the applicability of non-invasive recording and spectral analysis in the unanesthetized, freely moving preparation. These data suggest that the spectral analysis of heart rate may provide a method for the non-invasive estimation of vagal tone to the heart. (Supported by MH K02-00054, NSF SER 76-18255, MH-15128, HEW PHS RR 7030).

157 ANALYSIS OF THE ROLE OF CALCIUM IN RHYTHMIC HYPERPOLARIZATIONS INDUCED BY THEOPHYLLINE IN BULLFROG SYMPATHETIC NEURONS. S.M. McCort* and F.F. Weight. Laboratory of Preclinical Studies, National Institute on Alcohol Abuse and Alcoholism, Rockville, Md. 20852

In previous investigations, theophylline was found to prolong the duration of a calcium sensitive potassium conductance in the spike after hyperpolarization (Busis & Weight, Nature 263, 434, 1976). In that study, using sucrose gap recording, theophylline also produced an increase in baseline noise. In this study we investigated the cellular origin of that "noise" by recording intracellularly from neurons in the ninth or tenth paravertebral sympathetic ganglion of the bullfrog. Superfusion with a Ringer's solution containing 5 mM theophylline induced a slow depolarization of the membrane. The depolarization occured over 5-15 min. and was usually 10-15 mV in amplitude. In addition, spontaneous hyperpolarizations were observed in approximately 60% of the neurons. The amplitude of these hyperpolarizations varied between 5-20 mV and the duration between approximately 30 sec. - 45 sec. The hyperpolarizations reoccured rhythmically approximately every 3-6 min. During the spontaneous hyperpolarizations membrane resistance was decreased.

Since theophylline potentiates a calcium sensitive potassium conductance in the spike after hyperpolarization of these neurons, we analyzed the role of calcium in the spontaneous hyperpolarizations. Superfusing the ganglia with a calcium-free Ringer's solution resulted in a decreased frequency or a complete abolition of the rhythmic hyperpolarizations. Dantrolene also decreased the frequency or completely abolished the occurance of the rhythmic hyperpolarizations. In muscle, dantrolene has been found to block the intracellular release of calcium ions (Desmedt and Hainaut, J. Physiol., 265 565, 1977). These data suggest involvement of both extracellular and intracellular release of calcium ions in the generation of theophylline induced rhythmic hyperpolarizations.

158 CHANGES IN THE ORGANIZATION OF THE MICTURITION REFLEX PATHWAY (MRP) IN THE CAT FOLLOWING TRANSECTION OF THE SPINAL CORD. Richard J. Milne* and William C. deGroat, Dept. Pharmacol., Univ. of Pittsburgh, Pittsburgh, PA 15261

Previous studies showed that micturition in cats with an intact neuraxis was dependent upon a sacral spinobulbospinal reflex. This report describes the changes in the MRP in chronic spinal cats 1 to 14 weeks after spinal cord transection at T_{13}. Reflexes were recorded from postganglionic fibers on the surface of the bladder in response to stimulation of afferents in the pelvic nerve. In chronic spinal cats, reflexes occurred at long latency (150-180 msec) and only at stimulus intensities which activated C fiber afferents. The reflex, which was usually sufficiently intense to evoke bladder contractions, could be elicited when the bladder was distended with fluid or completely empty. It occurred only ipsilateral to the site of stimulation and was not affected by bilateral transection of the hypogastric nerves or the sympathetic chain. The reflex was abolished by mechanical stimulation of the anal canal.

In cats with an intact neuraxis reflexes were detected at shorter latencies (95 to 140 msec) contralateral as well as ipsilateral to the site of stimulation at stimulus intensities which activated Aδ fibers. In 60% of these animals (7 of 11 experiments) a longer latency reflex (180-200 msec) was also observed when the stimulus intensity was increased above the threshold for C-fiber afferents, at least 7 times the threshold for A-fiber afferents. This late reflex was also observed contralateral as well as ipsilateral to the site of stimulation. Neither the early nor the late reflex could be elicited when the bladder was empty. Neither reflex was affected by bilateral transection of hypogastric nerves and sympathetic chain, but both were abolished by spinal section at T_{10}. 24 to 48 hours after cord transection at T_{13} a very weak reflex which corresponded in latency and threshold to the C-fiber reflex could be elicited with trains of stimuli to the pelvic nerve.

We conclude that in chronic spinal cats the MRP undergoes a reorganization which is characterized by: (1) functional disconnection of the A-fiber component of the peripheral afferent limb, and (2) a change in the central pathway from a primarily supraspinal reflex distributed bilaterally to a spinal reflex distributed ipsilaterally. The reemergence of a strong spinal reflex one week after cord transection accounts for the development of automatic micturition in chronic spinal animals. (Supported by NIH Grant 07923-11, and an Overseas Research Fellowship from the Medical Research Council of New Zealand to RJM).

159 HYPOTHALAMIC AND SPINAL ACTIVATION OF PRE-CELIAC SYMPATHETIC ACTIVITY:Time Analysis and Influence of Hexamethonium. S.M. Morrison* and D. Whitehorn. Dept. of Physiology, Univ. of Vermont, Burlington, VT 05405

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

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

In most preparations DLC stimulation produced a time course of PCSA similar to that evoked from PFPH; a decline in PCSA occuring during the 2nd and 3rd seconds of DLC stimulation as blood pressure rose.

When HEX (25 mg/kg) was given i.v., resting pressure fell to about 70 mm Hg. and evoked pressure changes from PFPH or DLC were eliminated. In most animals, spontaneous PCSA increased (10-30%). Evoked activity in the first second of PFPH or DLC stimulation was relatively unchanged while activity in the 2nd and 3rd seconds increased as compared with pre-HEX responses. Since evoked activity in the 2nd and 3rd seconds of DLC stimulation remained after ipsilateral hemisection of the cord rostral to C1, the decline in PCSA in the 2nd and 3rd seconds in pre-HEX responses probably is due to baroreceptor reflex influences acting at the spinal level.

In some preparations, HEX produced a fall in spontaneous PCSA and the entire response to DLC stimulation was reduced by 30-70%. Pre-HEX PCSA response levels could be restored by increasing the intensity of DLC stimulation.

We conclude that time course analysis can be applied to PCSA responses obtained with DLC stimulation, allowing measurement of both sympatho-excitation and reflex inhibition acting at the spinal level.

160 SUPERIOR CERVICAL GANGLION DECENTRALIZATION IN THE ADULT RAT. ITS LONG TERM EFFECTS ON THE IRIS NERVE TERMINALS. Catherine Mytilineou and Maria C. Papaconstantinou*. Dept. of Neurology, Mt. Sinai School of Medicine, New York, N.Y. 10029.

Adult rats (70-80 days old) were subjected to unilateral superior cervical ganglion decentralization and the effects on the adrenergic nerve terminals were studied in the iris. There was no change in the norepinephrine (NE) content of the iris up to 8 weeks after decentralization. The number of the nerve terminals in the iris did not change and their uptake properties remained normal as determined by the ^{3}H-NE uptake by the irides from untreated and reserpine pretreated animals. However when irides innervated by a decentralized ganglion were depolarized in vitro by high extraneuronal K^+ concentration (100 mM), they released significantly higher amounts of previously accumulated ^{3}H-NE than the control irides. This effect was not evident until two weeks after decentralization but remained present up to 16 weeks, the longest time studied. Catecholamine histofluorescence of the irides indicated a change in the distribution of NE with an increase in the fluorescence intensity of the varicosities and a decrease in the intensity of the inervaricose spaces in the nerve plexus of the decentralized irides.

Our results demonstrate that, in the rat, prolonged isolation of the peripheral adrenergic neurons from the central nervous system and interruption of firing does not affect the NE content or uptake properties of the nerve terminals. However, decentralization does induce changes, possibly in the distribution of the neurotransmitter inside the nerve terminals, which result in increased release of ^{3}H-NE by K^+ depolarization. (Supported by United States Public Health Grant NS11631).

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

If changes in epinephrine (E) and norepinephrine (NE) reflect some common central mechanism of autonomic function, plasma levels of the 2 catecholamines should relate well with each other under a variety of different conditions. To examine this issue, we sampled plasma from an indwelling intravenous line in recumbent humans every 15 min for either 4 hr (n=9) or 8 hr (n=3). Subjects in the first group arrived in the laboratory 1 hr before the start of sampling while subjects in the second group slept overnight in the laboratory. Deproteinated plasma was frozen and subsequently assayed for norepinephrine and epinephrine by a radioenzymatic method. NE and E levels in the first group both decreased in a similar fashion over time (median r of log transformed data = .75, $p < .01$). Because this pattern of relatively high early values was seen in subjects studied in both the morning and afternoon, we concluded that the downward tendency was related to our experimental procedure. This was verified in our second group of subjects who showed no consistent changes in NE and E over time. To further study the relation between NE and E, we detrended data by computing deviations from a linear regression cast through each subject's log-transformed E or NE values. Correlations between NE and E residuals fell in 2 groups: those with a poor relation (r ranging from .10 to .43, n = 6), and those with a strong relation (r ranging from .60 to .88, $p < .01$, n = 6). Time series statistics were then applied to both NE and E residuals in the group studied for 4 hr. Peak power ranged from frequencies of 34-188 min (median = 107 min) for NE and from frequencies of 50-188 min (median = 94 min) for E. Our conclusions from these results are that (a) some common process is responsible for tonic stimulation of E and NE because of the invariable downward slope in both substances over time, probably related to our experimental design; (b) superimposed on this tonic factor are phasic fluctuations in both neuro-humors with a median ultradian rhythm of about 1.5 hr; and (c) humans can be divided into 2 groups based on whether or not their ultradian rhythms of E and NE correlate tightly. Thus E and NE correlate well with each other in some but not all conditions; this suggests that central control of release of these neuro-humors may be due to activation of anatomically discrete but interrelated areas.

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

Electrical stimulation of the fastigial nucleus (FN) in the cerebellum of dogs results in the widespread activation of sympathetic efferents. FN-evoked potentials are present in preganglionic fibers of the T_2 white ramus communicans and the splanchnic nerve. FN stimulation evokes a marked pressor response, an initial tachycardia which is buffered by baroreceptors, and an increase in plasma renin activity. The object of this study was to determine the effects of spinal lesions on evoked potentials in the splanchnic nerve and on the pressor response. The right or left FN was located with a stimulating electrode in dogs anesthetized with chloralose (115 mg/kg). The splanchnic nerve was cut and evoked potentials were recorded from multifiber units in the central end. Lesions were made successively in different segments of the spinal cord. The fastigial pressor response was unaffected by L_1 spinal transection, moderately attenuated by lesions placed in the T_5 dorsolateral funiculi and T_6 spinal transection, and abolished by C_8 transection. This indicates that sympathetic efferents affecting blood pressure leave the spinal cord primarily in the upper thoracic segments (T_1 to T_5). FN stimulation also increased the activity of multifiber units in the splanchnic nerve. At the end of pressor stimulation, the spontaneous discharge of some splanchnic units was inhibited during maximal and declining pressure presumably via the baroreflex. This inhibition was attenuated or abolished by transection of the T_6 cord. The splanchnic potentials were relatively unaffected by L_1 transections although sometimes the spontaneous activity and evoked response were attenuated but without changes in the conduction latency. The effect of subsequent spinal lesions on the FN-evoked response indicate multiple pathways of splanchnic preganglionic fibers. Some units were unaffected by mid-thoracic lesions suggesting that the preganglionic fibers leave the cord in the upper white rami then descend in the sympathetic chain. The evoked response of other units was abolished after mid-thoracic lesions suggesting that splanchnic fibers also leave the lower thoracic cord. Still other multifiber units were attenuated by mid-thoracic lesions suggesting that fibers exit from the upper and lower thoracic segments. All evoked responses were abolished after C_8 transection. In conclusion the fastigial pressor response is conveyed primarily by fibers departing from the upper thoracic cord. Splanchnic fibers may depart from various segments of the thoracic spinal cord. Splanchnic multifiber units were excited by FN stimulation and sometimes inhibited by a convergent descending baroreceptor pathway. (Supported by NIH grant HL05670 and the Oklahoma Heart Association).

163 SOCIAL INTERACTION, STRESS AND CATECHOLAMINES IN RATS S. PASHKO, K. DETURCK* and W.H. VOGEL
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Male wistar rats were catheterized in the jugular vein and the silastic tubing protected with a steel spring which was supported by a swivel pulley and counterweight. The animal could move relatively freely and blood could be drawn without disturbing the animal. Plasma norepinephrine (NE) and epinephrine (E) were measured daily and found to vary only slightly over a 5 day period following surgery (range 200-400 and 150-300 pg/ml plasma, respectively). Repeated drawing of five samples of 0.25 ml blood over one hr did not significantly affect hematocrit and plasma NE and E. Isolated catheterized rats were placed together with male rats, female diestrous rats, amphetamine-treated rats (5 mg/kg, IP, 15 min prior to experiment) or mice for one hour and blood was drawn after 1, 5, 15, 30 and 60 min. The catheter was found not to draw unusual attention by the second animal. The presence of the male or female rat produced no marked behavioral changes and had no effects on plasma NE or E in the experimental animal. The presence of a mouse or amphetamine-treated rat produced aggressive and excited behavior in the catheterized rat. In the case of the amphetamine-treated rat, NE levels were significantly elevated at all times (from 194 pg/ml to 540, 523, 429, 425 and 418 pg/ml, respectively) with no change in E levels. In the case of the mouse, NE levels were significantly elevated at 1 and 15 min (from 218 pg/ml to 325 and 371 pg/ml, respectively). No change in E levels was found. This model seems quite suitable to study blood catecholamines (and other chemicals) during social interactions.

164 EVIDENCE FOR INVOLVEMENT OF A CNS CHOLINERGIC MECHANISM IN REFLEX-INDUCED VAGAL BRADYCARDIA IN THE CAT. Christine A. Petti,* Thomas P. Williams*, Cinda J. Helke, and Richard A. Gillis*. (SPON: F.G. Standaert). Dept. Pharmacol., Schs. Med. & Dent., Georgetown Univ., Washington, D.C. 20007.

To confirm earlier findings of others that a CNS cholinergic mechanism is involved in reflex-induced vagal bradycardia (e.g., Rozear et al.: Int. J. Neuropharmacol. 7: 1, 1968), experiments using chloralose-anesthetized cats were performed whereby baroreceptors were activated with i.v. bolus injections of phenylephrine and the effects of drugs that modify CNS cholinergic synaptic transmission were observed. Drugs tested were propantheline, neostigmine, and hexamethonium, and were all administered into the fourth cerebroventricle. Propantheline (0.2-0.5 mg), a muscarinic receptor blocking agent, administered to six animals exhibiting reflex vagal bradycardia prevented approximately 70% of the response without altering either the rise in arterial pressure induced by phenylephrine or the responsiveness of peripheral muscarinic receptors. Neostigmine (0.2-1.2 mg), an inhibitor of cholinesterase, administered to five animals exhibiting reflex vagal bradycardia significantly enhanced the response without altering either the pressor effect of phenylephrine or the responsiveness of peripheral muscarinic receptors. Central administration of propantheline counteracted the enhancing effect of neostigmine. Administration of an agent known to block nicotininc receptors (hexamethonium) had no effect on phenylephrine-induced bradycardia. These results suggest that a CNS cholinergic synapse comprises part of the reflex vagal pathway, and that muscarinic receptors mediate transmission at this synapse.

165 LOCALIZATION OF NEURONS IN THE RAT SPINAL CORD WHICH PROJECT TO THE SUPERIOR CERVICAL GANGLION. T.R. Rando*, C.W. Bowers*, and R.E. Zigmond. Dept. Pharmacol., Harvard Med. Sch., Boston, MA 02115

Horseradish peroxidase (HRP) was applied to the proximal cut end of the cervical sympathetic trunk several millimeters caudal to the superior cervical ganglion in the rat. After survival times of two, three, six or nine days, the animals were sacrificed and the spinal cords processed to visualize the HRP using either diaminobenzidine or benzidine hydrochloride. The results from experiments at two, three, and six days were similar. All labeled neurons found in the spinal cord were in segments C8-T5 with 90% of the neurons in segments T1-T3. The total number of labeled neurons found in 6 experiments at a survival time of 6 days ranged from 1583-2129 (mean ± S.E.M. was 1842 ± 83). At nine days the mean number of labeled cells and the density of label per cell were reduced and the most caudal, labeled neurons were in segment T4. Labeled neurons were distributed among four areas of the spinal cord: the intermediolateral nucleus (76.5%), the lateral funiculus (20.8%), the central autonomic region (2.3%) and the intercalated nucleus (0.5%). The cells of the intermediolateral nucleus did not form a continuous column, but instead were found in clusters, several clusters being present per root. (Supported by U.S. Public Health Service Grant NS 12651).

166 MEDIAL POSTERIOR HYPOTHALAMIC LESIONS INHIBIT HYPERTENSION IN SPONTANEOUSLY HYPERTENSIVE RATS. Elinor Riley*, Kazuo Takeda*, and Ruben Bunag. Dept. of Pharmacology, Univ. of Kansas Medical Center, Kansas City, Kansas 66103.

Although evidence exists for autonomic hyperactivity and endocrine involvement in the etiology of hypertension in the Kyoto Wistar rat (SHR), the exact role of the nervous system is unknown.

Bilateral lesions (stainless steel electrodes, 0.2 x 0.5 mm exposed length, 2 mA x 5 sec DC anodal current, rectal cathode) or sham operations (electrode placement without current passage) were made in 55-day-old female SHR and their normotensive Kyoto Wistar-derived counterparts (WKY, both from Charles River Laboratories).

Systolic blood pressure, heart rate, and body weight were recorded weekly up to 16 weeks of age (9 weeks after lesion). The rats were housed in a limited access room (lights on 0700-1900 h) where measurements were also made (AM) using a tail cuff method with preheating and a 25 mm cuff.

Blood pressure did not change in WKY but was significantly lower in lesioned than in sham operated SHR throughout the experiment. Heart rate was reduced in both WKY and SHR. Although an initial weight loss in lesioned SHR was followed by normal weekly gain, body weights remained lower than those of controls.

	9 Weeks after Lesion		
Group (n)	Blood pressure	Heart rate	Weight
WKY sham (6)	108±11	431±8	202±6
lesion (6)	107±5	385±11+	200±6
SHR sham (9)	167±4	458±7	173±4
lesion (9)	127±7*	388±13*	151±4*

+ = p <.01 * = p <.001 t-test

When sympathetic nerve activity (splanchnic nerve) was recorded in sham and lesion SHR groups (urethane anesthesia) 10 weeks after lesion, basal nerve activity and changes in blood pressure and nerve activity in response to anterior hypothalamic stimulation were significantly reduced in lesioned SHR.

Our results suggest that hypothalamic lesions inhibit the rapid blood pressure elevation that normally occurs in SHR at the age of about 5-12 weeks and that this inhibition is possibly due to reduced sympathetic output. (Supported by NIH Grant HL 14560.)

167 DESCENDING CONNECTIONS FROM THE BRAIN TO THE SPINAL CORD IN THE RAT, AS STUDIED WITH HORSERADISH PEROXIDASE. Christopher Ross, David A. Ruggiero*and Donald J. Reis. Lab. of Neurobiol., Dept. Neurol., Cornell Univ. Med. College, New York, NY 10021.

With the objective of defining descending central autonomic pathways in the rat, we have injected horseradish peroxidase (HRP) into thoracic segments of the spinal cord and compared the distribution of retrogradely transported enzyme with that following injections at other levels. HRP (0.2 microliters of a 50% saline solution) was unilaterally injected into cervical, upper or lower thoracic, lumbar, or sacral levels of rat spinal cord. Following 48-hr survival, brains were sectioned and processed using diamino- or tetramethyl benzidine.

Injections at all levels resulted in HRP-labelled perikarya located in the solitary complex, the spinal trigeminal nucleus, the periphery of nucleus cuneatus, vestibular and perihypoglossal complex, raphe nuclei, reticular nuclei ventralis, dorsalis, gigantocellularis (NGC), pontis caudalis et oralis, the locus coeruleus, subcoeruleus, parabrachial, dorsal tegmental, and pedunculo pontine (PPN) nuclei, red nucleus, nuclei of Darkschewitsch and the posterior commissure, zona incerta, perifornical area, entopeduncular and paraventricular nuclei, lateral hypothalamus and cerebral cortex. Labelled neurons in the locus ceruleus were restricted primarily to ventral regions, suggesting a topographic organization of this nucleus's efferent connections. The existence of labelled cells in the paraventricular and lateral hypothalamus confirms the previous report of direct hypothalamo-spinal connections in the rat (Saper et al, Brain Res. 117, 305). In contrast to other species, few if any afferents from fastigial nucleus and tectum project to spinal cord in the rat.

A striking connection (previously undescribed) from PPN to the cord, especially the cervical and lumbar enlargements, appears significant when related to the importance of PPN as a terminus of pallidal and precentral cortical efferents, and may represent a direct extra-pyramidal channel for signals derived from the basal ganglia to the spinal cord.

Projections predominantly to thoracic levels of the cord were observed from the lateral reticular nucleus, the paramedian reticular nucleus, portions of the nucleus gigantocellularis (pars dorsalis), and the nucleus parvocellularis, supporting physiological evidence that these nuclei may be of particular importance in central autonomic regulation.

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168 SEGMENTAL ORIGIN OF PREGANGLIONIC SYMPATHETIC AXONS EMERGING IN PARTICULAR RAMI OF THE GUINEA-PIG THORACIC CHAIN. Eric Rubin* and Dale Purves. Dept. of Physiol. and Biophys., Washington Univ. Sch. of Med., St. Louis, MO 63110

Sympathetic ganglion cells in the guinea-pig are selectively innervated in that each neuron is contacted by preganglionic axons arising from a preferred subset of the spinal cord segments that innervate the ganglion as a whole (Njå and Purves, 1977). Since selective innervation is a function of the segmental level of preganglionic axon emergence, a distinguishing feature of preganglionic neurons may be the rostro-caudal position of their cell bodies within the spinal cord. To examine this idea we have used the retrograde marker horseradish peroxidase (HRP) to identify the preganglionic neurons whose axons emerge from the cord at a particular level. About 1 mg of crystalline HRP was applied to a single cut ramus of one of the mid-thoracic sympathetic chain ganglia (T4-7) in adult guinea-pigs. After survival times of 2-3 days, transverse or longitudinal frozen sections of the spinal cord were cut, and the HRP reaction product developed with DAB or TMB. Transverse sections taken from the mid-portion of the segments above and below the one corresponding to the cut ramus failed to show any labeled cells (8 animals), while labeled preganglionic neurons were present in nearly every section from the segment whose ramus had been exposed to HRP. Serial longitudinal sections of the thoracic spinal cord confirmed that the retrogradely labeled cells were almost completely confined to a region approximately one segment long, which in each case was at the level of the cut ramus (9 animals). About 500-700 neurons were labeled in each animal (uncorrected counts). We conclude that preganglionic sympathetic axons in the guinea-pig emerge from the cord at the level of their parent cell bodies. Our finding conflicts with several recent reports, and possible reasons for this discrepancy will be discussed.

Njå, A. and Purves, D. J. Physiol. (Lond.) 264:565-583, 1977.

169 CENTRAL PROJECTIONS TO A MEDULLARY TONIC VASOMOTOR CENTER IN RABBIT, DEMONSTRATED BY TRANSPORT OF HORSERADISH PEROXIDASE. David A. Ruggiero*, Mamoru Kumada, and Donald J. Reis (SPON: C.R. Noback). Lab. of Neurobiol., Dept. Neurol., Cornell Univ. Med. College, New York, NY 10021.

The pressor response to cerebral ischemia or to brainstem distortion (Cushing reflex) appears mediated by a restricted region of dorsal medulla overlapping, in part, the parvocellular (NPvc) and dorsal gigantocellular (NGCpd) reticular nuclei. Since lesions of caudal portions of this area result in a profound hypotension (Kumada et al, 1979 in press), we have proposed it represents the so-called tonic vasomotor center. We sought to establish afferent projections to this region by use of a modified horseradish peroxidase (HRP) technique (Ruggiero et al, 1977). HRP was injected into sites of medulla (NPvc and NGCpd) of rabbits from which low intensity electrical stimulation elicited an appropriate cardiovascular response. In general, there was a differentiation of afferent projections into these structures; several projections, however, overlap both nuclei. Afferents to NPvc derive predominantly from dorsolateral frontal cortex (FC), central amygdala, lateral hypothalamic area (LHA) (dorsomedial to the peduncular portion of the internal capsule), ventral tegmental area, substantia nigra pars reticularis (SNpr), ventromedial zona incerta, ventral central grey (CG), nuclei of Darkschewitsch and the posterior commissure, cuneiform nucleus (CuN), lateral perirubral field (PRF), locus ceruleus (LC), dorsal subceruleus (SCd), ventral medial parabrachial nucleus (PBN), group h, nucleus pontis oralis, NPvc and reticularis dorsalis (RD), vestibular complex (VC), spinal trigeminal (STN) and mesencephalic nucleus of V, and lamina VII of cervical spinal cord (CSC). Afferents to NGCpd derive from dorsal FC, LHA, posterior hypothalamic area, perifornical nucleus, CG, Edinger-Westphal and anterior median complex, interstitial nucleus of Cajal, ventromedial LC, SCd, dorsal tegmental nucleus, VC, STN, nucleus pontis caudalis, NPvc, RD, reticularis ventralis, fastigial nucleus and lamina VIII of CSC. Minor projections to both or either of these nuclei arise from bed nuclei of stria terminalis and anterior commissure, lateral preoptic, dorsomedial and entopeduncular nuclei, SN pars compacta, medial PRF, sub-CuN, lateral PBN, Köllicker-Fuse nucleus, solitary complex and most lamina of CSC. The medullary vasomotor area therefore appears as a site of convergence of afferent fibers from widely dispersed segments of CNS. While many are from regions known to influence cardiovascular activity, projections from somatomotor, general sensory and limbic regions are present as well. Such convergence would be consistent with the view that the region is a focus for integrating cardiovascular activity with movement and behavior.

170 ADRENAL CATECHOLAMINES IN THE SODIUM SENSITIVE, HYPERTENSIVE RATS (DAHL RATS). J.M. Saavedra, R. Del Carmine*, R. McCarty*, V. Weise*, and J. Iwai* (SPON: G. Gilad) National Institute of Mental Health, Bethesda, Md. 20205.

The effects of high (8%) and low (0.4%) sodium diet in adrenal catecholamines (CA) have been studied in the sodium resistant and the sodium sensitive Dahl Rats.

When kept under low sodium diet, the sodium sensitive rats have increased dopamine-ß-hydroxylase (DBH) and phenylethanolamine-N-methyltransferase (PNMT) activities with respect to the sodium resistant animals, indicating an increased synthesis of CA.

The effects of a high sodium diet were opposite in both strains. High sodium resulted in a decrease in the synthesis of CA in the sodium resistant rats (decreased tyrosine hydroxylase (TH), DBH and CA). In contrast, the sodium sensitive rats responded with increased CA synthesis (increased TH, PNMT and CA).

These results show that there are genetic differences in the CA metabolism in adrenal glands, between both strains, and also in the response of adrenal CA metabolism in each strain after a high sodium load.

The increase in CA synthesis in sodium sensitive rats after high sodium diet, coincides with clinical hypertension. These results suggest that adrenomedullary catecholamines may be implicated in the development and maintenance of the hypertension in the Dahl rats.

171 VAGAL CARDIAC INNERVATION: COMPARATIVE CONTRIBUTIONS OF THE DORSAL MOTOR NUCLEUS AND THE NUCLEUS AMBIGUUS DETERMINED BY LIQUID SCINTILLATION COUNTING. James Schwaber, Susan Wray*, and Gerald Higgins*. Department of Anatomy, The University of Vermont College of Medicine, Burlington, Vermont 05405.

Vagal cardiac efferents have been reported by different laboratories to have their central origin in either the dorsal motor nucleus (DMN) or the nucleus ambiguus (NA), the location apparently depending upon the species. Alternatively, subpopulations of cardiomotor cells may be present in both nuclei, each potentially having different projections and functions. We have approached this question by injecting ^{3}H proline into each nucleus and assaying the heart by liquid scintillation counting for significant levels of axonally transported radioactivity.

First, to locate each nucleus, vagal efferent neurons were retrogradely labeled with HRP. Small injections were then stereotaxically placed in 19 rats and 10 cats. Following survival times of 2-17 days the medulla was processed using the autoradiographic method to determine the injection sites. The vagus nerve and its branches and cardiac terminals were dissected and processed for counting.

Bilateral injections of both nuclei revealed the vagus to have an extensive cardiac innervation, including: the major vessels rostral to the heart, the left atrium rostrally near the aorta and pulmonary arteries and on the posterior surface, the right atrium along the SA node, the interatrial septum extending to the vicinity of the AV node, and some areas of the ventricles. The innervation of the major vessels and left atrium appears to remain largely superficial to the myocardium in the layer of visceral epicardium, whereas the innervation to the regions of the SA and AV nodes and the interatrial septum extends more deeply into adjacent planes of connective tissue.

Injections confined to a single nucleus show that the DMN and NA both contribute to the cardiac innervation in the cat and the rat. There are differences in the cardiac termination of each nucleus, the DMN of the rat, for example, predominantly projecting to the regions around the major vessels and the left atrium, and to a much lesser extent to the region of the SA node. In contrast the right nucleus ambiguus appears to preferentially innervate the region of the SA node. We conclude that both the DMN and the NA may project to the heart as distinct subsets of cardiomotor neurons in the rat and the cat. (Supported by VHA Grant 5-26528).

172 CARDIAC-RELATED DISCHARGE OF NEURONS IN THE MEDIAL PARABRACHIAL NUCLEUS OF THE CAT DURING SLEEP-WAKING STATES. Gary C. Sieck* and Ronald M. Harper (SPON: Thelma Estrin). Department of Anatomy and Brain Research Institute, University of California School of Medicine, Los Angeles, CA 90024.

The discharge of single neurons in the nucleus parabrachialis medialis (NPBM) was examined in unanesthetized, unrestrained cats across sleep-waking states. Adult cats were anesthetized with sodium pentobarbital, and electrodes were implanted for monitoring EEG, EOG, EKG, lateral geniculate nucleus EEG, and dorsal hippocampal EEG. A bundle of 10 fine-wire electrodes (62 µ insulated nichrome), together with a miniature microdrive, were implanted stereotaxically in the NPBM. Recordings were initiated in a total of six cats after they had recovered from these surgical procedures. Sleep-waking states were assessed using standard physiological and behavioral measures. Cardiac relations in NPBM neuronal activity during each sleep-waking state were determined by calculating cross-correlation functions between neuronal discharge and a reference event at the peak of the 'R' wave of the EKG. Different bin widths were used in the cross-correlations to assess phase relationships between the neuronal spike train and the EKG reference. Cardiac relations were observed in approximately 25% of the 60 NPBM neurons examined. The extent of the phasic cardiac modulation of NPBM neuronal activity varied between cells and sometimes across states for individual cells. In some NPBM neurons, cardiac relationships in discharge appeared during only 1 or 2 sleep-waking states. Generally, cardiac relationships in neuronal activity during quiet sleep (QS) were absent or reduced compared to waking (AW) and REM sleep states. Most NPBM neurons that showed cardiac relations increased their discharge 0-50 msec after the peak of the 'R' wave. In other NPBM cells, an increase in activity occurred 200-250 msec after the 'R' wave. Compared to waking, mean discharge rates of NPBM neurons decreased slightly during QS, and increased during REM sleep. Mean heart rates were comparable during AW and REM sleep states, and were reduced during QS. We propose that cardiac modulation of NPBM neuronal discharge may reflect the influence of cardiac afferent feedback, possibly via the n. tractus solitarius (NTS). The reduced phasic modulation of NPBM neuronal activity during QS may result from forebrain inhibitory influences on this area.

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173 BRAINSTEM AREAS MEDIATING THE HYPOTENSIVE EFFECTS OF MUSCIMOL. D. W. Snyder, J. A. Boccagno* and M. J. Antonaccio. Squibb Inst. Med. Res., Dept. Pharmacol., Princeton, NJ 08540 USA

Muscimol (M) a GABA receptor agonist, acts centrally to decrease blood pressure (BP), heart rate (HR) and sympathetic renal nerve discharge (RND) (Antonaccio and Taylor: Eur. J. Pharmacol. 46: 283, 1977). The present study defines the areas within the brain that are mediating the hypotensive effects of M. In chloralose anesthetized cats, M was perfused into the lateral ventricle and the perfusate was collected from either the cisterna magna (ICV) or the cerebral aqueduct (supramedullary area SMA). To differentiate the sites of action in the medullary region, M was administered into either the caudal or anterior region of the 4th ventricle. Administration of M (0.003-0.03 µg/kg/min) for 30 min ICV produced dose related decreases in BP, HR and RND with the highest dose of M producing maximum reductions of 40%, 30% and 80%, respectively. When the largest dose of M was prevented from reaching the 4th ventricle, (perfusion of SMA) a reduction in BP and HR of <15% occurred. Administration of M (0.03 µg/kg/min) into the caudal region of the 4th ventricle failed to alter BP, HR or RND. However, administration of clonidine (1.0 µg/kg/min) for 10 min, an α agonist, to the same area of the medulla led to a 30% fall in BP and 20% fall in HR. In contrast to the lack of effect of M in the caudal region of the medulla, perfusion of M (0.03 µg/kg/min) into the anterior region of the 4th ventricle produced maximum decreases in BP and HR. Pretreatment with the GABA receptor antagonist, bicuculline methiodide administered into the cerebral aqueduct in a dose which produced virtually no change in BP, HR or RND (0.5 µg/kg/min) blocked or markedly reduced the effects of M administered ICV. These results indicate that the major sites mediating the hypotensive actions of M are localized in the anterior region of the medulla and/or on the anterioventral surface of the medulla. The actions of M were blocked by pretreatment with the GABA receptor antagonist bicuculline suggesting the hypotensive effects are mediated by activation of GABA receptors. Furthermore, the medullary site of action of M is markedly different from that of clonidine which lowers BP and HR by activating central α adrenergic receptors. There appears to be a supramedullary site which contributes to the hypotensive effects of M.

174 EFFECT OF SYMPATHETIC BACKGROUND ON THE NEGATIVE CHRONOTROPIC RESPONSE TO VAGAL STIMULATION. Sherry L. Stuesse, Mathew N. Levy*, and Harrison Zieske*. Division of Investigative Medicine Mt. Sinai Hospital, Cleveland, Ohio 44106.

The time in the cardiac cycle at which a vagal stimulus is delivered is an important determinent of the chronotropic response. Thus, at some point in the cardiac cycle, a vagal stimulus will be maximally effective; at another point in the cardiac cycle it will be minimally effective. The effect of sympathetic stimulation on the sinus node chronotropic response to vagal stimulation was determined in open-chest, anesthesized dogs. Both stellate ganglia and the cervical vagi were decentralized and stimulated distal to the ligations. A factorial experimental design was used. The stellates were stimulated at one of three constant frequencies while one burst of stimulation was delivered to the vagi each cardiac cycle. The time of vagal stimulation was varied.

A background level of stellate stimulation significantly affected the following parameters ($P<.01$): the maximum and minimum amounts of slowing during vagal stimulation and the times in the cardiac cycle at which the vagal stimulus was maximally or minimally effective. However, stellate stimulation did not significantly alter the range over which a negative chronotropic vagal response could be produced nor did it shift the time from the minimum chronotropic response to the subsequent P wave (St to P interval) even though the total cardiac cycle was shortened.

175 A POSSIBLE ROLE OF OPIATE RECEPTORS IN THE PRESSOR RESPONSE TO ANGIOTENSIN II. J.E. Szilagyi* and C.M. Ferrario. Research Division, Cleveland Clinic Foundation, Cleveland, Ohio 44106.

Previous inability to produce a differential effect of angiotensin II (AII) infused via the vertebral arteries (VA) when compared to the intravenous (i.v.) route of administration was attributed to the depressant effects of pentobarbital anesthesia. A centrally mediated pressor response (PR), however, was unmasked when chloralose anesthesia was used in combination with morphine. The recent demonstration of opiate receptors in the same regions of the medulla where AII acts to facilitate central sympathetic activity caused us to wonder whether the action of morphine in facilitating the PR to AII represented some interaction between the hormone and this opiate system. The following study was designed to investigate a possible interrelationship between central effects of AII and opiate receptors in the brain by observing the central and peripheral pressor effects of AII and norepinephrine (NE) before and after treatment with naloxone (NX). Eight male mongrel dogs were anesthetized with chloralose (60 mg/kg, i.v.) after pre-medication with morphine (2 mg/kg, i.m.). Arterial pressure (MAP) was monitored via a femoral artery catheter. For infusions, a jugular vein (i.v.) was cannulated, while small catheters were placed in both vertebral arteries (VA) without interruption of blood flow. AII (10,20 ng/kg/min) and NE (100,200 ng/kg/min) in 0.9% saline were infused in random order for 3 minutes into the VA or jugular vein. The dogs were then injected with 0.8 mg NX i.v. and the response to AII and NE determined again. At the largest dose treated AII (20 ng/kg/min) infused VA caused a 22.1 ± 3.5 (SE) % increase in MAP. The same dose given i.v. produces a 18.6 ± 3.5% increase in MAP. NE (200 ng/kg/min) infused VA and i.v. caused a 13.5 ± 2.4% and a 17.2 ± 4.8% increase in MAP respectively. Ten minutes after a dose of NX the PR to AII VA (20 ng/kg/min) was reduced by 61 ± 5.1 % ($p < 0.01$) while those to NE were unchanged. The depression of PR to AII VA was not due to a generalized decrease in vascular reactivity since PR to the same dose of AII i.v. was not significantly changed after NX (18.6 ± 3.5% vs 11.9 ± 3.4%). These data show that administration of NX in previously morphinized dogs specifically antagonized the magnitude of the centrally mediated PR to AII. A specific mechanism is suggested by the lack of similar effects to central administration of NE or i.v. NE and AII. There is, thus, reason to suspect that there exists a previously unrecognized interaction of endogenous opiates in the medulla in mediating the action of AII at the level of the area postrema, the site of action of centrally administered AII.
Supported in parts by NHLBI grant #HL-6835 and American Heart Association Fellowship, Northeast Ohio affiliate.

176 FULMINATING HYPERTENSION PRODUCED BY LOCAL INJECTION OF KAINIC ACID INTO THE NUCLEUS TRACTUS SOLITAKII IN THE RAT. W. Talman*, M. H. Perrone*, N. Doba, and D. J. Reis. Laboratory of Neurobiology, Department of Neurology, Cornell University Medical College, New York, New York 10021

Kainic acid (KA) is a glutamic acid analog, which in low doses stimulates and in high doses may destroy intrinsic neurons in the brain purportedly via interaction with glutamic acid receptors. We sought to determine the effects of local administration of KA into the nuclei of the tractus solitarius (NTS), (the site of termination of baroreceptor afferents) on arterial pressure (AP) and heart rate (HR) in the rat. Rats were anesthetized with halothane and cannulas inserted in the ventral artery of the tail to record AP. Vehicle (0.3ul) containing KA (0.6-60ng) or, as control, 30ng of ascorbic acid was injected, under direct vision, bilaterally into the NTS or adjacent regions of brainstem. At the onset of injection, KA, in any dose, produced transient hypotension, bradycardia, and apnea with rapid return to baseline. After termination of anesthesia, KA elicited a dose-dependent elevation of AP, HR, and pulmonary edema (PE). At a dose of 0.6ng, KA did not change AP from control (114±2 mm Hg ; 349±24 bpm; n=5, n.s.). Modest hypertension and tachycardia followed a dose of 6ng KA. At doses of 15-60ng, hypertension and tachycardia were accompanied by PE and death within 30 minutes (e.g. 15ng: AP to 157±8 mm Hg; HR to 450 ±38 bpm; n=5 p<.01-.001). KA (80ng) injected into the nucleus gracilis or pressor areas of dorsal medulla did not alter AP or HR. Rats unilaterally injected with higher doses, while exhibiting transient hypertension and tachycardia for 3 hr, recovered and survived. No histological changes in NTS were noted 2 weeks after unilateral injection of 60ng of KA. Such injections failed to alter activity of choline acetyltransferase or tyrosine hydroxylase in microdissection of NTS. Since KA does not damage neurons in NTS, the data suggest that the drug produces hypertension by blocking activity of neurons in NTS which mediate baroreceptor reflexes. It raises the possibility that glutamate may be a neurotransmitter in the baroreceptor reflex arc.

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177 PROJECTION AND CONNECTIONS OF VASCULAR AFFERENTS IN THE FELINE CENTRAL NERVOUS SYSTEM. F. J. Thompson, C. D. Barnes, K. A. Fields, D. A. Lerner, J. R. Wald. Dept. of Neuroscience, University of Florida, Gainesville, FL 32610, and Dept. of Physiology Texas Tech. School of Medicine, Lubbock, Texas.

Little is known regarding the physiology or functional connections of vascular afferents which potentially form a vast source of sensory input. Afferent fibers from a segment of femoral vein were selected to provide a model to study the properties and connections of vascular afferents. The study reported here was undertaken to examine the electrophysiology and projection pattern to the spinal cord of vascular afferents electrically activated in a segment of femoral vein. These experiments were carried out in decerebrate-spinal cats. Stimulation of the femoral venous afferents elicited potentials which were recorded from the dorsum of the ipsilateral lumbar and sacral spinal cord, and from the lumbar dorsal rootlets. These potentials enabled determination of the following findings:

1. The fibers activated in the femoral vein segment course into the spinal cord via the saphenous nerve, and the fourth, fifth, and sixth lumbar dorsal roots.
2. Transection of the spinal cord (T_{10}) "releases" venous afferent elicited synaptic activities in the spinal cord subsequent to the initial slow negative cord dorsum potential.
3. Compound action potentials recorded from the rootlets of the fifth and sixth lumbar segments were characterized by two peaks. Conduction velocity calculations in four animals produced mean values of 57.8 and 44.3 meters/sec respectively for these two peaks.
4. Strength-duration relationships of the dorsal root fibers activated by femoral venous afferent stimulation revealed a rheobase current of 130 μa and chronaxie of 210 μsec. Paired pulse stimulation revealed an absolute refractory period of 1.1 msec.
5. Stimulation of the femoral venous afferent elicited dorsal root potentials recorded from the ipsilateral and contralateral lumbar rootlets.
6. Conditioning stimulation of the femoral venous afferents elicited facilitation followed by inhibition in both flexor (common peroneal) and extensor (gastrocnemius, deep posterior) monosynaptic test reflexes.

In a subsequent series of of experiments, the distribution of femoral venous afferent projections to the sensorimotor cortex was determined. A multiple representation on the sensory and motor cortex was observed.

178 RESPIRATORY RELATED MODULATION OF HEART RATE DURING SLEEP AND WAKING STATES IN THE CAT. Robert B. Trelease*, Gary C. Sieck*, and Ronald M. Harper. Department of Anatomy and Brain Research Institute, UCLA, Los Angeles, CA 90024.

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

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179 DO SUPERFICIAL MEDULLARY NEUROVASCULAR ELEMENTS HAVE RESPIRATORY CHEMOSENSOR FUNCTION? Trouth, C.O., Patrickson, J.W., Kang, Y.H. Holloway, J.A., Moolenaar, G.M. Dept. Physiology & Biophysics, College of Medicine, and Dept. of Zoology, Howard University, Washington D.C. 20059.

The response characteristics of spontaneously firing neurons on the ventrolateral surface of the medulla oblongata in 11 cats anaesthetized with chloralose urethane were analysed in order to identify receptors involved in the chemical drive to respiration. The discharge frequency of chemosensitive units increased from 7 to 19 impulses per second when mock CSF-pH superfusing the ventral surface was changed from 7.4 to 7.0 and decreased to 2 imp/sec when pH was changed to 7.8. The activity of these same units increased as the CO_2 tension of the inspired gas was increased, however, HCl and $NaHCO_3$ administered intravenously had no effect on the activity of these neurons. Histological examination of the site at which neurons were identified which responded specifically to mock CSF-pH changes and increased inspired CO_2 revealed: 1) Thickened marginal glia with processes projecting into the subarachnoid space. 2) Neurons dispersed within the thickened glial patches - some lying less than 10 um from the surface. 3) Neurons (Neurovascular elements) within the walls of superficial arterioles (branches of the basilar artery) and venules. Are these Neurovascular elements the central respiratory chemoreceptors? (Supported by NSF Grant HES 75 - 09024 and NIGMS Grant 1 TO 2 GM 05010 - 01)

180 PREOPTIC-ANTERIOR HYPOTHALAMIC AREA AS MEDIATOR OF BRADYCARDIA RESPONSES IN RABBITS. J. Wallach, H. Ellenberger*, N. Schneiderman, D. Liskowsky*, R. Hamilton and M. Gellman*. Dept. of Psychology, Univ. of Miami, Coral Gables, Fl. 33124.

Electrical microstimulation (10 s trains, 100 pulses/s, 0.5 ms pulses, <300 μA, threshold~110 μA) of 15 sites in the preoptic-anterior hypothalamic area (PAHA) of 15 Urethane-anesthetized rabbits elicited bradycardia (M=-32bpm, SD=±9) and a depressor response (M=-12 mm Hg, SD=±3). The bradycardia was due to vagal excitation as it was abolished by bilateral vagotomy. Single-pulse (0.1 ms duration) stimulation of aortic nerve (AN) activated 14 neurons recorded extracellularly within PAHA indicating that neurons in the region receive barosensory input. Mean onset latency for cells activated by AN stimulation was 25 ms (SD=±14). In order to examine the decending pathways mediating the bradycardia responses elicited by stimulation of PAHA, extracellular recordings were made from dorsomedullary neurons that were activated by single-pulse stimulation of both PAHA and AN. Nine of the 15 neurons receiving barosensory input were also driven by single-pulse stimulation of PAHA. These units were histologically located in dorsal vagal nucleus (DVN) or nucleus tractus solitarius (NTS). Although the mean onset latency to diencephalic stimulation was relatively short (M=10 ms, SD=±2) these neurons did not follow repetitive stimuli faithfully. Therefore, the functional connections between PAHA and dorsal medulla are probably not monosynaptic. Four of the above neurons located in DVN were also antidromically-invaded by stimulation of ipsilateral vagus nerve, and therefore were preganglionic and probably cardioinhibitory. Unilateral injections of horseradish peroxidase (HRP, 30%, 0.03-0.1μl) were made in dorsomedial NTS and DVN in 17 rabbits. Following a 48 hr survival period, the brain tissue processed in 3 different chromagens displayed cell body labeling in the anterolateral hypothalamic area, paraventricular n., dorsomedial hypothalamic n., zona incerta, central n. of amygdala, and central gray. None was observed in preoptic region or anterior hypothalamic n. The anterolateral hypothalamic area revealing cell body labeling was the most caudal and lateral region of PAHA from which (a) neurons were activated by AN stimulation, (b) bradycardia and depressor responses were obtained to train stimulation and (c) cardiac-related neurons in DVN and NTS were activated by single-pulse stimulation. However, since single-pulse stimulation in anterolateral hypothalamus only activated units in the dorsal medulla oligosynaptically, it appears that the functional and anatomical pathways studied were parallel but not identical (Supported by NSF Grant BMS 78-15403 and by a grant from Florida Heart Association).

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

Daniel J. Williford*, Joseph A. DiMicco*, & Richard A. Gillis*. (SPON: R.J. Kellar). Dept. of Pharmacology, Schs. of Med. & Dent., Georgetown Univ., Washington, D.C. 20007.

Bicuculline administered into the lateral cerebroventricle of chloralose anesthetized cats produces increases in arterial pressure and heart rate. To determine whether this effect is localized in forebrain areas, bicuculline in doses of 1, 5 and 25 ug was administered into either the third and lateral ventricles with cannulation of the cerebral aqueduct or into the fourth ventricle of vagotomized cats. Administration into the third and lateral ventricles resulted in dose-dependent increases in pressure and rate, with the maximal response being 63 + 11 mm Hg and 44 + 9 beats/min., respectively. These same doses had no significant effect on heart rate and only a slight effect on pressure when administered into the fourth ventricle. Administration of the GABA receptor agonist, muscimol, into the third and lateral ventricle in a dose that had no effect alone on pressure and rate (10 ug) prevented the cardiovascular effects of bicuculline. In contrast, administration of the centrally active antihypertensive agent clonidine (31 ug) had no effect on bicuculline-induced increases in pressure and rate. In addition, muscimol (10 ug) when administered at the time of the peak pressure and rate responses of bicuculline restored these indices of cardiovascular function to normal. Clonidine (31 ug), however, administered in the same fashion had no effect. These results suggest that a tonically-active GABAergic system exists in the region of the forebrain and exerts inhibitory control over sympathetic activity influencing arterial pressure and heart rate.

AXONAL TRANSPORT

182 A POSSIBLE NON-NEURONAL TRANSFER OF ^{3}H-PROLINE-LABELED MOLECULES FROM THE DORSAL COLUMN NUCLEI TO THEIR TERMINAL TARGETS IN CATS. K. J. Berkley and H. H. Molinari. Dept. Psychol., Fl. St. Univ., Tallahassee, FL 32306.

Several investigators have found that ablations of the dorsal column nuclei (DCN) in cats produce degeneration in the inferior olive (IO). This projection involves neurons because degenerating synaptic terminals are observed at the ultrastructural level in IO following these ablations. It is not surprising, therefore, that injections of ^{3}H-leu into DCN produce dense labeling in IO, since neurons at the injection site are also heavily labeled.* Unexpectedly, however, ^{3}H-pro injections into DCN also produce dense labeling in IO, despite the fact that neurons at the ^{3}H-pro injection site appear to be, at best, only very lightly labeled 24 hrs after the injection.*

There are several possible explanations for these unexpected findings. (1) ^{3}H-pro-labeled neurons were missed due to insufficient sampling of IO-projecting neurons. The ^{3}H-pro samples, however, were taken from areas in DCN which are known to contain IO-projecting neurons and in which small ^{3}H-pro injections still produce heavy labeling in IO. (2) ^{3}H-pro-labeled molecules may be transported very rapidly out of the DCN neurons, precluding their visualization at a 24 hr survival time. The same labeling pattern is seen, however, even at a 20 min survival time. (3) Since the methods used here allow visualization only of bound amino acids, it may be that ^{3}H-pro is unseen in DCN neurons because it is taken up and transported from the perikaryon in its free form before being bound. It is generally agreed, however, that amino acids are incorporated into molecules within the perikaryon.

These considerations suggest that the paucity of neuronal labeling by ^{3}H-pro in DCN as seen in the present experiments does not reflect an artificial situation. If so, then some ^{3}H-pro-labeled molecules must be transferred to IO (as well as to other terminal targets of DCN) by a non-neuronal mechanism. One such mechanism might involve glial cells. Consistent with this suggestion is the fact that cells small enough to be glia are labeled in DCN when HRP is injected in IO. Both astrocytes and oligodendrocytes could be involved in this transfer mechanism. Most astrocytes and their processes are heavily labeled following ^{3}H-pro injections along with some oligodendrocytes and myelin.* Astrocytes with very long processes are known to exist and the fibers which project to IO are myelinated. Thus, it appears that molecules labeled by tritiated amino acids may be transferred from one location to another by glial as well as by neuronal mechanisms.

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*Molinari and Berkley, 1979, this volume.

183 EVIDENCE FOR MULTIPLE SOMATIC POOLS OF INDIVIDUAL AXONALLY TRANSPORTED PROTEINS. Robert W. Berry. Dept. Anat., Northwestern Univ., Sch. Med., Chicago, IL 60611.

Although the existence of a large somatic storage pool of secretory material is a common characteristic of neurosecretory cells, it is not known if secretory proteins are withdrawn from this pool at random for axonal transport and secretion, or if cellular mechanisms exist that route newly-synthesized proteins either to storage or transport. These alternatives can be tested by using neurons L11 and R15 of Aplysia, which synthesize and commit to axonal transport large quantities of a specific class of low molecular weight presumptive neurosecretory proteins (LMW proteins). Pulse-chase studies in which the LMW proteins are identified by SDS gel electrophoresis indicate that they leave the soma by a biphasic process: a rapid phase of decay (halflife 1-2hr) is followed by a slower phase (halflife >10hr). These kinetics are not due to the presence of more slowly turning-over contaminating species, since the same results are obtained when the LMW proteins are identified by migration on gel systems which separate proteins by both molecular charge and molecular weight. Two additional tests indicate that total LMW protein turns over at the slow rate: Exposure to vinblastine for 6hr blocks decay without affecting synthetic rate, but has no detectable effect on somatic LMW content as assayed by Coomassie staining of SDS gels. Furthermore, the ratio of labeled LMW protein to labeled higher molecular weight species is the same after 18hr of exposure to labeled precursor as after a 2hr exposure, whereas if total LMW protein had a 1-2hr halflife, the ratio should have declined significantly over this period. These data are not consistent with the single-pool model. Rather, they indicate that following synthesis these proteins are partitioned into at least two kinetically distinguishable somatic pools, one of which is rapidly subjected to axonal transport, and the other of which represents a slowly turning-over somatic storage pool.
(Supported by NIH grant NS-11519.)

184 AXONAL TRANSPORT OF CHOLESTEROL. William D. Blaker*, Arrel Toews and Pierre Morell. Biol. Sci. Res. Ctr. and Dept. of Biochem., Univ. of North Carolina, Chapel Hill, NC 27514.

Twenty-two-day-old rats were injected intraocularly with [^{3}H] acetate and killed 1 hr to 35 days later. Cholesterol was isolated from the retinas, optic tracts, lateral geniculate bodies and superior colliculi. Radioactivity was rapidly incorporated into retinal cholesterol with near maximal labeling present one hour after injection. [^{3}H] cholesterol was lost from the retina with an apparent half-life of 15 days, with transported radioactivity accounting for 40% of the decrease.

Transported labeled cholesterol (contralaterally corrected for systemic background labeling) was present in the optic tract by two hours and in the superior colliculus by three hours, consistent with a fast rate of transport. Radioactive cholesterol accumulated in all visual structures throughout the 35-day period, but the rate of accumulation was maximal at about the time of arrival of the initial pulse of radioactivity and fell to low levels by 2-4 days. Colchicine treatment of the retina blocked transport of cholesterol but not its synthesis by the retina. The use of [^{3}H] mevalonate or [^{14}C] acetate also showed transported labeled cholesterol in all visual structures by 1 day.

The time course of accumulation of transported cholesterol is consistent with that of phospholipids, which are also rapidly transported (Toews, et.al., J. Neurochem. 32:1165-1173). Both showed an increase in the rate of accumulation at 4-5 days in optic tract and superior colliculus, indicating the fast transport of a phase of slowly released lipid. Differences between the transport time courses of cholesterol and phospholipid are probably due to differences in metabolism rather than differences in modes of transport. Labeled phospholipid decreased in all visual structures after 5 days while cholesterol continued to rise, reflecting the greater metabolic stability of cholesterol. Cholesterol showed a greater preferential deposition in the axons relative to nerve endings when compared to phospholipids.

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185 BATRACHOTOXIN BLOCKS SLOW AND RETROGRADE AXONAL TRANSPORT IN VIVO. R.J. Boegman, R.J. Riopelle and E.X. Albuquerque. Depts. of Pharmacology and Medicine, Queen's University, Kingston, Canada, K7L 3N6, and Dept. of Pharmacology, University of Maryland, Baltimore MD 21201.

Batrachotoxin (BTX) causes membrane depolarization and has been shown to block fast axonal transport both in vivo and in vitro at concentrations of 1.86×10^{-12} and 1×10^{-9} moles respectively (Boegman and Albuquerque, Fed. Proc. 62 158 1978; Ochs and Worth, Science. 187 1087 1975). It is not clear how the toxin blocks fast axonal transport since there does not appear to be a direct relationship between membrane excitability and the transport process. In an attempt to define the site at which BTX acts in blocking axonal transport we examined slow axonal transport and retrograde axonal transport in BTX-poisoned nerves in vivo. The accumulation of cholineacetyltransferase (CAT) proximal to and Nerve Growth Factor (NGF) distal to the site of BTX (3.7×10^{-11} moles) injection was used to monitor slow and retrograde axonal transport respectively. Slow transport was blocked within 1 day and remained so for up to 7 to 10 days when recovery was evident by the decreased accumulation of CAT at the site of injection. The amount of CAT accumulating proximal to the injection site was, however, only 1/2 that observed following nerve ligation. Retrograde transport of ^{125}IβNGF was blocked within 6 hrs of injection into the foot pad with accumulation of the protein distal to the site of BTX injection. The degree of block was as complete as that observed following nerve ligation. Neither BTX nor TTX influenced uptake or transport of ^{125}IβNGF when injected into the nerve or into the foot pad 30 minutes prior to NGF injection. In contrast to BTX, Tetrodotoxin did not block either slow or retrograde axonal transport. These results suggest that BTX interacts with a component in the axon possibly tubulin or Ca++ which is required to maintain fast, slow and retrograde axonal transport.

Supported by Canadian and U.S. Muscular Dystrophy Foundations and the Medical Research Council of Canada.

186 NERVE SPECIFIC ENOLASE AND CREATINE PHOSPHOKINASE ARE TRANSPORTED AS PART OF THE AXOPLASMIC MATRIX (SLOW COMPONENT b). Scott T. Brady* and Raymond J. Lasek (SPON: R. Plonsey). Dept. Anat. Sch. Med., Case Western Reserve Univ., Cleveland, OH. 44106.

Slow Component b (SCb) is a complex constellation of proteins moving in the anterograde direction along the axon at a rate of 3-4 mm/day. Materials associated with SCb have been shown to move separately from the microtubule-neurofilament network (Slow Component a) and to include both actin and clathrin (Garner and Lasek 1978 Trans.Am. Soc. Neurochem. 9 200, Black and Lasek 1977 Soc. Neurosci. Abstr. 7 29). Pulse labelled SCb is known to move for weeks or months as a discrete wave. These properties of SCb suggest that it represents a distinct cellular structure which may be termed the axoplasmic matrix, although some 60% of the proteins in SCb are easily solubilized in physiologic buffer. These easily solubilized proteins are commonly thought to be diffusible proteins in the cell and may include the enzymes of glycolysis and related intermediary metabolic pathways. Marangos et al (1975 Biochim. Biophys. Acta 392 75) reported the axonal transport at 6 days but not at 6 hours of a brain specific soluble protein later found to have enolase activity. Since this nerve specific enolase (NSE) [E.C. No. 4.2.1.11] is the nerve isozyme of the glycolytic enzyme, we have examined the transport of NSE and a second enzyme associated with energy metabolism, creatine phosphokinase (CPK) [E.C. No. 2.7.3.2].

^{35}S-methionine was injected into the eyes of adult guinea pigs. The animals were sacrificed 6 days later when the only labelled proteins in the optic nerve are associated with SCb. The optic nerve was homogenized and aliquots subjected to two dimensional electrophoresis. NSE was purified by a modification of the procedure of Marangos et al (1975 J. Biol. Chem. 250 1884) and rabbit brain CPK was obtained from Sigma (St. Louis, Mo.). Fluorographs of the two dimensional gels showed at least 50 proteins transported in SCb. NSE (pI 5.5, mw 48000) and CPK (pI 6.3 mw 43000) were found to have labelled homologues in SCb which behaved identically upon two dimensional electrophoresis. We conclude that NSE and CPK are transported in SCb. It has been suggested that SCb proteins are tightly associated in a highly ordered complex because they move in a coherent manner and are associated specifically with SCb and not with any other transport components [i.e. Slow Component a and Fast Component] (Tytell and Lasek 1978 Soc. Neurosci. Abstr. 8 37). If the easily solubilized proteins NSE and CPK are associated with this complex, which we term the axoplasmic matrix, then the common view that these and other metabolic enzymes are diffusible in the axon requires reevaluation.

187 **AGRANULAR RETICULUM-LIKE CISTERNS INVOLVED IN THE NEURONAL TRANSPORT OF ACID HYDROLASES AND HORSERADISH PEROXIDASE (HRP).** **M. W. Brightman* and R. D. Broadwell (SPON: S. P. Wise). Lab. Neuropath. and Neuroanat. Sci., NINCDS, NIH, Bethesda, MD 20205.**

Studies with HRP as a tracer have suggested that the agranular reticulum is associated with the anterograde and retrograde axonal transport of peroxidase. We have reported (J. Comp. Neurol. 185:31, '79) that in the hypothalamo-neurohypophysial system of the hydrated mouse, HRP-labeled cisternal profiles with an appearance and width (400-700Å) similar to the agranular reticulum were minimally involved in the retrograde axonal transport of the HRP. Similar profiles labeled with HRP or containing acid hydrolase were more prevalent during anterograde axonal transport, but only in hyperosmotically stressed mice. We proposed that these cisterns are part of the lysosomal system and that anterograde transport of HRP is associated with the movement of acid hydrolases, presumably from perikaryal secondary lysosomes. We report here additional evidence that these organelles in the soma participate in the anterograde movement.

In supraoptic perikarya from normal, hydrated mice injected with HRP into the blood or cerebral ventricles, very few agranular reticulum-like cisterns, attached or unattached to secondary lysosomes, contained peroxidase. The number of HRP-labeled cisterns and dense bodies increased when the animals were hyperosmotically stressed by having them drink 2% NaCl 5-8 days prior to HRP injection. Supraoptic cell bodies from osmotically stressed mice not injected with HRP were incubated for acid hydrolase activity using trimetaphosphate or β-glycerophosphate as substrate . Acid hydrolase-positive lysosomes and 400-1000Å wide cisterns proliferated in these perikarya. Some of the cisterns were confluent with secondary lysosomes. Peroxidase-exposed perikarya incubated for both HRP and acid hydrolase activity from the salt-treated animals contained secondary lysosomes and a few cisterns which exhibited reaction products of both enzymes.

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

188 BATRACHOTOXIN BLOCKADE OF AXOPLASMIC TRANSPORT: STUDIES OF A DIRECT TOXIN EFFECT ON PURIFIED MICROTUBULES. George B. Brown and Lelland C. Tolbert, Neurosciences Program, University of Alabama in Birmingham, Birmingham, AL 35294.

Ochs and Worth (Science, 187:1087, 1975) have shown that the steroidal alkaloid batrachotoxin (BTX) is 5,000 to 10,000 times more potent than the vinca alkaloids in blocking fast axoplasmic transport in the cat, and that this effect is not due to the well-known depolarizing action of the toxin on excitable cells. We have purified tubulin from rat brain using three cycles of polymerization/depolymerization and examined the binding of a radiolabeled BTX derivative, ^{3}H-batrachotoxin benzoate (^{3}H-BTX-B), to this protein. At a concentration of 0.1 μM, ^{3}H-BTX B did not bind to tubulin in either the depolymerized or polymerized state over a time period of 2.5 hr. Ochs and Worth reported that 0.1 μM BTX produced a block of axoplasmic transport in 2.35 hr. We also conducted a pilot study to examine the effect of BTX on the polymerization of tubulin. Purified tubulin was subjected to polymerizing conditions (0.1 mM 2-(N-morpholino) ethanesulfonic acid, 1 mM EGTA, 1 mM GTP, 0.5 mM $MgCl_2$, 4 M glycerol, pH 6.4, at 37°C) either in the presence or absence of 1 μM BTX. Polymerization was allowed to continue for 1.6 hr and the amount non-polymerized protein remaining was determined. Relative to the sample without toxin, BTX inhibited polymerization by approximately 20%. The observed lack of ^{3}H-BTX-B binding to tubulin contrasts with the apparent direct action of BTX on tubulin polymerization in our initial study. Although BTX-B is known to bind at the same sodium channel site as BTX, this may not be the case with respect to toxin action on axoplasmic transport. The effect of batrachotoxin benzoate on axoplasmic transport should be tested in vitro.

189 DIFFERENTIAL UPTAKE OF HORSERADISH PEROXIDASE ISOENZYMES BY CULTURED NEUROBLASTOMA CELLS. Kwan Y. Chan, Richard H. Haschke and Ann H. Bunt. Depts. of Ophthalmology and Anesthesiology, University of Washington, Seattle, WA 98195.

Neurons of the central visual pathways of the rat have been shown to take up horseradish peroxidase (HRP) at the axon terminal and transport the protein retrogradely in a selective way (Bunt and Haschke, 1978: J. Neurocytol. 7, 655-78). Cultured mouse neuroblastoma cells (clone N18) have been used to develop an in vitro system for further studying the selectivity of protein uptake. The cells, while differentiating in serum-free medium, were incubated with HRP isoenzyme C (HRP-C) and A (HRP-A). Ultrastructural morphometry of labeled organelles was performed on cells fixed and processed for cytochemistry after incubating with 2 mg/ml of HRP for 5 min and also for 5 min followed by an hour of wash. The number of vesicles labeled by HRP-C was 1.5 times that of HRP-A during initial endocytosis, and the number of dense bodies labeled by HRP-C was 1.8 times that of HRP-A in the pulse-chase experiment. However, to overcome the limitations of morphometry the rate of uptake of HRP isoenzymes in unfixed cells was quantified by a spectrophotometric assay using o-dianisidine as substrate. To measure rates of intracellular decay of HRP activity after endocytosis, the cells were incubated with 0.5 mg/ml of HRP for 2 h and subsequently washed for various periods. After lysing the cells in 0.05% Triton X-100, HRP activity was measured directly in the lysate. The data showed that the two isoenzymes decayed at different rates inside the cells, with a half-life ($t_{½}$) of 8.7 h for HRP-C and 1.9 h for HRP-A. The decay rate constant (k) can be calculated from equation $k = \frac{\ln 0.5}{t_{½}}$. To find the rate of uptake of HRP, the cells were incubated with 0.2 mg/ml of HRP for various periods, washed 5 times and lysed. The amount of HRP internalized by the cells for a given period (t) was measured (X'_t) and corrected for lysosomal decay to obtain the initial value (X_t), by using the equation $X_t = \frac{X'_t \cdot kt}{1-e^{-kt}}$.

Over the 7-hour period studied, HRP-C was taken up by N18 cells at a rate 2.2 times that of HRP-A. Over the HRP concentration range of 0.2-0.7 mg/ml, HRP-C was incorporated into cells 2-3 times faster than HRP-A. At higher concentrations (2 to 6 mg/ml) the rate of uptake of these isoenzymes appeared to become similar. Experiments are in progress to assess the effects of various compounds on the uptake of proteins by these cells.

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190 CALCIUM LOCALIZATION IN MAMMALIAN NERVE FIBERS IN RELATION TO ITS REGULATION AND AXOPLASMIC TRANSPORT. S.Y.Chan, S.Ochs, and R. Jersild,Jr.: Depts. of Physiol., Anat., and The Biophysics Prog., Indiana University Medical Center, Indianapolis, IN 46223.

A specific Ca^{2+} requirement for axoplasmic transport was shown using a desheathed cat sciatic nerve preparation (Ochs, Worth & Chan, Nature, 270: 748, 1977). A block of transport was seen following incubation of nerves in a Ca^{2+}-free or low Ca^{2+}medium with normal transport restored by adding Ca^{2+}. The block in Ca^{2+}-free media was considered due to a depletion of free Ca^{2+} from the axons. On the basis of the transport filament model, the level of free Ca^{2+} in the axon regulates a calcium binding protein (calmodulin) which activates the Ca^{2+}-Mg^{2+}-ATPase utilizing ATP needed to drive the transport filaments. The level of free Ca^{2+} in the axon is regulated by mechanisms present in the mitochondria and endoplasmic reticulum (ER) which sequester and release Ca^{2+}, keeping it close to about 10^{-7}M. To show the presence of Ca^{2+}, K^{+}-pyroantimonate which binds to Ca^{2+} to form an electron-dense deposit, was used to stain thin sections of nerve for EM. Nerves were incubated in media containing normal levels of Ca^{2+} or media with raised or lowered levels of Ca^{2+}. Incubation was carried out at 38°C, pH 6.7 and the nerves bubbled with 95% O_2 + 5% CO_2. The nerves segments were then fixed in 5% glutaldehyde and 1% osmium with 2% K-pyroantimonate added. Electron-dense granules were seen in the EM sections of nerves incubated for 3 hrs or more in normal media containing Ca^{2+}. The granules were found scattered throughout the axoplasm with some attached to the axolemma, to the surface of the mitochondria, and some within the ER. Evidence that the granules contained Ca^{2+} was obtained from their wash-out from thin sections exposed to 4 mM EGTA. Preliminary studies using X-ray microanalysis also showed Ca^{2+} present in the granules. Experiments with Na^{+} and Mg^{2+} showed very much less binding to pyroantimonate. When incubated in a Ca^{2+}-free medium for 3 hours or more, the nerves were depleted of their granular deposits. Conversely, when desheathed nerves were placed in in vitro media containing 20 mM Ca^{2+} or more, a larger number of granules was seen throughout the axoplasm, in the ER, inside the matrix and attached to the outside of the mitochondria. Granules were also seen in the nodes of Ranvier which could be related to the exchange of Ca^{2+} at the nodal membrane. The increased deposits seen in the nerves exposed to high levels of Ca^{2+} were depleted from nerves returned to a low Ca^{2+} medium for at least ½ hr. This was most marked for the mitochondria and ER. Such changes indicate that these organelles act to regulate the level of free Ca^{2+} in the axons by sequestering and releasing Ca^{2+}. Supported by NIH grant #RO1 NS 8706-10 and NSF grant #BNS 77-24176.

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Withdrawn by author

192 NEW TECHNIQUES FOR REVEALING THE CYTOSKELETON OF AXOPLASM AND THE EFFECTS OF CA^{++} UPON ITS ORGANIZATION. Mark H. Ellisman and Keith R. Porter. Dept. Neurosci., Sch. Med., UCSD, La Jolla, CA 92093.

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

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

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193 TRANSNEURONAL TRANSPORT OF HORSERADISH PEROXIDASE IN THE RAT CILIARY GANGLION. P. Gomêz-Ramos*, E.L. Rodriguez-Echandia* (SPON: F. Reinoso-Suarez). Dept. Morfología, Inst. Invest.Oftalmol. Castroviejo, Fac. Medicina, Univ. Autónoma.Madrid 34.Spain.

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

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

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

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

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

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194 AXONAL TRANSPORT OF PHOSPHOLIPIDS IN RAT SCIATIC NERVE. Robert M. Gould, Raymond S. Sinatra*, Warren Spivack*, Mark Berti*, Henry M. Wisniewski*, Thomas Lindquist*, and Nicholas Ingoglia. N.Y.S. Inst. for Bas. Res. Ment. Retard., Staten Island, NY 10314, and Dept. of Physiol. and Neurosci., N.J. Med. Sch., Newark, NJ 07103.

Using EM autoradiographic techniques we have demonstrated that tritiated inositol, locally injected into mouse and rat sciatic nerve is actively incorporated into phospholipid within the axoplasm. In contrast, we have found no evidence for an axon-based incorporation of choline into phosphatidylcholine. At last years meeting we presented evidence that inositol transferase, the enzyme required for de novo phosphatidylinositol formation is axonally transported. Choline phosphotransferase required for de novo phosphatidylcholine synthesis is probably not axonally transported. Buildup of this enzyme at the site of nerve ligation was shown to be largely a result of increased metabolism of local Schwann cells, responding to the trauma.

In this present report we examine the axonal transport of the phospholipids in rat sciatic nerve following the injection of tritiated choline and inositol into the spinal cord (according to the procedure of Lindquist and Ingoglia, Brain Res. 161 (1979) 95-112). At the time of injection the left sciatic nerve was crushed at its emergence from the sciatic notch. At 6, 12, and 18 days following the injection and crush animals were sacrificed by intracardiac perfusion with 4% glutaraldehyde in Millonig's phosphate buffer. The spinal cord, ventral roots (L_4 & L_5) and both sciatic trunks were carefully dissected. Both nerves were sectioned into two mm lengths and alternate sections were taken for autoradiographic and biochemical analyses.

The distributions of radioactive lipids along the nerves varied. With both precursors a greater percentage of the label was found more distal at the longer survival times. In both normal and crushed nerves phosphatidylcholine was transported to a greater degree than phosphatidylinositol. Toews et al (J. Neurochem. 32 (1979) 1165-1173) made a similar observation with transport in rat optic nerve using ^{32}P-phosphate as precursor. There was not much difference in the distribution of lipid along the control and crushed nerves.

Grain localization in more distal segments (after 30 days exposure) is much more pronounced with choline than inositol. With choline label is localized over axons and the myelin sheaths suggesting as pointed out by Droz et al (Brain Res. 155 (1978) 342-355) that some transported lipid can enter the myelin sheaths. Some label is also present in the growing tips and regenerating fibers. (Supported by grants NS-13980, AM-20541, and NEI-02887 from NIH).

195 DYNEIN CROSSBRIDGING OF BRAIN MICROTUBULES AND ITS ATP DISSOCIATION. Leah T. Haimo* and Joel L. Rosenbaum. Dept. Biol., Yale Univ., New Haven, CT 06520.

Dynein, the ATPase enzyme responsible for flagellar beating, binds to brain microtubules and causes their ATP dependent crossbridging. When dynein, obtained from Chlamydomonas flagella, is combined with purified brain tubulin and the preparation warmed to 30 C, microtubules are immediately formed as analyzed by an increase in turbidity of the tubulin suspension and by the observation of microtubules by dark field optics. Moreover, these microtubules appear aggragated when compared to microtubules assembled in the absence of dynein. Examination of these aggragated microtubules by electron microscopy reveals them to be crossbridged by a regular array of dynein-like arms; no such arms are observed on microtubules to which dynein is not added. Moreover, the periodicity of these arms along the in vitro assembled microtubules is 23.5nm, equal to the spacing of dynein arms along flagellar microtubules. Addition of ATP to the aggragated microtubules results in an immediate decrease in turbidity of the microtubule suspension, and observation of this preparation by dark field microscopy indicates that the microtubules are no longer aggragated.

The ATPase activity of microtubules polymerized in the presence of dynein is 0.1-0.2 umole Pi/min/mg which is slightly less than that of flagellar axonemes, 0.2-0.3 umole Pi/min/mg. On the other hand, microtubules polymerized in the absence of dynein have no measureable ATPase activity. Furthermore, SDS gel electrophoresis indicates that dynein sediments with microtubules on a sucrose gradient.

In favorable electron microscopic preparations, the dynein arms are observed to bind to the in vitro assembled brain microtubules at an angle. Dynein can, therefore, be used to determine the polarity of microtubules in situ.

These observations demonstrate that dynein can bind to brain microtubules, induce their crossbridging, and result in ATP dependent microtubule sliding. These data are the first demonstration that cytoplasmic microtubules can bind dynein in a manner similar to that of flagellar microtubules and supports the notion that cytoplasmic microtubule-mediated movements might result from dynein induced crossbridging between adjacent microtubules.

196 CALMODULIN ACTIVATION OF BRAIN AND NERVE Ca^{2+}-Mg^{2+}-ATPASE. Z. Iqbal, B.P. Garg*, and S. Ochs. Depts. of Physiology, Neurology and the Medical Biophysics Program. Indiana University Medical Center, Indianapolis, Indiana 46223.

In the transport filament model for the axoplasmic transport, the energy required for the transport of materials is supplied through the hydrolysis of ATP by a calcium-dependent adenosine triphosphatase (Ca^{2+}-Mg^{2+}-ATPase) which had been found present in the nerve (Khan and Ochs, Brain Res. 81: 413-426, 1974). Calcium has also been shown to be required to maintain transport by the block of transport in desheathed nerves placed in a Ca^{2+}-free medium in vitro and the addition of Ca^{2+} reversing the block (Ochs, Chan, and Worth, Nature 270: 749-750, 1977). We have also demonstrated that Ca^{2+} is fast transported in association with a calcium binding protein (CaBP) in nerve (Iqbal and Ochs, J. Neurochem. 31: 409-418, 1978). The CaBP shows similarities to the calcium-dependent regulatory protein (CDR) as regards its electrophoretic mobility on acrylamide gels containing SDS and its isoelectric point (3.9) on isoelectrofocusing. CDR, which is now known as calmodulin, was first observed as a modulator of adenylate cyclase and cyclic nucleotide phosphodiesterase (PDE) of brain and has recently been found to activate Ca^{2+}-Mg^{2+}-ATPase isolated from RBC and sarcolemma. In this communication we now report that peripheral nerve calmodulin can stimulate microsomal Ca^{2+}-Mg^{2+}-ATPase isolated from neural tissue. Cat brains were homogenized in 0.32 M sucrose and the post-mitochondrial supernatant was centrifuged at 105,000 g for 1 hr. The microsomal pellet obtained in this preparation contains an appreciable amount of calmodulin. This was determined by; (a) electrophoresis on acrylamide gels containing SDS, (b) activation of PDE, and (c) the binding to it of ^3H-trifluoperazine (TFP). Washing the microsomes with 10 mM Tris-HCl, pH 7.5 containing 0.1 mM EGTA was not effective in releasing calmodulin from the microsomes. Incubation of the microsomes in 0.05% Triton X-100 was relatively more effective in dissociating calmodulin from the microsomes. The pellet obtained after the treatment of the microsomes with Triton X-100 had Ca^{2+}-Mg^{2+}-ATPase activity which was stimulated 1.5 - 3-fold by the calmodulin prepared from peripheral nerve. This stimulation was found to depend on the level of Ca^{2+} concentration in the incubation medium. Furthermore, addition of TFP blocked the stimulation of Ca^{2+}-Mg^{2+}-ATPase by calmodulin. Similar observations were made using the Ca^{2+}-Mg^{2+}-ATPase obtained from sciatic nerve. These results suggest that under physiological conditions where Ca^{2+} levels are low, approximately 10^{-7} M, calmodulin is required to activate Ca^{2+}-Mg^{2+}-ATPase, an integral part of the axoplasmic transport mechanism. Supported by NIH grant # RO1 8706-10 and NSF grant # BNS 77-24176.

197 EFFECT OF RESERPINE ON AXONAL TRANSPORT OF PROTEINS AND GLYCOPROTEINS IN NORADRENERGIC NEURONS IN THE RAT BRAIN. Barry E. Levin, Neurology Serv. and Dept. of Neurosciences, VA Med. Ctr., East Orange, NJ 07019 and N. J. Med. School, Newark, NJ 07103

The injection of radiolabeled precursors into the locus coeruleus (LC) of the rat is followed by the sequential appearance in the hypothalamus of 2 waves of rapidly transported glycoproteins (wave I:72-192 mm/d and wave II:24 48-mm/d), one intermediately (wave III:13-20 mm/d) and one slowly transported (wave V:1.4-2.9 mm/d) wave of proteins travelling in noradrenergic neurons. Reserpine, which disrupts storage of catecholamines and markedly affects the metabolism of LC noradrenergic neurons, also affects axonal transport and turnover of proteins in these neurons. Rats were pretreated with Serpasil phosphate (Ciba), 5 mg/kg IP, at intervals of 1-21 days prior to [^3H] fucose (waves I and II) or [^3H] leucine (waves III and V) injection into the LC. Reserpine pretreatment was associated with significant decreases to 25-40% of control LC levels in [^3H] glycoprotein and protein content during the first 2 days following injection. When axonal transport was corrected for these and subsequent changes in LC protein turnover to give a net transport value, distinctly different effects of reserpine pretreatment on the various waves were evident when compared to vehicle injected or uninjected controls. In general, axonal transport in all four waves was decreased at some point during the first four days after reserpine injections, varying from complete to partial blockade. Transient decreases in transport also occurred at 7 and 14 days after reserpine in waves I and II, while wave V was decreased from 8-10 days. Transport in wave III was significantly depressed from days 6 through 10. Transport in all waves had returned to control levels by day 21 after reserpine injection. That these changes in transport were due to decreased quantities of transported proteins rather than a change in transport rates was shown by the failure of transported [^3H] proteins to appear in serial hypothalamic sections at various times other than those at which such waves would usually have appeared in the untreated animals. The differential effects of reserpine on axonal transport of proteins and glycoproteins in these neurons appear to reflect a re-ordering of the priorities of the cell in response to the changes in the metabolism of catecholamines and their synthetic enzymes caused by reserpine.

198 IMMUNOFLUORESCENT LOCALIZATION OF AXONALLY-TRANSPORTED POLYPEPTIDES. Joel Levine* and Mark Willard*. (SPON:D.G.Amaral). Dept. Anat.&Neurobiol., Wash.Univ.Sch.Med., St. Louis, MO 63110.

We have used immunofluorescent techniques to determine the destination of two axonally-transported polypeptides. These polypeptides, designated 26 and 27, were previously shown to move down axons of rabbit and guinea pig retinal ganglion cells at a velocity of approximately 50 mm per day, i.e. slower than the most rapidly moving group of axonally transported polypeptides, but faster than at least three other groups. Twenty-six and twenty-seven (molecular weights 260,000 and 245,000, respectively) have an electrophoretic mobility similar to that of erythrocyte spectrin, smooth muscle filamin and several other actin-binding proteins.

In order to raise an antibody against 26 and 27, we purified these polypeptides from an extract (0.6M KCL) of guinea pig brain by means of gel filtration and preparative SDS gel electrophoresis and injected the denatured purified polypeptides into rabbits. The resulting antibody was specific for 26 and 27 by the criterion of immunodiffusion; in addition, it precipitated only 26 and 27 from detergent extracts of guinea pig brain and from extracts of the optic nerve and tract containing radioactively labeled transported proteins.

We used the antibody to locate 26 and 27 in frozen sections of nervous tissue by indirect immunofluorescence. In sections of guinea pig spinal cord and brainstem containing axons cut in cross-section, the major fluorescent profiles were ring-like, indicating that 26 and 27 are most concentrated near the axolemma. The fluorescent profiles probably cannot be entirely accounted for by staining of the myelin sheath since large motor neurons displayed a fine rim of fluorescence around unmyelinated structures, e.g. the perikarya, large dendrites and axon initial segment. Immunoprecipitation experiments showed that the 26 and 27 antigens are present in other tissues, including kidney, liver, cardiac muscle and cultured fibroblasts. When cultured guinea pig fibroblasts were stained with the antibody, the fluorescence appeared in linear arrays and bands across the cells. In all cases, the immunofluorescent staining was specific for the 26 and 27 antigens since similar fluorescent profiles were absent when tissues and cells were stained with pre-immune serum, pooled serum from non-immunized rabbits and serum which had been adsorbed with 26 and 27. These results strongly suggest that 26 and 27 are located peripherally in nerve cells, most likely closely associated with the plasma membrane; judging from the results with fibroblasts, they are disposed in a non-uniform, highly organized fashion.

199 A NEW PROCEDURE FOR MICROELECTROPHORETIC DELIVERTY OF HORSERADISH PEROXIDASE. E.R. Marchand and J.N. Riley. Dept. Neurosciences, Univ. California San Diego, La Jolla, CA 92093.

The horseradish peroxidase (HRP) retrograde tracing method has been used extensively as a neuroanatomical tracing technique. One of the major drawbacks of the method is the extensive diffusion of the enzyme when injected into the CNS. While more discrete deposits can be made with the microelectrophoretic procedure developed by Graybiel and Devor (1974), analysis of local connections is obscured by diffusion of the enzyme. We have developed a procedure which allows extremely small deposits of HRP to be made in which there is little secondary diffusion of the enzyme.

The basic elements of the procedure are: (a) dilute solutions (1.5%-5%, w/v) of fluorescein isothiocyanate-conjugated HRP (F-HRP, Polysciences) in 0.1 M Tris·HCl buffer, pH 8.5, are loaded in glass micropipettes having internal tip diameters of 4-12 μm; (b) constant cathodal current (2-4 μA) is passed for 10-60 min to eject the F-HRP. Unlike unlabeled HRP, F-HRP-filled electrodes will carry constant cathodal current for extended periods of time; (c) following survival times of 24-30 hrs, animals are perfused with 5% dextrose in 0.05 M phosphate buffer followed by 2% glutaraldehyde solutions containing 5% and 10% sucrose in the same buffer; (d) frozen sections are processed according to a modified benzidine dihydrochloride procedure.

We have found that the use of F-HRP has several advantages for making electrophoretic deposits compared to unconjugated HRP, such as Sigma Type VI. First, extremely small deposits are made reliably. Deposits as small as 125 μm result in detectable retrograde and anterograde transport. Second, the secondary diffusion of F-HRP is significantly less than with unconjugated HRP, even when the injection sites are of comparable size. Third, the use of a dilute F-HRP solution minimizes any potential problem from leakage along the electrode tract.

Details of the procedure will be presented as well as examples of results obtained with the method is studies of the afferents to the suprachiasmatic nucleus and arcuate nucleus of the hypothalamus of the rat.

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200 THE ANTEROGRADE TRANSPORT OF HRP IS AN ACTIVE PROCESS. M-M. Mesulam and E.J. Mufson. Beth Israel Hospital, Boston, MA.

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

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

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

The validity of the anterograde transport of HRP can be shown in cases with combined intraocular injections of HRP and ^{3}H-amino acids by the similarity of the termination patterns demonstrated by both tracers. Furthermore, in other experiments in rats and monkeys, we have demonstrated all the known efferents of various injection sites with HRP histochemistry. However, these experiments also showed that HRP methods more sensitive than the traditional diaminobenzidine procedure are required for the reliable visualization of efferent neural connections.

In conclusion, the anterograde displacement of HRP is probably as significant and active a process as its retrograde transport. Although caveats are still applicable to its interpretation, it is clear that the active anterograde transport of HRP may be used for tracing efferent connections. (Supported by NS-09211).

201 DIFFERENCES IN GLIAL AND NEURONAL LABELING FOLLOWING ^{3}H-LEUCINE AND ^{3}H-PROLINE INJECTIONS IN THE DORSAL COLUMN NUCLEI OF CATS. H. H. Molinari and K. J. Berkley. Dept. Psychol., Fl. St. Univ., Tallahassee, FL 32306

When ^{3}H-leu is injected into the dorsal column nuclei (DCN) of cats, most large and some small cells are labeled. When ^{3}H-pro is injected, only small cells are labeled and the neuropil labeling between cells becomes heavier and more homogeneous.

In order to characterize these labeled elements more precisely, injection sites from the DCN clusters region were examined using electron microscopic autoradiography. Following ^{3}H-leu injections, most neurons were densely labeled. About 50% of the astrocytes and oligodendrocytes were also labeled with a density about equal to or less than the neurons. Scattered labeling of the rest of the neuropil was related predominantly to dendrites and axons. In contrast, following ^{3}H-pro injections, all neurons were, at best, only lightly labeled while glial cells were heavily labeled. In addition to a marked increase in the density of grains over labeled glia, there was an increase in the percent of labeled astrocytes to about 85%. These increases were reflected in the rest of the neuropil by an increase in the proportion of grains associated with astrocytic processes and myelin and a decrease in the proportion associated with dendrites and axoplasm.

Since many astrocytes are located adjacent to neurons in the DCN clusters region, it was possible that the probability of glial cell labeling might depend upon its proximity to these neurons. At both ^{3}H-pro and ^{3}H-leu injection sites, however, glial labeling did not appear to depend upon the proximity of the glial cells either to neurons, blood vessels or other glial cells.

The reasons for the preferential uptake of ^{3}H-pro by glial, and not by neuronal, elements in DCN is not yet known. Since such an uptake pattern suggests that proline might function as a neurotransmitter, various physiological manipulations which might be expected to alter the labeling patterns of neurotransmitters were performed prior to injecting ^{3}H-pro into DCN. Such manipulations included: a) depolarization of axon terminals in DCN either by increasing the K^{+} concentration or by electrically stimulating the dorsal columns (DC), b) removal of synaptic terminals by ablating DC and the somatosensory cortex, or c) antidromic activation of the cluster neurons. None of these manipulations changed the light microscopic labeling pattern. Thus, it appears that proline probably does not function as a neurotransmitter in DCN. This conclusion is supported by the fact that putative neurotransmitters in DCN, such as glycine, glutamate and GABA, all produced light microscopic labeling patterns unlike that of proline when their tritiated forms were injected into DCN.

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202 COMPARISON OF PROTEINS TRANSPORTED IN DIFFERENT CNS TRACTS. Stephanie S. Padilla*, L. Jack Roger*, Arrel D. Toews, Jeffry F. Goodrum* and Pierre Morell. Biol. Sci. Res. Ctr. and Dept. of Biochem., Univ. of North Carolina, Chapel Hill, NC 27514

A comparison was made of the [^{35}S]-methionine labeled proteins axonally transported in each of three central nervous system tracts in the rat [i.e., 1. retina to superior colliculus, 2. dorsal lateral geniculate body (dlgb) to visual cortex, and 3. substantia nigra to "striatum" (nuc. caudatus, putamen, and nuc. accumbens)]. Injection of the isotope into the retina, dlgb or substantia nigra was followed by sacrifice at time points designed to coincide with the arrival of the fast (3-4 hr), intermediate (16-48 hr), and slow (6-21 days) waves of transport in the respective projection sites. After dissection of the injection and projection sites and solubilization of the tissue, proteins were fractionated on a discontinuous (3% stacking gel) 7.0-15% polyacrylamide gradient slab gel in buffers containing sodium dodecyl sulfate. The [^{35}S]-methionine labeled proteins were visualized by fluorography of the dried slab gels.

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

In contrast, time-dependent changes were observed in the projection sites; each transport wave contains characteristic proteins. In all three tracts the labeled proteins transported in each wave are very similar in electrophoretic pattern quantitatively. The only distinctive differences observed were labeled protein of MW≈27,000 (arriving in the intermediate and slow waves) absent in the superior colliculus but present in the visual cortex and striatum; and a protein MW≈53,000 (arriving in the slow wave) absent in the visual cortex but present in the striatum.

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

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203 **ON THE MECHANISM OF ACTION OF PYRIDINETHIONE ON THE NERVOUS SYSTEM.** Mohammad I. Sabri* and Peter S. Spencer. Dept. Neurol. & Neurosci., Alb. Ein. Coll. Med., Bronx, NY 10461

The zinc salt of 1-hydroxy-2(1H) pyridinethione, commonly known as zinc pyridinethione (ZPT), is a broad spectrum bacteriocide and fungicide used as a antidandruff ingredient in shampoos. Oral administration of ZPT, or percutaneous absorption of the sodium salt of 1-hydroxy pyridine--2-thione (NaPT) results in a syndrome of progressive hindlimb paralysis in rodents. Mendell and Sahenk (1) have shown that ZPT interferes with the axonal transport system and produces distal axonopathy in rats. The biochemical mechanisms involved in the pathogenesis of toxic damage seen in ZPT or NaPT neuroapthy are obscure. We have proposed a working hypothesis that chemically unrelated neurotoxins producing distal axonopathy or "dying-back" might be inhib-iting enzyme systems required for the maintenance of intermediary metabolism (2). A blockade of metabolism at one of those sites would have profound effects on energy supplies, causing nerve cells with their high energy demands to undergo pathological changes.

We demonstrated earlier that neurotoxic hexacarbon compounds such as 2,5-hexanedione and methyl n-butyl ketone inhibit the glycolytic enzymes glyceraldehyde-3-phosphate dehydrogenase (DL-glyceraldehyde-3--phosphate: NAD oxidoreductase; phosphorylating; E.C. 1.2.1.12; -GAPHD)(3) and fructose-6-phosphate kinase (phosphofructokinase; ATP:D-fructose-6-phosphate-1-phosphotransferase; E.C. 2.7.1.11; PFK) (4). Acrylamide and carbon disulfide, which also produce distal axonal degeneration, inhibit glycolysis at the same sites, suggesting a common metabolic block. Data reported in this study indicate that NaPT also inhibits these enzymes in crystalline form as well as in nervous tissue. Thus 0.4mM NaPT inhibited crystalline GAPDH by about 30% after 20 min at 37°C in vitro, and at 3mM concentration about 80% activity was lost. A comparable inhibition was found when crystalline and nervous tissue PFK was preincubated with NaPT. The enzyme activities were preserved by dithiothreitol, suggesting sulfhydryl groups are the target sites of toxic action. By contrast, succinic dehydrogenase (succinate:(acceptor) oxido reductase; E.C. 1.3.99.1) activity in isolated rat brain mitochondria was not affected by 0.4mM NaPT; crystalline lactic dehydrogenase (L--lactate:NAD oxidoreductase; E.C. 1.1.1.27) remained unchanged even after preincubation with 25mM NaPT.

In summary, these data point to the causation of toxic neuropathies of the distal axonopathy type and provide a basis to test the biochemical properties of other compounds which precipitate this disease.

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204 AFFERENT PROJECTIONS TO THE MEDULLA OBLONGATA IN THE CAT. Patricia M. Saxton and J. Siegel. Inst. for Neuroscience and Behavior, Univ. of Delaware, Newark, DE 19711.

The rostral to midcaudal extent of the medulla oblongata is known to mediate the integration of basic physiological processes including inhibitory motor effects and the coordination of the visual motor system. A previous report (Neurosci. Abst., Vol. III (213), 1977) described projections to the rostral gigantocellular tegmental field (FTG) using injections of 0.2 ul horseradish peroxidase (HRP) and incubating with standard diaminobenzidene (DAB) procedures.

We have since been using the more sensitive tetramethylbenzidene (TMB) chromagen for incubations after pressure injections in the FTG both rostrally at the level of the abducens n. and more caudally dorsal to the inferior olivary complex. Cells of origin are labeled bilaterally in the frontal eye fields (precruciate and lateral deep presylvian gyri), in the mesodiencephalic areas including the ipsilateral interstitial n. of Cajal and contralateral n. of the posterior commissure, contralateral superior colliculus, medial and inferior vestibular n., n. praepositus hypoglossi, n. incertus, and ventrolateral regions of the cervical spinal cord. All of these areas are closely associated with the eye-head coordination of the visual motor system. Cells of origin in the following ventral telencephalic areas are seen exclusively with the sensitive TMB procedures; bilaterally in the ventrolateral frontal cortex (ectosylvian, orbital, and coronal gyri), in the ipsilateral central n. of the amygdala throughout its rostral-caudal extent, the associated bed n. of the st. terminalis, and the substantia innominata of the rostral ventral forebrain. Labeled cells are also seen in the caudal diencephalon in the zona incerta, subthalamus, posterior and lateral hypothalamus; in the mesodiencephalic junction in the prerubral fields and ventral tegmental areas; in the mesencephalic tegmentum; and scattered through the reticular formation bilaterally throughout the medulla. This pattern of labeling is suggestive of a ventral tier of limbic behavioral inhibitory regions converging from the forebrain and other anterior regions onto cells of the caudal brainstem.

Both rostral and caudal medullary injections of 0.2 ul label similar areas of the brain, suggesting the diffuse projections from these areas throughout the extent of the FTG. However, preliminary experiments using smaller injections, 0.02 to 0.04 ul, reveal more specificity, particularly in the afferent projections from the superior colliculus and from the central n. of the amygdala and bed n. of the st. terminalis. (Supported by NSF Grant BNS 76-01652.)

205 EFFECTS OF CHANGES IN THE COMPOSITION OF EXTERNAL SOLUTIONS ON STRUCTURE AND AXONAL PARTICLE TRANSPORT IN AMPHIBIAN AXONS. Richard S. Smith. Department of Surgery, University of Alberta, Edmonton, Alberta, Canada. T6G 2G3

Desheathed sciatic nerves from *Xenopus laevis* were incubated for varying periods of time in salines with and without divalent cations. Single axons were sampled from the nerve bundles and these were viewed by darkfield microscopy to detect axonally transported particles. Nerve bundles treated similarly, but undissected, were fixed for structural studies.

Nerves incubated in salines containing (mM): NaCl, 112; KCl, 3.0; CaCl, 2.0; $MgSO_4$, 2.0, and buffer, displayed an active retrograde particle transport and a normal structure for at least 12 h. In solutions with no Ca^{2+} or Mg^{2+} and with 2 mM EGTA the axons showed, over a period of 4h, a slowing or arrest of particle transport concomittant with gross structural changes. The structural changes consisted of collapse of the axis cylinder and a beading of some of the fibers. Ultrastructural examination showed that in these fibers the axolemma contained defects which may represent holes or local deformations. Addition of 2 to 5mM Mg^{2+} to the solution protected the axons from the structural changes and particle transport continued normally.

Nerves in isotonic NaCl or Na glutamate with EGTA showed slight collapse of the axis cylinder at 4h with continued particle transport. In isotonic KCl with EGTA the fibers became beaded by 4h; the axis cylinders did not collapse and particle transport was active. Nerves in isotonic K glutamate with EGTA remained normal in structure and retrograde particle transport was observed at periods up to 24h.

It is concluded that the abnormality of particle transport in nerves bathed in conventional physiological salines from which divalent cations are omitted is secondary to the structural changes in the axons. Solutions free of divalent cations which caused no or minimal structural changes in the axons caused no abnormality of particle transport. This finding cannot, however, be interpreted to mean that divalent cations play no role in axonal transport.

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206 ON THE IMPORTANCE OF PROTEINSYNTHESIS FOR AXONAL TRANSPORT
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These experimental results provide evidence that protein synthesis is necessary for the loading not only of amino acids but also of nonprotein precursors onto the transport system. Dorsal root ganglia of R. catesbeinana were incubated in (32P)-phosphate, (35S)-Met, or (3H)-Leu, and transport was allowed to proceed in the attached sciatic nerve for up to 20 hours. Liquid scintillation analysis revealed that the transport of all three isotopes was blocked when the ganglia were exposed to cycloheximide or puromycin before the isotope was added. If cycloheximide or puromycin were added after the isotope, the amount of activity transported increased as the delay between the addition of isotope and of inhibitor was increased. In the case of (32P), label was found in the TCA soluble and chloroform-methanol fractions.

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

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207 IMMUNOCYTOCHEMICAL LOCALIZATION OF PLASMA PROTEINS IN NEURONAL PERIKARYA.
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It is well known that exogenous protein tracers are transported intraaxonally to neuronal perikarya following their uptake by axonal terminals. Foreign proteins shown to be retrogradely transported include horseradish peroxidase[1] bovine albumin[2] and tetanus toxin[3] and nerve growth factor[4]. This led us to speculate that there may be movement of naturally occurring plasma proteins into axonal terminals and along axons to cell-bodies in the central nervous system. Thus we undertook to search for normal plasma proteins in the cell-bodies of neurons whose axons project outside the blood-brain barrier using the peroxidase anti-peroxidase (PAP) immunocytochemical technique[5].

The medullae of male rats were rapidly frozen in isopentane and liquid nitrogen. Cryostat sections (10 µm) were fixed for 10 minutes in 95% ethanol at room temperature. The following steps were then carried out at room temperature (1) incubation of sections for 30 mins. in rabbit antiserum to whole rat serum at various dilutions in Tris-buffered saline (TBS)(pH7.6) (2) washing in 3 changes TBS (3) application of goat antibody to rabbit IgG diluted 1:10 with TBS for 30 mins. (4) washing as above (5) treatment with rabbit PAP complex diluted 1:50 with TBS (6) washing as above (7) incubation in hydrogen peroxide and 3,3'-diaminobenzidine in Tris-buffer (pH7.6) for 15 mins. (8) finally sections were washed, dehydrated, cleared and mounted.

Reaction product, indicative of the presence of serum protein, was seen in neurons of hypoglossal nucleus, nucleus ambiguous and dorsal motor nucleus of vagus. A diffuse staining was also present in the neuropil of the area postrema, a region in which the blood-brain barrier to protein is known to be absent[6]. Appropriate control sections were unstained confirming that staining by the complete method truly demonstrated the sites of attachment of primary antiserum to antigenic proteins normally present in the serum of rats.

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

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208 FAST AXOPLASMIC TRANSPORT OF TUBULIN AND TRIAD PROTEINS
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Two axoplasmic transport mechanisms are usually considered present in nerve fibers, one giving rise to a fast, the other to a slow downflow. Alternatively, in the "unitary" view, one transport mechanism accounts for both the fast and slow rates with the slow rate resulting from those materials dissociating from the transport mechanism early on (Ochs, J. Physiol. 253:459, 1975). The L5 dorsal root ganglia or ventral cord of rats were injected with high specific activity (^{35}S)-methionine as a protein precursor, and the axoplasmic transport of labeled proteins in their sciatic nerves studied. The nerves, taken after periods of downflow of 4 hours to 33 days, were cut into 5 mm segments, each segment delipidated in chloroform: methanol (2:1) and solubilized in 8 M urea, 5% 2-mercaptoethanol, 1% SDS in 60 mM Tris-HCl, pH 6.8. The solubilized proteins of successive nerve segments were each electrophoresed on SDS polyacrylamide gels, stained, scanned and prepared for fluorautoradiography. The slow transported proteins contained the prominently labeled "triad" neurofilament proteins and α and β tubulin subunits (Hoffman and Lasek, J. Cell Biol. 66:351, 1975). Additionally, a smaller amount of tubulin and "triad" proteins were found to be fast transported in the crest, at close to 410 mm/day. The fast transported tubulin and "triad" proteins amounted to about 5% that of the slow downflow. The tubulin and "triad" proteins in the crest were higher in amount than in the plateau region just behind the crest. The difference in the specific activity of tubulin in the crest and the plateau was more pronounced when a postganglionic ligature was placed 2 hrs. after the injection of ^{3}H-leucine into L7 dorsal root ganglia of cats and sciatic nerves taken after 5-7 hrs. of downflow. The nerve portions corresponding to the crest and the plateau regions were prepared for analysis of tubulin by the temperature dependent assembly-disassembly procedure and the amount of radioactivity in tubulin determined by gel filtration, acrylamide gel electrophoresis and isoelectrofocusing. These procedures also demonstrated that a small amount of tubulin was transported at a fast rate. We consider the fast and slow transport of these proteins to be explained on the basis of the unitary concept. As the transport filaments move down the fiber, most of the tubulin and "triad" proteins dissociate from the transport system leaving only a small amount to continue further down the fiber. The pool of tubulin and "triad" proteins deposited in the axoplasm can then participate in the turnover of the subunits comprising microtubules and neurofilaments respectively. Supported by NIH grant PHS RO1 NS 8706-09, NSF grant BNS 75 03868-A03.

209 GLIA-AXON PROTEIN TRANSFER: A SELECTIVE PROCESS WHICH SUPPLEMENTS THE SUPPLY FROM THE NEURON SOMA.
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In the squid giant axon, there is considerable evidence that some of the proteins synthesized by the adaxonal glial cells are actively transferred into the axon, but little is known about these putative transferred proteins. To obtain a better understanding of their properties and functions, we compared them with the major proteins of axoplasm (AXM) and the proteins synthesized by the glial sheath and the stellate ganglion (representing proteins synthesized principally in the soma). Proteins synthesized by the sheath and ganglion were labeled by incubating 3-4 cm of ligated giant axon and the isolated ganglion in 0.3 ml of artificial seawater with 0.5 mCi ^{3}H-leucine for 60-270 min at 19-21°C. At the end of the incubation, the axon and ganglion were rinsed and the AXM, containing labeled transferred proteins, was extruded. The empty sheath, AXM, and ganglion were each homogenized in solubilization buffer (5% β-mercaptoethanol, 8 M urea, and 10mM Tris-HCl, pH 7.3). All homogenates were centrifuged at 140K x g - 30 min to remove insoluble material and frozen until analyzed by combined iso-electric focusing and SDS-polyacrylamide gel electrophoresis (2D-PAGE).

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

About 19 proteins in the 2D-PAGE pattern of AXM were visibly stained with Coomassie blue, indicating that they are major axonal proteins. Eleven of these stained proteins were coincident with labeled transferred proteins, which suggests that some of the transferred proteins are identical to major axonal proteins. Because the ganglion is the primary source of these major axonal proteins, we then compared the proteins synthesized in the ganglion with the transferred proteins and found that several of the transferred proteins, which were identical to major axonal proteins, were also made in the ganglion. These observations show that some of the major proteins of axoplasm, presumably supplied principally by the soma, are also selectively transferred to the axon from the adaxonal glial cells. Therefore, we propose that glia-axon protein transfer may serve to supplement a specific fraction of proteins supplied to the axon via axonal transport from the soma.

210 HORSERADISH PEROXIDASE DETERMINATION OF LATERAL PREOPTIC AREA INNERVATION. M. J. Wayner, F. C. Barone, S. L. Scharoun*, W. Woodson, Jr.*, J. E. Zrebiec*, R. Guevara-Aguilar and H. U. Aguilar-Baturoni. Brain Research Lab, Syracuse Univ., Syracuse, NY 13210 and Dept de Fisiologia, Facultad de Medicina, U.N.A.M., Mexico.

Horseradish peroxidase (HRP, Sigma Type IV, 25%) was applied microiontophoretically to the lateral preoptic area (LPA) of male hooded rats. After 24 hr animals were perfused intracardially, the brain was removed and sectioned in the frontal plane. Sections were processed with DAB for the brown reaction and were lightly counterstained with cresyl violet. Labeled neurons were identified and photographed under the microscope in light and dark fields and sequential sections were examined. The HRP ejection sites were approximately 500 μm in diameter within the LPA. Anterior to the ejection site a large number of labeled neurons and axons were located in the LPA and medial forebrain bundle (MFB). Many labeled axons and cell bodies were found along the striahypothalamic tract dorsal to the ejection site. Labeled soma were found in the globus pallidus and ventral caudate putamen. In addition, labeled axons and soma were observed along the stria terminalis and diagonal tract of Broca into the lateral septal nucleus. Axons were also labeled in the anterior commissure. Labeled neurons were located ventral and lateral to the ejection site in the olfactory terberculum. Also, cell bodies were labeled in the medial preoptic nucleus, medial to the ejection site. Similar results were observed posterior to the ejection site. Many labeled neurons and axons were located in the LPA and the lateral hypothalamus along the MFB into the mesencephalon. Labeled cell bodies were also identified in adjacent areas including the zona incerta, medial hypothalamic regions, and the amygdaloid nuclei. These results, based on the retrograde movement of HRP, indicate that the primary presynaptic inputs into the LPA are from neurons which are located along the MFB, in the striahypothalamic tract, and lateral hypothalamic area. Other significant inputs occur from the lenticular nucleus and various limbic structures including the septum, amygdala, and hypothalamic areas adjacent to the MFB.

211 THE IDENTIFICATION OF AN AXONALLY TRANSPORTED PROTEIN BY MEANS OF IMMUNE-AFFINITY ELECTRON MICROSCOPY. Mark Willard*, Carolyn Simon*, Celia Baitinger*, Joel Levine* and Pate Skene*. (SPON: B. Stanfield). Dept. Anat. & Neurobiol., Wash. Univ. Sch. Med., St. Louis, MO 63110.

We have used immune-affinity electron microscopy to demonstrate that an axonally transported polypeptide (H) is associated with the 100 Å filaments of neurons. H is a 195,000 molecular weight polypeptide which, together with at least two other polypeptides (45 and 46, molecular weights = 145,000 and 73,000, respectively) is axonally transported at a velocity of 0.7-1.1 mm per day in the rabbit retinal ganglion cells. At least four other groups of proteins are transported at more rapid velocities in these neurons.

In order to determine which organelle contains H, we employed the following strategy: first, H was purified from rabbit spinal cord and an antibody directed against H was obtained by injecting the purified protein into goats. Second, Formvar-coated electron microscope grids were incubated with the anti-H IgG in order to coat the Formvar with the specific antibody. Third, the grids were incubated with polyglutamic acid to block nonspecific adsorbtion sites on the Formvar. Fourth, the resulting "immune-affinity grids" were used to specifically adsorb organelles containing H-antigens from extracts of spinal cord. Finally, the grids were negatively stained and observed by electron microscopy.

The only organelles which appeared to be specifically adsorbed to anti-H coated immune-affinity grids were filaments of approximately 100 Å diameter; in some cases the anti-H IgG-coated grids adsorbed more than 100 times as many of these filaments as did control grids which had been incubated with the same concentration of IgG from a non-immunized goat. The capacity of the anti-H IgG to adsorb filaments onto grids was completely blocked when the IgG was preincubated with H.

These experiments provide immunological evidence that H, a polypeptide which is axonally transported in the most slowly-moving transport group (group V) of the rabbit retinal ganglion cells, is associated with the 100 Å neurofilaments. Group V therefore may be largely concerned with the movement of neurofilament-associated proteins, as was originally proposed by Hoffman and Lasek for a similar transport group (SCa) in the rat sciatic nerve. Our experiments also serve to demonstrate the potential usefulness of immune-affinity electron microscope grids as a general approach for determining the organelle association of certain proteins.

BASAL GANGLIA

212 CAUDATE NUCLEUS INVOLVEMENT IN SPATIAL ORIENTATION. L. Abraham and M. Potegal. Dept. of Physical & Health Ed., Univ. of Texas, Austin, TX 78712 and N.Y. State Psychiatric Inst., New York, N.Y. 10032.

Investigation of rostral vestibular projections has led to the suggestion that the caudate nucleus may mediate egocentric spatial orientation behavior through the use of proprioceptive afference (Potegal, Acta Neurobiol. Exp. 32: 479, 1972). This hypothesis was tested with a spatial task allowing only such proprioceptive cues. Eighteen thirsty male rats (Long-Evans, 300-350g.) were trained to retrace a path leading to a water spout, following passive movement away from the spout in an opaque cart. This task required discrimination of the correct spout from eight spouts equally spaced around a chamber devoid of visual, auditory, tactile, and olfactory directional cues. All animals were trained to criterion in eight days (three consecutive successful trials over a path of five body lengths). The path contained one right angle turn, randomly assigned left or right, and the path from the spout was randomly set at one of three initial directions. Following training the animals were divided into three groups matched for performance. Two groups received bilateral radiofrequency lesions (0.1mm dia.); the third group received no lesion and served as a sham operation control group. Lesions in one group were placed in the posterior portion of the caudate nucleus, and lesions in the second group were placed in the hippocampus, an area implicated in some spatial tasks but which has not been reported to receive direct vestibular or other proprioceptive projections. Following a period of post-operative recovery, all animals were retrained for eight days. Comparison of pre-operative to post-operative scores revealed improved performance for the sham and the hippocampal groups. The caudate group, however, had significant performance deficits. These deficits were evident in maximum path length achieved and in rate of task acquisition. Analysis or righting reflexes (as a measure of vestibularly-based spatial function) revealed the caudate group was significantly different from the other groups in angle of head inclination at landing. Thus the caudate lesions apparently disrupted vestibular orientation. These findings support the hypothesis that the caudate nucleus employs vestibular afference in the performance of spatially-oriented behavior based on passive movement. Also, the involvement of the hippocampus in spatial orientation may be restricted to the use of exteroceptive cues for orientation to the environment, while the caudate mediates the use of proprioceptive cues for egocentric spatial orientation.

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213 MORPHOLOGICAL EVALUATION OF NEOSTRIATAL SLICES USED FOR ELECTROPHYSIOLOGICAL EXPERIMENTS. Il Jin Bak, Ulrich Misgeld* and Molly H. Weiler*. Dept. Neurol. and Pharmacol., Sch. Med., UCLA, Los Angeles, CA 90024.

The refinement of the brain tissue slice technique for neurophysiology has made it possible to measure electrical activity including field potentials and extra and intra-cellular unit activity in the rat neostriatum. Such studies combined with neurochemical experiments indicate that relatively thin (300-400 μm thick) neostriatal slices maintain electrical activity and also a high value of acetylcholine (5.08 $\pm$ .53 n mol (mg protein)$^{-1}$ (n=3) in the slice. On the other hand relatively thick neostriatal slices (700 μm thick) showed limited electrical activity and lower concentration of acetylcholine (2.67 $\pm$ .25 n mol (mg protein)$^{-1}$(n=4) in the slice. A morphological examination of these thin and thick slices was done at the light and electron microscopic levels.

In thin slices, most of the preserved cells were localized in an inner layer which measured an average of 150 μm and was sandwiched between 75 μm thick outer layers which contained more degenerating cells. In the central zone, intact cells varied in size, measuring from 10 μm to 20 μm in diameter. No conspicuous swelling of the cells was noted. Among the numerous well preserved nerve cells there were very few degenerating cells similar to that seen in vivo. In the central zone about 90% of the nerve cells were preserved.

In contrast to the thin slice, thick slices of the neostriatum demonstrated numerous vacuoles throughout the tissue. These thick slices did not display any layering of normal and abnormal tissue. Large vacuoles which probably originated from glial cell processes often engulfed the degenerating nerve cell bodies. Many nerve cells showed signs of degeneration, including cells which had swollen cytoplasm containing large cytoplasmic vacuoles. Such swollen cells measured more than 20 μm in diameter. Among the counted neurons, almost 70% were degenerating and 30% were intact.

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

214 LIGHT AND ELECTRON MICROSCOPIC ANALYSIS OF VARIOUS NEOSTRIATAL NEURONS INTRACELLULARLY LABELED WITH HRP: II. PATTERNS OF AXONAL DISTRIBUTION. G.A. Bishop, H.T. Chang, and S.T. Kitai, Dept. Anat., Michigan State University, E. Lansing, MI 48824.

In our previous study (Preston et al., 1979) the axons of medium spiny neurons, intracellularly stained with horseradish peroxidase (HRP), were traced from their cell of origin in the neostriatum of rats to the globus pallidus (GP) or internal capsule (IC), thus positively identifying this cell type as a striatal projection neuron. In the present study, the same technique has been used to examine specific details of the axons of several neuron types of the rat neostriatum. Recording, injection and histological processing procedures have been described previously (Preston, et al., 1979).

The axons of at least three different neostriatal neurons have been analyzed. The axons of medium spiny neurons arose from the soma or primary dendrite thinning abruptly to approximately 0.5 μm as they entered a fiber fascicle to leave the nucleus. Three to four collaterals arose from the axon within 250 μm of the soma, each of which branched repeatedly forming an extensive collateral network mainly confined to the dendritic field of the cell of origin. No further collaterals were observed to arise from the axons of these neurons until they entered GP where 1-3 fine collaterals arose from the parent axon as it coursed toward the IC. Electron microscopic analysis revealed that these axons are myelinated. A second type of medium spiny neuron (similar to the spiny II of DiFiglia et al. in monkey (1976) had an axon arising from a primary dendrite which entered a fiber fascicle and coursed caudally. It could not be followed beyond the border of the neostriatum. Six collaterals arose from the axon at distances of 100-300 μm from the soma. In contrast to the collaterals of the first type of medium spiny neuron described above, the collaterals of the latter cell branched infrequently, each branch extending for at least 500 μm away from the cell of origin. This axon is currently undergoing electron microscopic analysis. Finally the axon of a large aspiny neuron (soma diameter 35 μm), arising from the soma, became myelinated at a distance of 50 μm. This axon did not appear to give rise to local collaterals. In conclusion, different neostriatal neurons exhibit unique branching patterns of their local collaterals.

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215 AUTORADIOGRAPHIC DEOXYGLUCOSE STUDIES OF APOMORPHINE EFFECTS IN HALOTHANE ANESTHETIZED RATS. Lucy L. Brown, Leslie I. Wolfson,* Dept. Neurol., Albert Einstein Coll. of Med., Bronx, N.Y. 10461.

Previous autoradiographic ^{14}C deoxyglucose (DG) studies (Brown and Wolfson, 1978) found that apomorphine increased glucose utilization (GU) in several extrapyramidal system nuclei: the substantia nigra reticulata (SNR), subthalamic nucleus (STN), globus pallidus and striatum. The substantia nigra compacta (SNC) was apparently unaffected. The localized GU increases were observed in awake rats which were either freely-moving or restrained in a plaster cast. In the present study, anesthetized rats were used to observe the effect of apomorphine on extrapyramidal system nuclei in animals with no movement and no demand for movement. Male Sprague-Dawley rats (250-300 g) were anesthetized with halothane (~1%) mixed with oxygen. Seven animals were injected with apomorphine (I.P., 5 mg/kg) and 5 minutes later were injected with 50 μCi ^{14}C DG through a previously placed femoral vein catheter. The animals were maintained with anesthesia for 30 min., decapitated, and the brains were prepared for autoradiography following the methods of Sokoloff et al. (1977). Seven animals were anesthetized with halothane but not treated with apomorphine. Autoradiographic data (both visual inspection and preliminary densitometric data) show that the anesthesia alone produced a large decrease in GU in the sensorimotor cortex and nucleus accumbens; the additional treatment with apomorphine did not change the GU of these areas. In the extrapyramidal system, the halothane treatment produced a small decrease in GU; however, the additional apomorphine treatment markedly increased GU in SN and STN, but not in globus pallidus and striatum. But of particular interest, in the halothane-treated rats without apomorphine there was a striking increase in GU over awake rats in the SNC region. This increase in the halothane rats was markedly attenuated by apomorphine. Such an attenuation with apomorphine parallels the well-documented decrease in neuronal firing rate of SNC cells with apomorphine (Bunney and Aghajanian, 1973, 1976). This effect of apomorphine to decrease GU in the compacta region only when GU is especially high may reflect a presynaptic potentiation of DA on GABA inhibition. The presynaptic action would be functional only when GABA inhibition is submaximal, in the halothane anesthesia state. However, several other interpretations are possible. In summary, under halothane anesthesia, apomorphine increases GU in the STN and SNR similarly to the awake state, but affects GU in other nuclei and subnuclei differently. Supported by NIH grant NS 09649.

216 EFFECTS OF NEONATAL MEDIAL FOREBRAIN BUNDLE (MFB) LESIONS ON DEVELOPMENT IN CATS. II. PREVENTION OF AMPHETAMINE-INDUCED SLOWING OF THE SPONTANEOUS FIRING RATES OF CAUDATE NEURONS BY NEONATAL MFB LESIONS. N.A. Buchwald, M.S. Levine, C.D. Hull, L. Erinoff* and E. Garcia-Rill. Mental Retardation Research Center, School of Medicine, UCLA, Los Angeles, CA. 90024.

We have recently described some of the behavioral, neurochemical and electrophysiological effects of neonatal MFB lesions made in kittens of 9-21 days of age (Levine et al., Neurosci. Abst. 3 (112): 1977; Levine et al., Neurosci. Abst. 5: 1979). The present experiment assessed the neurophysiological effects of amphetamine treatment on the spontaneous firing rates of caudate neurons and determined if these effects could be blocked by neonatal MFB lesions that interrupt the nigrostriatal pathway and severely deplete the caudate nucleus of dopamine. Juvenile cats of 7-12 months of age received 3 dosages of d-amphetamine sulfate (1,2,4 mg/kg i.p.). Time between injections varied from 10 days to several weeks. The spontaneous firing rates of caudate neurons were measured several weeks to several months after the last amphetamine injection. The cats were then sacrificed and the dopamine content of the caudate nuclei determined. In intact cats amphetamine produced a slowing in the spontaneous firing of caudate neurons. Average interspike interval (ISI) in amphetamine-treated cats was about 3800 msec compared to about 1800 msec in intact cats. In cats that had received neonatal MFB lesions reducing caudate dopamine content by about 75%, amphetamine treatment did not slow the firing rates of caudate neurons. Average ISI for amphetamine-treated cats with MFB lesions was about 2000 msec while that for cats with MFB lesions but no amphetamine treatment was about 2200 msec. This result was not a nonspecific effect of neonatal brain-damage since amphetamine produced a decrease in spontaneous firing rates of caudate neurons in a group of cats that had ventral thalamic lesions as neonates (Mean ISI=3900 msec). In addition to its effects on the spontaneous firing, amphetamine treatment produced a 40% depletion of caudate dopamine in unlesioned cats. These results indicate that in the developing cat, a relatively mild dosage regime of amphetamine produces long-lasting neurophysiological and neurochemical effects. Furthermore, the neurophysiological effects can be blocked by neonatal MFB lesions that interrupt the nigrostriatal pathway.

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217 THE BASAL GANGLIA-TECTAL PATHWAY: ITS ROLE IN VISUALLY GUIDED BEHAVIOR IN THE PIGEON. Nellie M. Bugbee* and William Hodos. (SPON: Roger M. Brown) Department of Psychology, University of Maryland, College Park, MD 20742.

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

The present study investigated the function of this pathway in pigeons. Pre-operatively, subjects were trained to: 1) discriminate visual stimuli differing in color, intensity or pattern; 2) track a target which changed position continuously (ie, peck grain mounted on a revolving drum); 3) track a target which changed position intermittently (ie, to rapidly peck a sequence of response keys which were illuminated in a random order); and 4) peck stationary targets (grain mounted in the same positions as the response keys). Subjects were also examined on a series of neurological tests and assessments of normal locomotion patterns.

Following training, the basal ganglia-tectal pathway was interrupted in 10 subjects by placing electrolytic lesions in SpL; in 8 other subjects, control lesions were placed in nucleus rotundus, nucleus ovoidalis, or nucleus pretectalis.

Subjects with bilateral interruption of the basal ganglia-tectal pathway showed a severe and seemingly permanent deficit in their ability to track targets which changed position in space. In the task in which the target shifted position intermittently, subjects continued to peck the response keys, but took much longer to complete a response sequence than normal or control subjects. In the task in which the target moved continuously, most subjects were totally incapable of pecking the grain even when it moved at the lowest velocity (8cm/sec). These same subjects were able to detect and accurately peck stationary targets, and to discriminate visual pattern, intensity and color. Motor behavior, including flight, walking and head movements were normal in these subjects, as were neurological reflexes related to posture and locomotion. Control subjects were not impaired on any tasks.

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

218 AFFERENT CONNECTIONS TO THE SUBTHALAMIC NUCLEUS OF RAT STUDIED WITH HORSERADISH PEROXIDASE. G.A. Campbell*, M.J. Eckardt*, and F.F. Weight (Spon: G.C. Salmoiraghi). Laboratory of Preclinical Studies, National Institute on Alcohol Abuse and Alcoholism, Rockville, Md. 20852.

Investigations using the ^{14}C-deoxy-D-glucose technique have found that the dopamine agonist apomorphine and d-amphetamine produced the greatest increase in metabolic activity in the subthalamic nucleus (STH) (Wolfson and Brown, Soc. for Neurosci. Abst. 2:510, 1976 and Wechler, et al., J. Neurochem. 32:15, 1979). In addition, iontophoretic administration of dopamine to STH neurons has indicated the presence of neurons sensitive to dopamine (Campbell and Weight, Soc. for Neurosci. Abst. 4:442, 1978). These studies suggest the possibility of dopaminergic input to STH. The current study was undertaken to identify afferent connections to the STH using the horseradish peroxidase (HRP) technique.

Male Sprague-Dawley rats (140-160 gms.) were anesthetized with sodium pentobarbital (60 mg./kg.), placed in a stereotaxic frame, and surgically prepared by removal of skull, dura, and cortex over the electrode site. In some experiments, single-barrel micropipettes were used, while in others four-barrel micropipettes were used to permit passage of a negative holding current through the HRP barrel during descent and withdrawal. Electrodes were inserted stereotaxically using the atlas of Konig and Klippel. Four percent HRP (Sigma Type VI) in pH 8.5 NaCl-Tris buffer was ejected using pulsed positive currents (1 sec. pulses, freq. 0.5 hz, amplitudes up to 500 nA for time periods of 45 to 90 min.). After survival periods of 24 to 36 hrs., the brains were processed histologically using the tetramethylbenzidene technique of Mesulam (J. Histochem. and Cytochem. 26:106, 1978).

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

219 CELLS OF ORIGIN OF DIFFERENT PALLIDAL EFFERENTS ARE SEPARABLE. David A. Carter* and Derek van der Kooy (SPON: F. Coceani) Toronto General Hospital and Department of Anatomy, University of Toronto, Toronto, Canada

Experiments in the cat have demonstrated entopeduncular nucleus (EP) cells projecting to either ventrolateral-ventral anterior nuclei of thalamus (VAL), centromedian-parafascicular nucleus (CM-Pf), lateral habenula (HBL) or nucleus tegmentipedunculopontis (TPP). The reports showed little or no topographical specificity of these neurons within EP. Carter and Fibiger (1978) suggested that the EP projection to TPP in the rat arose from a more ventral site within the EP region than did the projection to the thalamus. In order to re-examine this problem the fluorescent retrograde double labeling technique reported by van der Kooy, Kuypers, and Catsman-Berrevoets (1978) has been utilized. Fifteen rats had 0.1-0.3 ul DAPI - Primuline injected into one EP terminal field and the same amount of Evans Blue injected into another EP terminal field. The EP terminal fields included VAL, HBL, and the TPP region. Injections into HBL labeled virtually all EP neurons within the rostral two-thirds of this nucleus, but resulted in minimal labeling in its caudal aspects. This caudal region, however, contained a circumscribed group of cells labeled after injections into VAL. The TPP region injections resulted in heavy labeling in zona incerta, lateral hypothalamus, and a band of cells which runs ventral to EP to mushroom into the central nucleus of the amygdala. These findings suggest that different pallidal outflows represent functionally and anatomically distinct basal ganglia processes.

220 LIGHT AND ELECTRON MICROSCOPIC ANALYSIS OF VARIOUS NEOSTRIATAL NEURONS INTRACELLULARLY LABELED WITH HRP: I. SOMA-DENDRITIC MORPHOLOGY. H.T. Chang, G.A. Bishop and S.T. Kitai. Dept. of Anat. Michigan State University, E. Lansing, MI 48824.

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

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

In conclusion, we have positively identified the morphological characteristics, at both the light and ultrastructural levels, of at least three different neuronal types in the rat neostriatum and recorded differences in their intracellular responses to extrinsic inputs. (Supported by USPHS Grant NS 14866 and in part by NIH BRSG RR 05772-04).

221 MODIFICATION OF CIRCLING BEHAVIOR IN SHAKER-1 MUTANT MICE WITH HALOPERIDOL AND APOMORPHINE. John M. Cooke* and M.K. Wolf, Deptartment of Anatomy, University of Massachusetts Medical School, Worcester, MA 01605

Shaker-1 mutant mice, like other "circling" mutants, lack inner ear function and have a set of abnormal behaviors including hyperactivity, vertical head-tossing and running in circles (Deol, M.S., Proc. Roy. Soc. B, 145: 206, 1956). Similar circling behavior can be elicited by administering dopaminergic agents to animals with lesions of the nigrostriatal dopamine pathway (Ungerstedt, U., Acta Physiol. Scand. Suppl., 367: 49, 1971). After determining that the baseline level of spontaneous circling activity in individual adult shaker-1 mice was relatively stable and reproducible, we tested their response to haloperidol and apomorphine, drugs which affect CNS dopamine function.

Eleven affected mice were observed in their home cages for a minimum of 15 periods of 3 or 5 minutes. The number of clockwise and counter-clockwise turns were counted manually. The average circling rate ranged from 25 to 58/min. Seven of the eleven mice exhibited a preference for counter-clockwise circling. One mouse circled preferentially counter-clockwise during all 23 observation periods with an average ratio of counter-clockwise to clockwise circles of 128:1.

Shaker-1 mice responded to injections of haloperidol with reduced rates of circling with the maximum suppression approximately 2 hours after injection. Circling was reduced to one-half the baseline rate by 0.075mg/kg, i.p., and abolished completely with doses higher than 0.2mg/kg. Mice injected with haloperidol circled with the same speed and agility observed in uninjected shaker-1 mice but simply spent less time circling. The hyperactivity and vertical head-tossing were also noticeably decreased but no attempt was made to quantify these changes. A few affected mice were injected with valium in doses ranging from 0.2 to 1.2mg/kg. Valium reduced circling but did not abolish it even at the highest dose. In addition, mice injected with the higher doses of valium showed noticeable impairment of muscular coordination during circling.

Apomorphine (0.2mg/kg, i.p.) increased the rate of circling by up to 100% with the maximal effect 45 to 60 minutes after injection. These preliminary results suggest that genetically induced circling behavior may have some features in common with the circling behavior produced by unilateral lesions of the dopaminergic nigrostriatal pathway.
(Supported by NIH Grant Number NS-11425)

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

The purpose of this study was to investigate the organization of corticostriate connectivity in kittens at early postnatal ages. We reported previously that projections from the medial and lateral precruciate cortex in young animals are prominent and differentially organized within the lateral half of the head of the caudate nucleus and dorsal aspects of the rostral putamen. Results from that study were based on Fink-Heimer staining of preterminal and terminal degeneration following selective destruction of the precruciate cortex. It was difficult to identify terminal degeneration with this argyrophilic method in young brains. Therefore, we decided to repeat the study by using the autoradiographic technique. Multiple injections of a mixture of tritiated leucine and proline (54-200 μCi) were made into the precruciate gyrus of twelve kittens, ranging in age from two to twenty-seven days. As with the Fink-Heimer technique, the precruciate projections to the head of the caudate nucleus were prominent and restricted to the lateral half. However, using autoradiographic tracing techniques, we observe that 1) the projections to the head of the caudate nucleus display a patchy distribution, 2) cortical projections extend caudally into the body of the caudate nucleus and remain patchy in character, 3) the projections to the putamen appear to be more prominent than observed with the Fink-Heimer method, and 4) contralateral corticostriate projections are organized similarly to ipsilateral projections. From the striatal areas with the greatest cortical terminal fields, cores of tissue were removed and processed for electron microscopy. The perinatal projections from the precruciate cortex to the caudate nucleus and putamen end mainly on dendritic profiles. With maturation, these projections shift to a predominantly axospinous connectivity.
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223 PHARMACOLOGY OF KAINIC ACID LESIONED RATS. B.I. Diamond, J.E. Comaty and R.L. Borison. Mt Sinai Hosp, Chicago, Il. 60608.

The pathology of Huntington's chorea (HC) involves the degeneration of striatonigral GABA neurons and the loss of intrastriatal cholinergic neurons. It has been demonstrated that the intrastriatal administration of kainic acid (KA) produces a lesion which closely mimics the biochemical changes in HC. Although this animal paradigm provides insight into the neuropathological and biochemical changes in HC, few studies have been conducted to test whether this model may be pharmacologically analogous to the human counter part. We now report studies on the pharmacology of the KA animal model. Subjects were white male Sprague-Dawley rats which received stereotactically placed injections of KA into the caudate-putamen necleus. Animals were rated for stereotyped behavior using a five point rating scale of ascending intensity of behavior. Administration of d-amphetamine (2.0 mg/kg), in a dose per se subthreshold for eliciting stereotypy, evoked moderate stereotypy (score 2.62 ± 0.21) in KA lesioned rats. In contrast, apomorphine (0.5 mg/kg) in a dose that elicits stereotypy, produced only a slight increase in exploratory behavior. In the remainder of our studies, we compared the effects of various drugs on KA lesioned animals receiving d-amphetamine (2 mg/kg). The dopamine receptor blocker haloperidol markedly antagonized stereotypy (0.74 ± 0.08), whereas the weak dopamine blocker clozapine (10 mg/kg) potentiated stereotyped behavior. In studying the cholinergic system, the anticholinergic trihexyphenidyl markedly enhanced stereotypy to 4.12 ± 0.26. The cholinesterase inhibitor physostigmine, at 0.25 and 0.5 mg/kg, decreased stereotypy scores to 1.54 ± 0.16 and 1.00 ± 0.14 respectively. The acetylcholine precurser, choline chloride, failed to affect stereotypy at 75 mg/kg, but lowered stereotypy score to 2.00 ± 0.18 when the dose was raised to 150 mg/kg. In contrast, the muscarinic cholinomimetic arecoline markedly potentiated stereotypy at a dose of 0.1 mg/kg, while failing to affect behavior at higher dosages (0.5 and 1.0 mg/kg). The purported GABA-mimetic baclofen at 10 mg/kg significantly decreased stereotypy, whereas at 5 mg/kg this effect was less marked. The benzodiazepine diazepam, which is reported to have GABA mimetic properties, potentiated stereotypy at 2 mg/kg and markedly antagonized this behavior (1.3 ± 0.11) at higher doses (5 mg/kg). In contrast, isoniazid (20 or 40 mg/kg), which blocks GABA catabolism, potentiated stereotypy. Our results indicate that the pharmacology of the KA animal paradigm closely mimics the human pharmacological responsiveness, and may be used for preclinical drug screening of agents which may be therapeutic in HD.

224 ON THE ROLE OF GLOBUS PALLIDUS AS A SOURCE OF GABA-ERGIC PROJECTIONS TO THE NIGRA. G. Di Chiara*, M.Morelli,* M.L.Porceddu *and M.Del Fiacco* (SPON:R.Collu)
Institute of Pharmacology, University of Cagliari, Italy.

It is well known that long GABA-ergic neurons project from basal ganglia to the substantia nigra. While it is generally aggreed that this pathway originates, at least in part, from the caudate-putamen, there has been much debate on the role of the globus pallidus. Although this role has been reaffirmed by various recent studies, the evidence provided until now is either indirect or derives from non-specific pallidal lesions. In order to reexamine this problem we studied in rats the effect on nigral GAD of pallidal and striatal lesions induced by local injections of kainic acid, which destroys neuronal perikarya but spares axons "en passage". Unilateral intrapallidal injection of kainic acid (0.75 /ug/5 /ug) resulted in complete loss of neuronal perikarya in most of the pallidus, in the MFB area, in the anterior thalamus adjacent to the internal capsule (reticular nucleus) and in the most ventral part of the caudate body. The head and the dorsal 2/3 of the body of the caudate were intact. This lesion resulted in a non-significative decrease (-8%)of nigral GAD on the lesioned side. Kainic-lesions of the dorsal caudate body instead, resulted in a dramatic (-52%) reduction of nigral GAD. Kainate-lesion of the caudate head resulted in a significative (-18%) reduction of nigral GAD. Kainate-lesions of the caudate tail failed to reduce significantly nigral GAD.
These data negate the existence of a GABA-ergic pallidonigral projection but confirm that the caudate head contributes only a minor component to nigral GABA-ergic afferents as compared to the dorsal caudate body.

225 ULTRASTRUCTURE OF THE MONKEY GLOBUS PALLIDUS. Marian DiFiglia, Tauba Pasik and Pedro Pasik. Dept. of Neurol., Mount Sinai Sch. Med., CUNY, New York, N.Y. 10029.

The neuropil of the medial and lateral segments of the globus pallidus was examined in serial sections. Results show that the dendrites of uniform diameter seen in Golgi impregnations (see Pasik et al., this volume) give rise to various types of spines which are usually postsynaptic, having either symmetric or asymmetric contacts. The largest of these elements contain multivesicular bodies, cisterns and a few dense core and small clear vesicles. Others are long and thin, and may penetrate an axonal bag which makes an asymmetric synapse covering the entire surface of the process except for the tip. In these cases there is an additional rod-like density occupying the core of the spine. Most dendrites, including those with varicosities, are covered by synapsing axon terminals and both are encapsulated by glial processes. Some dendrites are not surrounded by axons and only occasionally are postsynaptic.

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

226 LOW CSF BIOPTERIN LEVELS IN INHERITED DYSTONIA. Roswell Eldridge, Adrian Williams*, Robert Levine*, and Walter Lovenberg*, NIH, Bethesda, MD 20502

The hereditary torsion dystonias are a genetically heterogeneous group of disorders whose chief or sole characteristic is dystonia. Although the pathological and neurochemical basis of these dystonic states remains unknown, abnormal dopaminergic mechanisms have been invoked as L-Dopa therapy may be helpful and similar involuntary movements can be reproduced by administration of dopamine receptor blocking agents.

Tetrahydrobiopterin (BH_4) plays a critical role in controlling the rate of tyrosine and tryptophan hydroxylation. This substance, expressed as 'hydroxylase cofactor content', can be reliably measured in the cerebrospinal fluid (CSF) by a radioenzymatic method utilizing a cofactor dependent phenylalanine hydroxylation system. We report CSF BH_4 levels in 7 members of a family with dystonia.

Four sisters developed generalized dystonia between the ages of 8 and 10. Their mother and two brothers had later onset of focal dystonia. The family was of German, English, and Irish extraction and there was no history of consanguinity or of Jewish ancestry. The mother and two daughters were taking low doses of diazepam at the time we obtained CSF but none of the others were on medication. None had had thalamotomy.

Mean CSF hydroxylase cofactor levels for those with generalized dystonia was 3.0 pmol/ml (SE±0.2). This compared to a mean of 20.2 pmol (SE±1.0) for 32 controls of similar age ($p < .001$ by 't' test). For the three family members with focal dystonia, the mean was 7.2 pmol/ml (SE±2.1).

BH_4 is synthesized in many organs of the body including brain but crosses the blood brain barrier with difficulty. Hence, CSF values are likely to reflect brain levels. Since there is no known loss of aminergic neurons in the hereditary dystonias, the low CSF BH_4 observed in these family members may reflect a primary metabolic abnormality rather than neuronal atrophy.

227 EFFECTS OF UNILATERAL DESTRUCTION OF THE NIGRO-STRIATAL DOPAMINE SYSTEM ON LOCAL CEREBRAL GLUCOSE UTILIZATION IN ADULT RAT. A. Ferron*, M.H. Des Rosiers, C. de Montigny, O. Bosler*, T.A. Reader and L. Descarries, Centre de recherche en sciences neurologiques (Département de physiologie), Université de Montréal, Montréal, Québec, H3C 3J7, Canada

In the mammalian brain, dopamine (DA) could influence glucose utilization as a neuro-hormone regulating glycogen metabolism and/or -modulator of functional neuronal activity. To explore these possibilities, local cerebral glucose utilization (LCGU) was measured by means of the [^{14}C]deoxyglucose method, in conscious male albino rats subjected, 5, 15 or 60 days earlier, to pretreatment with desmethylimipramine (25 mg/kg i.p.) and unilateral injection of 6-hydroxydopamine (6-OH-DA, 8 µg in 4 µl) into zona compacta of substantia nigra. Radioenzymatic determinations of DA content in neostriatum indicated 84% and 90% reductions on the side of the lesion, 5 and 15 days after 6-OH-DA, respectively. Histofluorescence examination revealed that this depletion was imputable to almost complete destruction of the ipsilateral nigro-striatal DA system. However, at days 5, 15 and 60, there was no side to side difference in LCGU in caudate-putamen. In contrast, striking differences were observed in globus pallidus (GP) and habenula (HA). Indeed, 15 days after 6-OH-DA, LCGU was 50% higher in GP, and 30% higher in HA, on the side of the lesion. Whereas at 5 days, an increase was already present in HA, but not in GP, none was any longer detectable after 60 days, in either structure. Twelve to 16 days after 6-OH-DA, extracellular recording of GP neuronal activity combined with glutamate microiontophoresis was carried out in urethane anesthetized as well as unanesthetized decerebrate animals. No significant side to side differences in the number and firing rate of spontaneously active units or mean threshold current of glutamate needed to activate quiescent neurons were detected in GP. Since GP is the major output of the richly DA-innervated caudate-putamen but receives little DA input, its increased rate of glucose utilization represents an effect at distance, probably due to suppression of inhibitory influences mediated by DA in neostriatum. However, in the absence of concomitant changes in neuronal firing, it cannot be ascertained at present whether such a temporary effect on LCGU actually corresponds to an increase in oxidative metabolism or rather reflects qualitative shifts in the glycolytic cycle. Radioautographic identification of the cellular elements in which deoxyglucose-6-phosphate is accumulated might help to resolve these issues.
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228 PALLIDAL NEURONS BRANCHING TO THE THALAMUS AND TO THE MIDBRAIN IN THE MONKEY. M. Filion and C. Harnois*. Lab. Neurobiologie and Dept. Physiologie, Fac. Méd., Univ. Laval, Québec, Qué., Canada, G1K 7P4.

According to anatomical studies, the pallidothalamic projection is considered to be more important than the pallidotegmental projection. In a previous study[1], using antidromic activation of entopeduncular neurons in the cat, we have shown that at least 46% of these neurons branch to the thalamus and to the midbrain. We have undertaken a similar study in the monkey. Stimulation techniques were improved to activate the great majority if not all pallidofugal fibers in a given area, while minimizing current spread. Squirrel monkeys were anesthetized with sodium pentobarbital. Pallidal neurons were recorded extracellularly. Stimulation electrodes were separated by 1.5 mm and arranged in frontal stereotaxic planes. Two electrodes were placed in the rostral part of the ventral anterior thalamic nucleus and 3 others were placed 1.5 mm more caudally. A similar array of 3 electrodes was placed at the level of the midbrain pedunculopontine nucleus. Pulses of current were delivered alternatively between pairs of neighboring electrodes in the same plane and in each polarity. The arrays were moved in 0.5 mm steps in the vertical plane to map sites where pallidal neurons could be activated antidromically with weak stimulation current. Antidromic responses, identified according to criteria described previously[1], were recorded from nearly every neuron histologically located in the medial globus pallidus, but not from those located in the lateral globus pallidus. Out of 90 neurons, 76 (84%) responded to stimulation of both the thalamus and the midbrain. In these cases, the antidromic responses to successive stimulation of the two sites collided at stimulation intervals longer than the difference of the latencies of the responses plus a refractory period. This indicates that the stimulation activated two branches of a parent axon, and not the same branch at two sites. Similarly, it was shown that several pallidofugal fibers branch to at least two thalamic planes separated by 1.5 mm. Eleven neurons responded only to the thalamus and 3 responded only to the midbrain. Therefore, the great majority of medial pallidal neurons branch to both the thalamus and the midbrain.

(Supported by the MRC).

1- J. Comp. Neur. (1978) 181: 763-780.

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

Previous studies from our laboratory have implicated that suppression of caudate nucleus (CN) spontaneous activity by morphine (MO) may be achieved partly via a direct activation of the nigrostriatal dopaminergic pathway (Lee, Wong, and Chan, Neuropharmacol. 16:571, 1977) and partly through inactivation of a population of inhibitory neurons in the striato-nigral feedback loop (Finnerty and Chan, Neurosci. Abst. 4:443, 1978). Neurochemically, these inhibitory neurons, believed to be located in the substantia nigra (SN) zona reticulata, have been proposed to suppress the zona compacta (SNC) cells utilizing glycine as the transmitter agent. This work represents a further analysis of the mechanism(s) of suppressive action by MO on CN activity and the role of glycine in this process.

Experiments were performed on Charles River rats lightly anesthetized with sodium pentobarbital (50 mg/kg, i.p.). Spontaneous unitary activity was recorded from the CN via stereotaxically placed tungsten microelectrodes. Local microinjection to the SN, at a volume of 1 µl, was performed by means of a stereotaxically placed 27-gauge syringe needle attached to a microinjection device. Systemic injections were administered via a cannulated jugular vein.

MO (200 µg/kg) microinjected into the SN was found to suppress the spontaneous CN activity, which was effectively reversed by naloxone (1 mg/kg, i.v.). These results confirmed that MO may elicit its effects by directly activating the opiate receptors on the SNC cells.

Intranigral microinjection of the glycine antagonist, strychnine (STRY, 1 µg/kg), elicited two types of response in the CN in different animals. There was either an increase or a decrease in its spontaneous activity lasting 3-5 min. The presence of a tonic inhibition exerted on the SNC cells by neurons using glycine as the transmitter is at least partially implicated.

When MO (5 mg/kg) was administered intravenously immediately following the intranigral microinjection of STRY, an interesting phenomenon was unveiled. The suppressive effect of the opiate on CN activity was either abolished or greatly minimized.

It is concluded that MO may suppress CN spontaneous activity by directly activating the SNC cells. The mechanism(s) whereby MO effect is antagonized by STRY remains to be elucidated. Possibly, it may involve the inhibitory neurons in the striato-nigral feedback loop and may engage an interaction between glycine and MO in the SN.

(We acknowledge the generous supply of morphine sulfate by Eli Lilly & Co. and Naloxone HCl by Endo Laboratories.)

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

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

Lesions of the nucleus accumbens (N.Acc.) produced behavioral manifestations which tend to classify it functionally as a component of the limbic system. However, on the bases of neurogenesis and development, the N.Acc. more closely resembles the striatum. Current concepts hold that this nucleus represents a bridge between the limbic system and the basal ganglia since it has in common features typical of each. Anatomical studies have revealed efferents from the N.Acc. to the substantia nigra (SN) in monkey and rat. Recent radioautographic studies have demonstrated a projection from the N.Acc. to the SN pars reticulata (SNor) in cat. We have previously reported that stimulation of the N.Acc. evokes either inhibition or brief excitation followed by inhibition of extracellularly recorded spontaneously active or driven units in SNpr. The inhibitory period reached a peak 50-80 msec following the onset of N.Acc. stimulation and persisted for approximately 300 msec.

Pharmacological interventions designed to characterize the nature of this inhibition were performed in α-chloralose anesthetized, immobilized, and artifically ventilated cats. Stainless steel microelectrodes were used to record extracellular spontaneous or sural evoked unit activity in SNpr. Brief trains of stimuli were delivered to the N.Acc. and caudate nucleus (CN) approximately 80 to 100 msec preceding the sural shock if the unit was normally quiescent. Bicuculline, 0.01 to 0.1 mg/kg iv, consistently antagonized N.Acc. and CN elicited suppression of SNpr cells. Diazepam 0.5 mg/kg effectively reversed bicuculline actions. Inhibition of spontaneously active units as well as sural driven units was blocked by bicuculline. In contrast, strychnine, 0.1 to 0.5 mg/kg iv, failed to affect inhibition arising from either N.Acc. or CN. In one instance, strychnine enhanced CN induced suppression of a SNpr cell. These data suggest that, analogous to the GABA-ergic striatonigral pathway, inhibition of SNpr cells arising from N.Acc. utilizes GABA as its transmitter.

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231 GABA RECEPTORS IN RAT SUBSTANTIA NIGRA: CHANGES IN RESPONSE TO LESIONS AND CHRONIC DRUG TREATMENT. K. Gale* (Spon: D. Stoff) Dept. Pharmacol., Georgetown U. Sch. Med & Dent, Wash. DC 20007

Changes in ^{3}HGABA binding in substantia nigra (SN) were examined after 1)discrete electrolytic lesions of striatonigral projections 2) hemitransections anterior to SN 3) hemitransections posterior to SN and 4) chronic administration of haloperidol or chlorpromazine. Specific binding of ^{3}HGABA was determined in a frozen and Triton X-100 treated crude synaptosomal-mitochondrial membrane fraction. Both high (K_D=20 nmolar) and low (K_D=120 nmolar) affinity binding sites were observed; the high affinity sites accounted for >50% of the total specific binding in SN. This was in contrast to cerebral or cerebellar cortex where high affinity sites account for < 15% of the total specific ^{3}HGABA binding. 3 weeks after striatonigral electrolytic lesions (which decreased GABA content in SN by > 65%) high affinity ^{3}HGABA binding in SN was increased by 60%, with no significant change in amount of low affinity binding sites. Hemitransections anterior to SN resulted in a similar increase in ^{3}HGABA binding in SN. In contrast, hemitransections posterior to SN resulted in a 30-40% decrease in specific ^{3}HGABA binding in SN when measured at 3 weeks postop. Chronic (8 weeks) treatment with haloperidol (1 mg/ kg/day) or chlorpromazine (20 mg/kg/day) did not alter GABA receptors in caudate-putamen but caused a significant increase (35%) in ^{3}HGABA binding in SN. An increase in nigral GABA receptors may compensate for a decrease in the function of striatonigral GABAergic neurons. This in turn may be an indirect effect of dopamine receptor blockade in the caudate. Such a compensatory mechanism may play a role in the development of tolerance to the haloperidol-induced activation of tyrosine hydroxylase.

The decrease in ^{3}HGABA binding after transections posterior to SN suggests the presence of GABA receptors on neurons connecting the SN with caudal brain regions and may include a population of GABA receptors located on serotonergic axon terminals in SN (based on studies with 5,7-dihydroxytryptamine lesions of the raphe). Nigral GABA receptor stimulation (by unilateral intranigral injection of muscimol) induced postural asymmetry and contralateral circling even after complete hemitransections between the SN and forebrain, indicating that GABA receptors located on projections descending from the nigra may be important for motor control. Thus, we postulate that a modification of nigral GABA receptor activity, produced by drug- or lesion-induced changes in striatonigral neural function, may have impact not only on dopaminergic and other ascending pathways but also on descending projections from SN.

232 EFFECTS OF CAUDATE STIMULATION ON INTRACELLULARLY RECORDED VA-VL NEURONS. E. Garcia-Rill. Dept. of Anatomy, University of Arkansas for Medical Sciences, Little Rock, AR 72201.

A recent study (Garcia-Rill, et al., Brain Res. 1979) described a differential distribution of motor cortex (MCX) projections to the caudate nucleus (CN). The medial MCX (axial representation) projects to the entire lateral half of the head of the CN. The lateral MCX (forelimb representation) projects to a circumscribed area adjacent to the internal capsule and within the projection area of medial MCX. The present study was undertaken to determine whether a differential distribution of CN efferent projections is maintained (via the globus pallidus) at the level of the VA-VL nuclei of the thalamus. Post-synaptic potentials were recorded intracellularly from VA-VL neurons in locally-anesthetized, paralyzed cats. Stimulating arrays were placed medially in the CN (within the medial MCX projection area), in the lateral CN (within the lateral MCX projection area) and in the internal capsule (IC). Fastigial, interpositus and dentate nuclei were also stimulated, as well as medial and lateral MCX. CN stimulation elicited responses in 13% of VA-VL neurons. All of these neurons showed EPSP responses, followed by IPSPs in most cells. (Mean latency 15.9 + 2.3 (S.E. of the mean) ms; Mean threshold 0.17 + 0.07 mA). All of these responses were elicited by medial CN stimulation and could not be replicated by lateral CN, MCX or IC stimulation. IC stimulation produced no response, pure IPSPs or lower amplitude EPSPs (1 case) in this proportion of VA-VL neurons. However, 72% of the total sample of VA-VL cells showed EPSP-IPSP or IPSP responses to IC stimulation. (Mean latency of EPSP or spikes 1.7 + 0.43 ms, mean latency of IPSPs 10.0 + 1.7 ms; mean thresholds 0.14 + 0.03 mA for EPSPs and 0.09 + 0.01 mA for IPSPs. Cerebellar stimulation produced EPSP-IPSP sequences in 27% of VA-VL neurons. (Mean latency 5.6 + 1.8 ms, mean threshold 0.12 + 0.03 mA). The interpositus was found to preferentially influence over half of these neurons, while the rest were evenly divided between preferential fastigial or dentate responses. To date, no VA-VL neurons responding to CN stimulation responded at short (< 8 ms) latency to cerebellar stimuli. VA-VL neurons responding to CN stimulation were preferentially located anteriorly, in medial VA and medio-dorsal VL.

This study reveals a preferential excitatory influence from medial CN (which in turn receives axial and proximal limb MCX input) to the anterior VA-VL nuclei. Whether lateral caudate (or pure inhibitory) influences are "filtered" by the entopeduncular nucleus remains to be determined.

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233 ELECTROPHYSIOLOGICAL CHARACTERIZATION OF A PROJECTION FROM PEDUNCULOPONTINE NUCLEUS TO ENTOPEDUNCULAR NUCLEUS IN THE CAT. Teresa Gonya-Magee and Marjorie Anderson. Depts. of Physiol. and Biophys and Rehab. Med., University of Washington, Seattle, WA. 98195.

Earlier horseradish peroxidase studies in the monkey (Clausing, DeVito, and Anderson, 1977) demonstrated a potential synaptic input to the globus pallidus from neurons in the pedunculopontine nucleus (PPN). HRP injected into the globus pallidus area in cats also causes retrograde labeling of neurons in PPN. Electrophysiological experiments were designed to determine the synaptic effect of PPN stimulation on neurons in external globus pallidus (GPe) and entopeduncular nucleus (ENT), the feline homologue of the internal globus pallidus.

Chloralose-anesthetized cats were prepared for electrophysiological recording from GPe and ENTO cells, and arrays of tungsten electrodes were inserted stereotaxically for stimulation of the ventrolateral thalamus (VL) and PPN area of the brainstem. Glass micropipettes filled with potassium acetate and fast green were used for recording and marking. Antidromic activation from VL was used to determine the location of ENTO. Stimulation of PPN with trains of pulses of 300 uA or less evoked action potentials orthodromically (OD) in ENTO neurons at latencies of 6-9 msec. Antidromic (AD) responses also were noted in some neurons at latencies of 1.6 - 4 msec. Extracellular recordings from GPe neurons showed OD or AD excitation at latencies similar to those for ENTO cells. Stimulation at different positions in the midbrain-pontine tegmentum showed that excitation of ENTO and GPe neurons was evoked at lowest stimulus intensities from the PPN area, corresponding to the region containing labeled cells in HRP experiments. Both OD and AD excitation were obtained from the same general brainstem area.

Our results suggest that PPN is a source of excitatory input to the entopeduncular nucleus and globus pallidus and may be an important determinant of the activity of those neurons.

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234 NEURONS IN THE RAT SUB-THALAMIC NUCLEUS SEND AXON COLLATERALS TO BOTH THE SUBSTANTIA NIGRA AND GLOBUS PALLIDUS. Toshi Hattori and Derek van der Kooy. Dept. Anat., Univ. of Toronto, Toronto, Ont., Canada.

The rat sub-thalamic nucleus (STN) is a densely packed group of deeply staining (Nissl) neurons, situated immediately dorsal to the cerebral peduncle in the caudal diencephalon. Moving from rostral to caudal, the STN shrinks in the dorsoventral plane and expands in the mediolateral direction. Both multipolar and fusiform cells are seen in the STN, and they range from 10u - 25 u in diameter. Smaller size cells are more likely to be found medially in the STN. The total number of neurons in the STN on one side of the brain was estimated to be 9,500.

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

235 THE RELATIONSHIP BETWEEN THE ISLANDS OF CALLEJA AND THE STRIATO-PALLIDAL COMPLEX: A HISTOCHEMICAL STUDY. Joanna M. Hill* and Robert C. Switzer, III. Laboratory of Brain Evolution and Behavior, National Institute of Mental Health, Bethesda, MD 20205

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

The striatal component of the olfactory tubercle, as revealed by acetylcholinesterase (AChE) stained preparations, is distinct from the iron-positive areas, and extends from the superficial strata through cell bridges of Heimer to join the main body of the striatum. The ICal are located within this area and also contain AChE.

The intimate spatial relationship with the ventral pallidum and striatum, as well as the similarities in histochemistry to both of these areas, suggest a role for the ICal in striato-pallidal functions.

236 INCREASED DOPAMINE RECEPTOR SENSITIVITY AFTER ESTROGEN TREATMENT. R.E. Hruska and E.K. Silbergeld. Experimental Therapeutics Branch, NINCDS, NIH, Bethesda, MD 20205.

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

Behavioral responses were measured after the injection of 6-hydroxydopamine (6-OHDA). Male rats were injected into the caudate nucleus (A: 7.9, L: 2.6, V: -0.4 mm; Konig and Klippel) with 20 µg 6-OHDA in 2 µl of saline containing 1% ascorbate. One and two weeks after unilateral 6-OHDA injection, the transport of dopamine was reduced to 36% of the uninjected side, while choline transport was unaffected (101% of control). The rats rotated intensely (30-40 rotations/5 min) to the ipsilateral side when administered d-amphetamine (3 mg/kg). The rats also rotated to the contralateral side (10-15 rotations/5 min) when administered apomorphine (5 mg/kg). Estrogen treatment increased the duration of the rotation to d-amphetamine, suggesting an increased sensitivity of the dopamine receptors.

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

These results indicate a direct effect of estrogens on dopamine receptor sensitivity as measured both biochemically and behaviorally. Biochemically, there is an increase in the number of dopamine receptors, and behaviorally, there is an increased response (rotation or stereotypy) to d-amphetamine treatment. These findings suggest an important interaction between estrogens and dopamine, which is of relevance to therapeutics and disorders involving dopamine.

237 A REGIONAL ANALYSIS OF DOPAMINE-INDUCED DEVIATION IN THE ADULT RAT. J. N. Joyce and C. Van Hartesveldt. Psychol. Dept., Univ. of Fla., Gainesville, FL 32611

An imbalance in dopaminergic (DA) activity between the caudate-putamen nuclei (CPU) is widely believed to be reflected in postural asymmetry. However, although the CPU in the rat is regionally differentiated with respect to neuroanatomy, neurochemistry, and some behaviors, the possibility that the CPU might be regionally organized with respect to DA-induced postural asymmetry had not previously been explored. In addition, since prefrontal cortex receives a heavy DA innervation it might also play a role in DA-induced asymmetries. We implanted male Sprague-Dawley hooded rats intracerebrally with permanent cannulae and administered both DA (25 ug in .25 ul) and the vehicle alone (.9% saline, .25 ul) unilaterally on separate occasions. A series of sites throughout the CPU, the prefrontal cortex, and the tissue surrounding the CPU were tested. After injection the rats were placed in a circular enclosure in a sound-attenuated chamber and observed via a television monitor. The amount of time spent in an asymmetrical posture, time spent grooming on each side, number of rotations, and locomotor activity were measured. Regional differences within the CPU were found with the vehicle alone: injection of saline into the ventral CPU produced a strong ipsilateral deviation, while injection of saline into the medial-dorsal regions had little effect. Injection of DA into both regions resulted in contralateral deviation, but while the absolute contralateral deviation scores for ventral injection were less than those for the medial-dorsal region, the difference between the effect of the saline vehicle and the effect of DA injection was greater for the ventral region. Injection of DA into prefrontal cortex also induced contralateral deviation; DA injections into other areas surrounding the CPU did not produce postural asymmetries. For both the CPU and prefrontal cortex the grooming data parallel those for postural deviation. No activity changes were produced by DA injection at any site; circling was infrequently seen.

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

238 FUNCTIONAL PROPERTIES OF DORSAL RAPHE-SUBSTANTIA NIGRA PROJECTIONS IN THE CAT. Athanasios B. Karabelas*, and Dominick P. Purpura. (SPON: G.D. Pappas). Dept. of Neuroscience, Rose F. Kennedy Center for Research in Mental Retardation, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

Intracellular recordings were obtained from substantia nigra (SN), pars compacta (p.c.) and pars reticulata (p.r.) neurons identified by antidromic stimulation of medial forebrain bundle (MFB) and thalamic VL-VM nuclei respectively in barbiturate-anesthetized, flaxadilized cats. Stimulating electrodes were also placed in the dorsal raphé nucleus (DRN) which was approached by direct visualization of upper brain stem following limited cerebellectomy. Thalamic stimulation elicited 0.8-2.8 ms latency spike potentials in nigral neurons whereas MFB stimulation evoked 1.6-2.3 ms latency spikes. Criteria for antidromic invasion were met by findings of constant latency at threshold firing level, collision with preceding spontaneous discharges and absence of underlying EPSPs. Stimulation of DRN evoked orthodromic activity in all antidromically identified nigrostriatal neurons and 80% of identified nigrothalamic neurons. Orthodromic response latencies ranged from 1.8-6.0 ms. Most orthodromic responses recorded intracellularly consisted of EPSPs with 1-3 superimposed spikes. Although IPSPs were not observed with intracellular recording DRN stimulation frequently inhibited SN extracellular unit discharges as well as elicited diphasic excitatory-inhibitory sequences. As a further aid to identification of target cells in SN HRP-staining of impaled neurons was employed. The electrophysiological data obtained in this study establish the existence of short latency excitatory synaptic inputs from a sub-population of neurons in the dorsal raphé nucleus to cells in both SN p.c. and SN p.r. While there is uncertainty concerning monosynaptic inhibitory projections from dorsal raphé to nigral neurons multisynaptic inhibitory effects of raphé stimulation on SN cells are clearly demonstrable.

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

Choline acetyltransferase (CAT) activity, as a marker of cholinergic neurons has been measured in the neocortex after microinjections of kainic acid into the caudate nucleus and globus pallidus of the rat. Anterior, middle and posterior neocortical regions were dissected (Kelly & Moore, 1978, Exp. Neurol. 61, 479) and analyzed separately. Five days after injection of kainic acid (1.25 µg in 1 µl of saline, 0.25 µl/min) into the caudate nucleus CAT was significantly decreased in the anterior and middle neocortical regions. The amount of kainic acid spreading to the neocortex was assessed by including 0.1 µCi of [3H] kainic acid in the injection. Five minutes after the end of the injection, radioactivity in the neocortex was less than 10% of that remaining in the striatum. Direct injection of kainic acid (1.25 µg in 1 µl of saline over 100 sec) into the neocortex did not affect neocortical CAT though experiments with [3H] kainic acid showed greater cortical retention of radioactivity by this route than by intracaudate injection. Thus spread of kainic acid up the injection cannula is not responsible for the decrease of neocortical CAT. With smaller injections of kainic acid (0.625 µg in 0.5 µl, 0.25 µl/min) into the caudate nucleus or globus pallidus, decreases of CAT in anterior and middle neocortex were observed only after the pallidal injections. The results suggest that the direct projections to neocortex from neurons in the region of the globus pallidus, demonstrated by the horseradish peroxidase technique (Divac, 1975, Brain Research 93, 385), are cholinergic and sensitive to kainic acid.

240 IMMUNOHISTOCHEMICAL LOCALIZATION OF CHOLINE ACETYLTRANSFERASE IN VARIOUS REGIONS OF HUMAN CNS. Hiroshi Kimura*, Edith G. McGeer, Patrick L. McGeer and Frank Peng. Div. Neurol. Sci., Univ. of B.C., Vancouver, B.C., Canada V6T 1W5

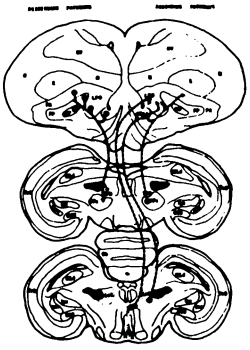
Antibodies prepared in rabbits against purified choline acetyltransferase (CAT) from human neostriatal tissue have been employed to localize CAT-containing neurons in the human CNS using the indirect (PAP) immunohistochemical method. Normal human brains, obtained from various corners' offices within 0.5-1 hr post-mortem, were removed from the skull and perfused via vertebral arteries with 6 l of 5% paraformaldehyde and 0.1% glutaraldehyde. Various brain regions were dissected out and post-fixed with 4% paraformaldehyde for 8 hr followed by washing with 15% sucrose in PBS for 48 hr. All steps were carried out at 2-4°C. Cryostat sectioned (20 µm) tissues were first treated with 0.05% Triton X-100 in PBS. Fab fragments of IgG from normal rabbit serum or from the rabbit anti-human CAT were then diluted with 0.01% Triton X in PBS and applied to the tissue for PAP immunocytochemical procedures. Specific staining of neuronal cell bodies was found in the caudate nucleus, putamen, globus pallidus, deeper layer of the cerebral cortex (the main motor area), and ventral horn motor neurons of spinal cord. CAT positive fibers were also found in the regions mentioned above as well as in the cerebellum, hippocampus, inferior olive and substantia nigra. The CAT specific staining obtained in the human spinal cord, cerebellum and hippocampus was in agreement with previous reports using other species of animals. In the neostriatal area, however, at least two different kinds of positively staining cell bodies were observed. These included some large or giant multipolar cells (> 25 µm) which possess large, roughly spherical or ovoid dendritic trees and were at one time thought to be efferent neurons but are, according to more recent evidence, very probably interneurons. Small multipolar cells (< 10 µm) with round shaped somata were also stained. The globus pallidus contained a high density of long positive fibers and a rather scattered population of large multipolar neurons.

241 DEMONSTRATION OF RECIPROCAL CONNECTIONS BETWEEN THE AVIAN PALEOSTRIATUM AND THE MIDBRAIN TEGMENTUM. Cheryl A. Kitt and Steven E. Brauth, Dept. of Psychol., Univ. of Maryland, College Park, Md.

In pigeons, equal parts mixtures of ^{3}H-leucine and ^{3}H-proline were placed in the dorsomedial paleostriatum augmentatum (PA) or lobus parolfactorius (LPO) of the paleostriatal complex. Light microscopic analysis revealed heavy silver grain accumulations over the central and dorsal portions of the pars compacta of the nucleus tegmenti pedunculo pontinus (TPc) and over the anterodorsal pars disseminata et dorsalis (TPD) of the nucleus tegmenti pedunculo pontinus.

Injections of horseradish peroxidase (HRP) into the TP area of the midbrain confirmed the existence of projections to this region from PA and LPO neurons. In addition, labeled cells were also observed in the paleostriatum primitivum (PP) and nucleus intrapeduncularis (INP) as well as the nucleus accumbens (Ac).

HRP injections placed in PA, LPO and Ac revealed that these neurons receive projections from the dopaminergic cells of TPc, the norepinephrine-containing cells of the locus ceruleus (LoC) and the serotonergic cells of the midbrain raphe (R). As found in prior studies PP and INP appear not to receive monoaminergic innervation. Taken together, these results indicate the existence of reciprocal connections between neurons of the avian paleostriatum and midbrain tegmentum comparable to those existing in mammalian forms between the striatum and substantia nigra area.



Projections from PA, LPO and Ac upon TPc and TPD appear comparable to projections of neurons in the mammalian striatal complex upon A8, A9 and A10 areas of the brainstem. TPc appears comparable to A8 and A9 and may contain components of A10 as well. The TPD regions appear most comparable to the pars reticulata of the mammalian substantia nigra. PA, LPO and Ac thus appear comparable to cells of the mammalian striatum while the larger cells of PP and INP appear most comparable to portions of the mammalian pallidal complex.

Interconnections of avian paleostriatal neurons and the midbrain tegmentum are depicted in this figure.

242 ALTERED GLUCOSE UTILIZATION OF BASAL GANGLIA CIRCUITRY FOLLOWING DEGENERATION OF ASCENDING DOPAMINERGIC NEURONS, AND APOMORPHINE-INDUCED REVERSAL. Michael R. Kozlowski* and John F. Marshall. Dept. of Psychobiology, Univ. of California, Irvine, CA 92717.

The 2-deoxy-D- ^{14}C glucose (2-DG) autoradiographic technique was used to determine (1) the effect of unilateral destruction of ascending dopamine (DA)-containing neurons on hemispheric glucose incorporation, and (2) the extent to which the DA agonist apomorphine (APO) reverses these denervation-induced changes.

Rats were given unilateral injections of 6-hydroxydopamine (6-OH-DA; 8 ug/4 ul) into the left ventral tegmental area (VTA) or lateral hypothalamus (LH), 30 min. after DMI (15 mg/kg, i.p.), which produced a 98% loss of ipsilateral neostriatal DA. One week postoperatively, 6-OH-DA-treated rats were given ^{14}C-2-DG (150 ug/kg, i.v.) 5 min. after receiving i.p. APO (0.25 mg/kg), its vehicle, or left striatal APO (5 ug). After 45 min. the rats were killed and the brains examined using X-ray autoradiography.

Six-OH-DA injected into the left VTA or LH decreased glucose incorporation into ipsilateral DA-innervated forebrain structures (neostriatum, amygdala, nucleus accumbens (NAS), olfactory tubercle (OT)). Labeling of structures receiving afferents from the neostriatum (globus pallidus (GP), entopedunclear nucleus (EN), substantia nigra pars reticulata (SNr)) as well as thalamic structures receiving afferents from EN or SNr (ventromedial nucleus (VM), ventral anterior lateral nucleus (VAL), far lateral habenula (FLH)) was increased.

Systemic or intrastriatal administration of APO attenuated or reversed most of these asymmetries. The left neostriatum was more heavily labeled, and other DA-innervated forebrain regions (amygdala, NAS, OT) showed symmetric labeling. APO administration also attenuated the denervation-induced increase in GP labeling. However, the APO further exaggerated the labeling in EN and SNr. A decreased labeling of thalamic structures receiving basal ganglia afferents (VM, VAL, FLH) was also observed. Due to the similarity in the pattern of changes observed after i.p. or striatal APO, its effect appears largely attributable to its action on denervated neostriatal cells. In confirmation, APO (i.p. or intrastriatal) increased the labeling of the ventral tip of the internal capsule, where axons of neostriatal efferents course. Most of the APO-induced changes were abolished by haloperidol (2 mg/kg, i.p.).

The ^{14}C-2-DG technique has proven a sensitive tool to study the metabolic activity of basal ganglia circuitry consequent to brain lesions and pharmacological treatments that alter neostriatal DA-ergic activity. The observed changes in labeling are largely consistent with the anatomy and electrophysiology of these structures. Further, the results provide information concerning the pathophysiology of DA-related movement disorders.

243 SENSORIMOTOR DEFICITS CAUSED BY GLOBUS PALLIDUS LESIONS. T. Labuszewski*, L.R. Edelstein, B. Federchuck*, D. Lederman*, T.I. Lidsky. Dept. Psychol., SUNY Stony Brook, N.Y. 11794.

There is a large literature demonstrating that rats with bilateral globus pallidus lesions show aphagia and adipsia lasting several days. In an attempt to understand the bases of these deficits, we used a fine-grained analysis of drinking behavior. Rats were trained to lick a spout recessed behind a plexiglass wall for 8% sucrose solution. Interlick interval, duration of tongue contact with spout, and postural adjustments were monitored. Pre-lesion, each animal showed characteristic bell-shaped lick duration and interlick interval distributions, which varied little from day to day. Globus pallidus lesions caused obvious disruptions of these rhythmic behaviors. This disturbance is in large part due to an inability to correctly position the mouth with respect to the spout and maintain that position for a burst of licks, activities which normally insure consistently efficient ingestion. After pallidal lesions, animals often miss the spout altogether. When they do find the spout, only the first few licks in a burst of licking are "on target". The head then drifts away from its "drinking position" and the animal spends much time licking at the surrounding empty space. The burst of licks is terminated after several unrewarded air-licks, the head and mouth are repositioned, and a new burst of licks initiated by the animal. The methods used by lesioned animals to find the recessed spout are reminiscent of someone finding their way in the dark by feeling. Some tap the spout with their noses, then move their heads into the proper drinking position; some tap it with their paws first. Normal animals show no obvious "spout finding" behavior. They walk up to the spout and simply begin licking. Lesioned animals will show more normal ingestion patterns, however, if ease of access to the drinking tube is greatly increased; although they often adopt bizarre head positions when they drink. This licking deficit seems to be qualitatively similar to the deficits in bar press behavior following unilateral basal ganglia lesions (Levine et al, JCPP, 1971 77: 282 293). Animals' ability to continue licking during speed controlled, smooth lateral movement of the spout was also examined. Preliminary data show that moving spout duration distributions also change after globus pallidus lesions.

These data are indicative of neither purely sensory nor purely motor deficits. Rather, basal ganglia damage seems to interfere with movements whose successful execution is dependent upon sensory feedback.

244 HETEROGENEITY WITHIN THE CAUDATE NUCLEUS OF THE CAT: BIOCHEMICAL AND HISTOCHEMICAL INVESTIGATIONS. John Lehmann H.C. Fibiger and A. Parent. Division of Neurological Sciences, University of British Columbia, Vancouver V6T 1W5, and (A.P.) Département d'Anatomie, Université Laval, Québec.

"Patchy" distributions of afferents to the caudate nucleus (CN) have been described. The recent finding (1) that acetylcholinesterase has a heterogeneous distribution in the CN emphasizes the importance of characterizing these discrete topographical units.

We report the results of assaying numerous small samples of cat caudate nucleus for tyrosine hydroxylase (TH), choline acetyltransferase (CAT), acetylcholinesterase (AChE), glutamate decarboxylase (GAD), and high-affinity glutamate uptake (Glu-up). The following significant correlations were obtained:

AChE, CAT:	r = .731	p<.001
CAT, TH:	r = .623	p<.001
AChE, TH:	r = .861	p<.001
TH, Glu-up:	r = -.650	p<.01
AChE, Glu-up:	r = -.681	p<.01

Subcellular fractionation studies have shown that all these enzymes are highly concentrated in the P_2 fraction; thus these enzyme values are interpreted as indices of terminal densities in given samples. The results suggest that dopaminergic and cholinergic terminals are concentrated in similar AChE-rich areas. AChE-poor zones appear to be preferentially innervated by corticostriatal fibers, insofar as high-affinity glutamate uptake can be considered a marker for this projection.

Since AChE is heterogeneously distributed in the caudate nucleus, it was of interest to determine if the putative cholinergic large aspiny neurons (2), which synthesize high levels of AChE, have a patchy distribution. In some coronal sections, the distribution of perikarya within squares of a superimposed grid deviate from the Poisson distribution, suggesting aggregation; however, this is not a consistent observation. More powerful stochastic methods will be presented which can be used to test for aggregation of discrete events in any histological material.

The density of AChE-positive perikarya in the CN of the cat is approximately half that of the rat; similarly, CAT activity in the CN of the cat is approximately half that of the rat. This correlation supports our previous suggestion (2) that the large aspiny neurons which stain heavily for AChE are the cholinergic interneurons of the striatum.

(1) Graybiel and Ragsdale, PNAS 75:5723, 1978.
(2) Lehmann, Fibiger, and Butcher, Neuroscience 4:217, 1979.
Supported by the Medical Research Council.

245 SOURCE AND TOPOGRAPHY OF THALAMIC PROJECTIONS TO THE CAUDATE-PUTAMEN IN THE RAT AS DEMONSTRATED BY THE RETROGRADE TRANSPORT OF HORSERADISH PEROXIDASE (HRP). J. F. Lentini* and G. M. Krauthamer. Dept. Anat., CMDNJ-Rutgers Medical School, Piscataway, N. J. 08854.

The posterior intralaminar thalamus, consisting of centrum medianum (CM), n. parafascicularis (Pf) and posterior n. centralis lateralis, is the source of a major projection to the caudate nucleus in the cat. There is, however, a long standing controversy with regard to the delineation of these nuclei.

In the rat, the existence of CM is not always recognized. The two most commonly used stereotaxic references for the rat differ widely in the size and location of CM and Pf. Hence, the stereotaxic coordinates defining these structures cannot be used with confidence in studies of the thalamo-striate projection system. To resolve this problem, we used HRP (TMB method) to define the intralaminar projection to the caudate-putamen. Large injections of HRP were used to define the extent of the thalamic input and small iontophoretic HRP deposits were used to determine its topographic organization. Clusters of retrogradely labeled cells were found surrounding the habenulo-interpeduncular tract (retroflex bundle) dorsally, ventrally, and laterally, but not medially. The projection was topographically organized. This delineation of CM agrees with that of the rat stereotaxic atlas of Albe-Fessard et al., but differs from that of the atlas of König and Klippel.

246 EFFECTS OF NEONATAL MEDIAL FOREBRAIN BUNDLE (MFB) LESIONS ON DEVELOPMENT IN CATS. I. RESULTS ON JUVENILE AND ADULT CATS. M.S. Levine, C.D. Hull and N.A. Buchwald. Mental Retardation Research Center, School of Medicine, UCLA, Los Angeles, CA. 90024.

We have previously described some of the effects of bilateral MFB lesions made in kittens of 9-21 days of age (Neurosci. Abst. 3 (112): 1977). These lesions interrupt many ascending and descending pathways and destroy parts of the lateral hypothalamus as well. Of specific interest in our experiments was damage to the nigrostriatal dopaminergic tract (NST). To determine the amount of destruction of the NST, caudate dopamine (DA) concentrations were measured. MFB lesions produced an average decrease of 75% in caudate DA. During the first 2 months postlesion, MFB lesioned kittens (N=30) gained less weight, were more hyperactive and perseverated more often in making incorrect responses in a spatial discrimination than their intact littermates (N=37) or littermates with control (ventral thalamic) lesions (N=16). The present experiments were designed to ascertain the long-term effects of neonatal MFB lesions. Studies were made of learning ability, responses to amphetamine challenges and of striatal neuronal firing rates in these cats at juvenile and adult ages. When the animals were 3-4 months old their ability to learn a visual discrimination was assessed in a modified T-maze. MFB lesioned cats learned the discrimination in slightly fewer trials (210 + 20, mean + S.E.) than their intact littermates (265 + 20) or their littermates with control lesions (260 + 35). When the meaning of the cues in the discrimination was reversed the MFB lesioned cats responded more frequently to the previously reinforced cue than animals in the other groups. The behavioral effects of d-amphetamine (3 dosages, 1,2 and 4 mg/kg i.p.) were assessed when the cats were 9-16 months of age. Amphetamine was used because in adult animals depletion of striatal DA markedly attenuates some of its effects. In all animals amphetamine produced autonomic responses consisting of pupillary dilation, piloerection and salivation. In intact cats and in cats with control lesions amphetamine also induced vertical and horizontal head movements. The frequency and intensity of these movements were significantly reduced in the cats with neonatal MFB lesions. A final set of experiments was carried out when the animals were 12-24 months old to determine if long-term changes in spontaneous firing rates of caudate neurons were induced by the neonatal MFB lesions. MFB lesions produced minimal alteration in the spontaneous firing of caudate neurons. The average interspike interval of caudate neurons of intact cats was about 1700 ms compared to about 2200 ms in cats with MFB lesions. Taken together these results indicate that early postnatal damage to the MFB produced a complex set of behavioral deficits during maturation but little alteration in the spontaneous firing of caudate neurons per se. Supported by HD-05958 and NS-12324.

247 CHOLINOCEPTIVE NEURONS IN THE ISOLATED CAUDATE. Luis A. Marco, John C. Torri*, and Alberto B. Santos*. Dept. of Psychiatry, Med. Univ. of S.C. and VA Hospital, Charleston, S.C., 29403.

Many intrinsic short-axon neurons in the caudate nucleus are thought to possess cholinergic receptor sites on the basis of histochemical and lesion studies but neurophysiological evidence is still lacking. The caudate of cats and rats was surgically isolated in situ on one side from the rest of the neuraxis and left with its connections intact on the other side. Single unitary activity was investigated in intact and isolated caudates at least 2 wks. after isolation by means of 5-barrel micropipette assemblies. Three barrels were loaded with acetylcholine (ACh), dopamine (DA), and glutamate (Glut) for iontophoresis and the other two with saline for recording and current neutralization. In both species, best unitary recordings were obtained with overall multi-barrel tip size of 6-7 μ and individual pore resistances of 15-20 MΩ. Numerous neurons in the immediate vicinity of the recording pore were silent in both species on the isolated side, they could not be triggered to fire impulse discharges by closely apposed electrical stimulation (within 1.5 mm of the recording site), and could only be activated by iontophoresis of Glut (a general inducer of cell firing) or ACh. ACh-sensitive units were induced to fire at currents between 10 and 30 nA. Some of these otherwise silent units reached peak-to-peak amplitudes up to 1.6 mV and firing rates up to 100 Hz. These effects were not current-dependent however because a) the ACh-activating effect often had delays of 0.5 min. or longer, and b) identical amounts of current from the neutralization or the DA barrel failed to elicit them. Some other non-spontaneous units were induced to fire only after ACh iontophoresis had been turned off, again following a long delay of silence. We have not observed differential responsiveness to DA between neurons in intact and isolated sides. Detailed analysis of neuronal populations is underway. Since terminal degeneration is complete 6 days after surgical isolation, the ACh-sensitive cells in the isolated caudates must be intrinsic or local circuit neurons. If the nigroneostriatal (NS) DA input is inhibitory on intrinsic ACh-containing caudate neurons, one would expect in the isolated caudate disinhibition of such intrinsic neurons which would release sufficient amounts of ACh onto postsynaptic cholinoceptive receptor sites in the next neuron in the synaptic chain of transmission. The fact that additionally iontophorized ACh was required to trigger ACh-sensitive neurons would suggest rather a deficiency in ACh release or an excitatory role for the NS DA system on these ACh-containing neurons. Further neurobiological and clinical significance of these results will be discussed. (Supported by NINCDS Grant # NS14712-02).

248 QUANTIFICATION OF DENDRITIC MORPHOLOGY IN THE DEVELOPING CAUDATE NUCLEUS OF THE CAT. J.P. McAllister*, R.M. Bradford*, M.S. Levine C.D. Hull and A.M. Adinolfi. Mental Retardation Research Center, School of Medicine, UCLA, Los Angeles, CA. 90024.

As the continuation of a series of investigations designed to quantify neuronal development of the basal ganglia, the present study has examined the dendritic growth of spiny neurons in the kitten caudate nucleus. Tissue from 3, 10, 19 and 114 day old kittens was impregnated using the Rapid Golgi method. Neurons from each age group were drawn using a camera lucida attachment and subsequent three dimensional analysis performed with the aid of a PDP 11/40 computer system. In this way, accurate information was obtained on a variety of dendritic parameters. Results from the four age groups were compared to determine how the dendritic morphology of caudate spiny neurons changes with development. Preliminary analyses from 66 neurons (3 day = 15; 10 day = 15; 19 day = 13; 114 day = 23) indicate that neither soma diameter (mean = 23.7μm) nor the number of branches per dendrite (mean = 7.7-9.9) change appreciably between 3 and 114 days of age. The average maximum radius of the dendritic field, however, enlarges from about 150μm to 220μm, but this growth is not significant until after 3 weeks of age. Average branch length increases from 60μm in the 3, 10 and 19 day groups to 85μm in the 114 day group. Similarly, the average dendritic length measures 494, 592, 440 and 783μm at 3, 10, 19 and 114 days, respectively. The lower values for average dendritic length and number of branches per dendrite (7.7) at 19 days may reflect impregnation differences in that all cells at that age were measured from a single animal. Nevertheless, the maximum values, which should compensate for dendrites which may appear prematurely shortened because they pass out of the section, yield dendritic field radii of 195, 196, 214 and 303μm at 3, 10, 19 and 114 days, respectively. Such linear growth occurs distal to the primary dendrite, because the lengths of the first branch do not increase significantly. In contrast, the total branch lengths comprising an entire dendritic field are relatively uniform, ranging from 2650 to 2990μm for the early age groups to about 3300μm at 114 days. This may correlate with the most striking finding, that the total number of dendrites per neuron appears to decrease with age. At 3, 10 and 19 days there are 6.1, 5.3 and 6.2 dendrites per neuron, respectively. This compares to 4.4 dendrites per neuron at 114 days. Loss of dendrites with age may be due to fusion of basal membranes at the point of origin to provide a common primary stem for two or more main secondary branches.

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249 LATERAL HABENULAR NUCLEUS: ORGANIZATION OF AFFERENT AND EFFERENT PATHWAYS. Russell L. McBride* (SPON: J. Sutin). Dept. Anat., Emory Univ., Sch. Med., Atlanta, GA 30322.

The lateral habenular nucleus (LHB) receives projections from the entopenduncular nucleus (feline homologue of the internal segment of globus pallidus) and lateral hypothalamus-preoptic region, and thus may be an integration site for limbic and subcortical motor systems. Evidence from anterograde and retrograde transport studies, however, suggests that there is at least some degree of segregation of afferent and efferent LHB pathways.

In anterograde experiments in cats, tritiated leucine (100 μCi/μl) was injected into regions projecting to LHB. Two days after injection cats were perfused and the brains processed with standard autoradiographic techniques. It was found that while the entopeduncular nucleus projects to the ventrolateral quadrant of LHB, anterior lateral hypothalamus projects to all of LHB except that region receiving input from the entopeduncular nucleus.

In retrograde transport experiments, a 50% solution of horseradish peroxidase (HRP) was injected into regions receiving projections from LHB. After two days survival, cats were perfused and brains processed with tetramethyl benzidine. Following injections into the central gray, near the dorsal tegmental nucleus and dorsal raphe nucleus, labeled LHB cells were located in the region receiving terminals from the anterior lateral hypothalamus; following HRP injection into the medial reticular formation lateral to centralis superior raphe nucleus, labeled LHB cells were located in the region receiving input from the entopeduncular nucleus and posterior lateral hypothalamus.

Preliminary electrophysiological evidence indicates that the output of the nucleus may be more homogeneous than suggested by the anatomical data. Extracellular recordings were made in barbiturate anesthetized cats from antidromically identified LHB projection neurons. Neurons, regardless of location in the nucleus, were inhibited for approximately 150 msec by either entopeduncular or lateral hypothalamic stimulation. The inhibition was frequently preceded by a 4 msec latency orthodromic action potential.

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

A number of studies have shown that neuronal aggregates or islands characterize the vertebrate neostriatum. To evaluate the possibility that these cellular territories change with age, the brains of C57Bl/6J mice 4, 10, or 27 months of age were fixed in Carnoy's solution and embedded in paraffin. Five micron coronal sections through the head of the caudate-putamen nucleus (the region of the nucleus rostral to the crossing of the anterior commissure), were stained with cresyl violet. Five sections from each animal were analyzed. The mediolateral extent of each section was divided into three zones--a lateral peripheral zone, a core or central region, and a medial peripheral zone. All large cells in each of the three zones were counted. To obtain data on the medium + small neuron population at each age, a 10 X 10 ocular grid calibrated at 400X magnification was placed over cellular islands in each region. All medium + small neurons in the field and all glial cell nuclei were counted. Two fields were selected and values added to give a single value each for medium + small neurons or glial cell nuclei per region. Only neurons with distinct nucleoli were included in the counts.

Possible sources of variance were ascertained using a three-level nested analysis of variance. Within cellular terriories, the number of medium + small neurons decreased with advancing age ($p < .05$). However, the total number of glial cell nuclei within these same territories showed no significant variation with age. Large neurons were also consistently present throughout the time period sampled in this study. In addition, it should be noted that, as indicated in an earlier report (Mensah, P. L., Brain Research, 137:53, 1977), large neuron variation was confined to regional variation within the head of the nucleus. At all ages, significantly more large neurons occurred in the core of central area than in either the lateral or medial peripheral zones ($p < .001$). These data suggest that physiological and biochemical peculiarities in aged mouse neostriatum may well be due to alterations in the medium + small cell population.

251 ELECTROPHYSIOLOGICAL ANALYSIS OF OLFACTORY TUBERCLE IN ISOLATED TURTLE BRAIN. Martha C. Nowycky* and Gordon M. Shepherd. (Spon: J.N. Crawley). Section of Neuroanatomy. Yale Univ. Sch. Med., New Haven Ct. 06510.

The functional organization of the olfactory tubercle has been studied in an isolated preparation of the turtle brain (see Nowycky, Waldow, and Shepherd, Neuroscience Absts. 4:583, 1978). The olfactory tubercle in the turtle may be considered as that cortical part of the striatum which receives direct projections from the olfactory bulb and also has a dense dopaminergic input from the midbrain (Parent, J. Anat., 114:379, 1973). The turtle olfactory tubercle shows rudimentary lamination of neuronal elements and in its depths is continuous with the underlying striatum.

Field potentials, extracellular spikes, and intracellular responses have been recorded in the olfactory tubercle following electrical stimulation of the following areas: olfactory nerve (ON), lateral olfactory tract (LOT), lateral forebrain bundle at the base of the striatum (LFB) and midbrain. Stimulation of ON, LOT, and LFB elicited a small negative field potential within 100 um of the surface, and, in deeper layers, a large positive field potential with a similar time course.

Extracellularly recorded single units which responded to ON stimulation were found to a depth of 600 um from the surface, i.e. well into the striatum. These cells frequently responded to LOT and LFB stimulation as well. The effects of various combinations of paired volleys were examined. A prominent effect was a long-latency facilitation of LOT and LFB test volleys following conditioning volleys in the same pathway. Another finding was a long-lasting suppression of ON test volleys following LFB conditioning.

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

252 EFFECTS OF CAUDATE OR FRONTAL CORTICAL ABLATIONS IN CATS AND KITTENS: PASSIVE AVOIDANCE. C.E. Olmstead and J.R. Villablanca. Depts. Psychiatry, Anatomy, and MRRC, UCLA Los Angeles, CA 90024.

A simple step-down passive avoidance task was used to evaluate the perceptual awareness of a fearful situation in 15 normal cats and in animals with extensive caudate lesions. The surgical groups were as follows: a) Adult lesions: 11 with bilateral caudate lesions (Md: 74%; range 36-90%). 8 with removal of the frontal cortices, 6 sham operated, and 5 unilateral acaudates. b) Kitten lesions (9-36 days of age): 8 bilateral acaudates (Md: 77%; range 44-93%), 8 bilateral afrontals, and 6 shams. All animals were tested as adults. On each of two days the animal was twice placed on a platform and the latency to step-down measured. Following trial 2 of day 2 the animal received a 10 sec. unescapable shock. Single step-down retention trials were carried out at 24 and 48 hrs. and 1 week following the one trial training. With the exception of two adult operated acaudates (28% and 61.2% removals), 1 unilateral acuadate and 1 adult operate which never stepped off the platform, all animals learned the avoidance task. A Friedman two-way ANOVA showed significant conditioning effect ($p < 0.001$). There were significant group differences only on trial 1 of day 1 ($p < 0.05$) where the adult acaudates stepped down significantly slower than both the intacts ($p < 0.05$) and the kitten acaudates ($p < 0.05$). For the adult acaudates there was a significant inverse correlation between lesion size and the step-down latency for both trials of the first pre-shock day and for the trial immediately preceding shock ($r_S = >-.64$, $p < .05$). Interestingly, partitioning the data for the adult operated acaudates according to the median caudate removal (73%.8%) showed significantly faster step-downs for the larger lesions on day 1-trials 1 and 2 ($p < 0.008$), but not on any of the test days. For the kitten acaudates no significant relationships were found between any of the parameters and lesion magnitude. These data show that 1) animals with total removal of the caudate nucleus or the frontal cortex learned one-trial passive avoidance as readily as sham operated and intact cats; 2) acaudate cats were distinguishable from the other groups only by slower step-downs on the first of the four habituation trials prior to shock; 3) cats made acaudate as adults a) extinguished more rapidly than either kitten acaudates or other control animals and b) showed an indirect relationship between lesion size and 1st trial habituation and extinction. We conclude that animals with extensive lesions of the caudate nuclei or frontal cortices are capable of avoiding an electric foot-shock. There are subtle changes in that avoidance behavior which may be attributed to the enhanced approach tendencies previously described in the acaudates (USPHS Grants HD-05958 and HD-04612).

253 DEMONSTRATION OF RECURRENT INHIBITION IN RAT NEOSTRIATUM. M.R. Park*, J.W. Lighthall*, & S.T. Kitai, Dept. Anat., Michigan State University, E. Lansing, MI 48824.

Recurrent inhibition has been demonstrated in rat caudato-putamen following direct stimulation, during intracellular recording, of a single neuron. Action potentials (AP) triggered by depolarizing square wave current pulses (2-5 msec; 0.5-5nA) conditioned a test EPSP evoked from stimulation of substantia nigra (SN). Reduction of the test EPSP amplitude, indicative of inhibition localized in the dendrites, occurred at interstimulus intervals of less than 20 msec. In nearly all cases where shunting of the test EPSP was observed, no corresponding hyperpolarizing potential was seen at the recording site. Time to peak of the test EPSP was not altered but its rate of decay was generally accelerated and the hyperpolarization following an SN EPSP often deepened. Reduction of the test EPSP by up to 35% was observed and was always less than that seen for inhibition resulting from solely extrinsic stimulation, as in double SN stimulation. To eliminate the possibility that the reduction of the test EPSP was due to an increase in conductance resulting from AP currents, two tests were performed. 1) When depolarizing pulses (250-350 msec) were injected triggering only a single AP, the depolarizing plateau following the AP remained flat, indicating no membrane conductance change. 2) If the conditioning AP was followed by a train of hyperpolarizing current pulses (1nA; 5.0 msec), there was no measurable conductance change. Neurons injected with HRP subsequent to testing for recurrent inhibition were recovered and identified as medium spiny neurons. (Supported by USPHS Grant NS 14886 and NIH BRSG RR 05772-04).

254 A GOLGI STUDY OF THE GLOBUS PALLIDUS IN MONKEYS. Pedro Pasik, Tauba Pasik and Marian DiFiglia. Dept. of Neurol., Mount Sinai Sch. Med., CUNY, New York, N.Y. 10029.

Examination of 15 Golgi-Kopsch series of monkey brains (M. mulatta) revealed at least two neuronal types in the globus pallidus. A medium to large neuron (12 x 18 µm to 15 x 40 µm) is the most frequently impregnated. Generally, four thick dendrites (about 6 µm in diameter) arise from the soma, branch sparsely and course at least 800 µm before terminating. Some of the dendrites are of even thickness, and others, sometimes belonging to the same neuron, are varicose. A single dendrite may have both characteristics in different regions. Dendritic spines and protrusions of various sizes and shapes appear on all dendrites and are much more frequent on those of uniform diameter, particularly in the more distal branches. Frequently, dendrites terminate in a complex arborization composed of 5 to 6 irregularly shaped, often recurving branches, which give rise to numerous pleomorphic appendages of different sizes. Such endings may have abundant spines. The above features were observed in both young and fully mature animals. The axons of these neurons almost always originate from the soma and do not impregnate in our series beyond 30 µm. Small neurons, about 12 µm in diameter, are seen infrequently. They have 3 or 4 dendrites of smaller caliber which exhibit varicosities and an occasional spine, and end after sparse branching about 150 µm from the cell body. The axon emerges from the soma or a proximal dendrite, is thin, and arborizes locally into a few fine beaded processes.

Two categories of extrinsic fibers are observed: (1) the radial fibers are seen in sagittal sections as axons of intermediate diameter (0.5 µm) coursing in discrete bundles from the neostriatum into the globus pallidus. Immediately after entering the pallidum they give rise to numerous fine branching collaterals (0.2 µm) oriented orthogonally to the main axon and parallel to the dendrites of medium to large neurons. The dendritic fields of the latter may span 5 or 6 bundles of radial fibers. Occasionally, a radial fiber terminates in a few fine processes within the outer pallidum. (2) Large caliber axons (1-1.2 µm) are followed up to 1 mm before terminating. Thin processes with large varicosities (2-3 µm) arise from them and follow a straight or curving path. The curved ones end in clusters of 10-20 boutons. These axons are seen in both segments of the pallidum and some of them appear to enter the inner segment from its ventral aspect.

The above findings confirm the results of Fox et al. (1974; 1975) and provide additional information concerning the dendritic patterns of pallidal cells, and features of the intrinsic and extrinsic axons.

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255 KAINATE-INDUCED NEURONAL DEGENERATION OF THE STRIATUM IMPAIRS SHORT-TERM MEMORY BUT NOT LONG-TERM MEMORY OF REWARDING EVENTS. Michele Pisa, Paul R. Sanberg and Hans C. Fibiger. Div. Neurol. Sci., Dept. Psychiatry, Univ. Brit. Col., Vancouver, Canada.

To assess the effects of neuronal degeneration in the striatum on short-term memory performance, rats with bilateral microinjections of kainic acid into the dorsal striatum (three nmoles in 0.5 ul of phosphate buffered saline, pH 7.4) were compared with vehicle injected controls in a reward-alternation runway task in which food was available in the odd but not in the even trials of daily sessions each consisting of a 12-trial sequence. Occurrence of speed alternation in this task, specifically running more slowly in the nonrewarded than in the rewarded trials of the sequence, is commonly interpreted to reflect memory of the recent consequences (reward or nonreward) of running. The rats were trained for 22 sessions with an intertrial interval (ITI) of 1 min, for 10 sessions with a 5-min ITI, and for 5 sessions in extinction. Separate measures were taken of the latencies in the start box, the alley and the goal box of the L-shaped runway. Throughout training with alternating reward the kainate-treated (KAT) rats ran significantly more slowly than the controls, irrespective of trial outcome. The rats of both groups ran significantly more slowly in the first, nonrewarded trial than in the second, rewarded trial of the sequence in all segments of the runway. This difference in speed was at least in part an effect of learning, because it strongly increased with practice. In the later trials of the sequence the control rats reliably alternated speed in all segments of the runway. In contrast, the KAT rats reliably alternated in the goal box only. On extinction, rate of suppression of the running response did not significantly differ among groups. Histology revealed severe neuronal degeneration of the dorsal striatum in all KAT rats. Some KAT rats also showed degeneration of the ventral striatum, and partial, unilateral or bilateral degeneration of the pyramidal neurones of the CA1 field of the hippocampus.

The present results indicate that striatal neuronal degeneration in rats does not affect long-term memory of invariant rewarding events, i.e. nonreward at the onset of the sessions. These lesions, however, possibly in combination with partial hippocampal damage, impair modulation of running performance reflecting short-term memory of variable rewarding events. In addition to these memory impairments, these lesions result in motoric alterations preventing running speeds comparable to those of control rats.

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256 STRIOSOMAL ORGANIZATION OF THE CAUDATE NUCLEUS: III. DISTRIBUTION OF AFFERENTS FROM THE FRONTAL CORTEX OF THE CAT. C.W. Ragsdale, Jr.*, and A.M. Graybiel (SPON: R.L.M. Faull). Dept. of Psychology, M.I.T., Cambridge, MA 02139.

Autoradiographic evidence suggests that the cortico-striate projection in cats and monkeys is organized in a complex pattern in which both grain-dense and grain-sparse figures can be recognized within the field of distribution of individual cortical areas. Since the grain-figures visible in autoradiograms have about the same dimensions as inhomogeneities in striatal acetylcholinesterase (ca. 500 um dia.), it seemed possible that the two patterns might be related. We have therefore compared them directly in the caudate nucleus of the cat.

Injections of ^{3}H-amino acids or ^{35}S-methionine were made into the pericruciate cortex of three cats. Up to 1m Ci of label was deposited in multiple closely spaced injections and the resulting injection sites involved parts of areas 4, 6 and surrounding cortical zones. Most sections were prepared for autoradiography, but at intervals sections were instead prepared by the acetylthiocholinesterase (AchE) method of Geneser-Jensen and Blackstad. Both processing techniques demonstrated marked inhomogeneities in the striatum. In the autoradiograms the caudate nucleus was massively labelled on both sides in a patterned manner confirming earlier accounts; in the AchE-sections irregularly shaped zones of low activity appeared against a matrix of dark stain. Autoradiograms were lined up with neighboring sections stained for cholinesterase. It was clear that while the inhomogeneities in grain-distribution in the autoradiograms were by no means always aligned with the zones of low AchE activity, there often was a match: sometimes the borders of the grain-dense patches corresponded to the borders of the pale AchE zones, sometimes grain-sparse and enzyme-poor zones were aligned. The grain-sparse areas corresponding to cholinesterase-poor zones appeared in the ventral half of the caudate nucleus both ipsilaterally (3 cases) and contralaterally (1 case). By contrast, instances of "filling in" of AchE-poor zones by grains in the corresponding autoradiograms were seen in the dorsal part of the caudate nucleus ipsilaterally (2 cases) and contralaterally (1 case). These last named alignments were especially prominent in one case (^{35}S) in which the cortical injection was centered in area 6.

We do not know how extensive such systematic correspondences may be for other cortical areas. Even for the fronto-striate projection quantitative analysis is difficult for technical reasons: in both the AchE staining and the autoradiography one must balance between prolonged processing that could mask some pale or grain-sparse zones, and brief processing that might fail to demonstrate the inhomogeneities adequately. Supported by NSF 78-10549BNS and NIH-1-R01-EY02886-01.

257 AN EXPERIMENTAL ANALYSIS OF THE AFFERENT PROJECTIONS TO THE CENTROMEDIAN AND PARAFASCICULAR NUCLEI IN THE CAT. G. James Royce, Michael F. Huerta, Joseph T. Weber and John K. Harting. Dept. of Anatomy, University of Wisconsin, Madison, WI 53706.

The method of horseradish peroxidase has been used to identify the various sources of afferent pathways to the centromedian and parafascicular nuclei in the cat. Following injections of HRP which involve only the centromedian-parafascicular region, an extremely wide distribution of labelled neurons is apparent within the cerebral cortex, brainstem and spinal cord.

Labelled neurons are present in approximately the rostral four fifths of the cerebral cortex. All labelled cells lie entirely within cortical layer V. Such cells occupy every neocortical gyrus, with the exception of the posterior lateral gyrus. The largest number of labelled neurons are present within the anterior sigmoidal gyrus and within the depths of the presylvian sulcus. A lesser, but considerable number of labelled neurons lie within the gyrus proreus, and the anterior sylvian, posterior sigmoidal and cingulate gyri. Scattered labelled cells can also be identified in the posterior sylvian gyrus, and in all of the ectosylvian and suprasylvian gyri.

This widespread distribution of afferent cortical cells is strikingly similiar to the pattern of efferent projectons from the CM-Pf complex, as revealed by autoradiographic methods (Royce '77). Further, it is interesting to note that the organization of afferent cells projecting to the CM-Pf nuclei is remarkably similiar to the pattern of afferent neurons which project to the head of the caudate nucleus (Royce, '77).

The superior colliculus contains many labelled cells. Such cells are restricted to layers IV-VII, with the following percentage distribution within each layer: layer IV, 62%; layer V, 19%; layer VI, 14%; and layer VII, 5%. All cell sizes are represented, however, most cells are small (less than 25 micra), while only a few cells are either medium-sized (25-39 micra) or large (greater than 40 micra).

The hypothalamus also contains HRP positive cells, especially the ventromedial nucleus and dorsal hypothalamic area. In addition, labelled neurons are present within the reticular thalamic nucleus, the entopeduncular nucleus, the periaqueductal gray, the principal sensory and spinal trigeminal nuclei, the vestibular complex, the fastigial nucleus of the cerebellum, all portions of the reticular formation, the locus ceruleus, the nucleus gracilis and the nucleus cuneatus, and lamina VII of the cervical spinal cord.

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258 STRIATAL KAINIC ACID LESIONS, CATALEPSY, LOCOMOTOR ACTIVITY AND HUNTINGTON'S DISEASE. Paul R. Sanberg,[†] Michele Pisa, and Hans C. Fibiger. Div. Neurological Sci., Univ. of British Columbia, Vancouver, B.C., Canada, and [†]Dept. Behavioural Biology, Australian National Univ., Canberra, A.C.T., Australia.

Microinjections of the neurotoxin, kainic acid (KA), into the striatum of rats produce biochemical, anatomical and behavioral changes similar to those seen in patients with Huntington's disease (HD)(1,2). The present study examined the effects of drug-induced cholinergic and dopaminergic manipulation on cataleptic and locomotor behaviors of rats with KA lesions of the striatum.

Three nmoles of KA in 0.5µl of phosphate buffered saline were injected into the rostral striata of male Wistar rats. Control rats received injections of the vehicle only. During behavioral testing the drugs, haloperidol (0.5, 1 & 2 mg/kg), pilocarpine (25, 50 & 100 mg/kg), amphetamine (1 mg/kg) and scopolamine (1 mg/kg) were given i.p. Catalepsy was measured as the latency to remove the forepaws from an 8 cm high bar, and locomotor activity was measured by photoactometers over two hours.

In the first experiment (about 1 month post-op) the KA lesioned (KAL) rats showed substantial *decreases* in haloperidol and *increases* in pilocarpine-induced catalepsy, compared to controls. At higher, normally subseizure threshold, doses of pilocarpine all KAL rats showed clonic convulsions. Catalepsy measured after vehicle injections did not differ between groups. In the second experiment the KAL rats showed *increased* locomotor responses to both amphetamine and scopolamine, compared to controls. No differences were observed following saline in either experiment.

The results showing enhanced sensitivity of KAL rats to the behavioral effects of some anticholinergic, and dopaminergic agents are similar to the effects observed in HD patients. The attenuation of the behavioral response produced by the neuroleptic, haloperidol, in these rats, supports the view that the striatum is the site of action of this agent. Furthermore, it indicates why HD patients exhibit only a minimal "transqualizing" effect, compared to other non-striatal damaged neurological patients, following the common use of haloperidol. Finally, the increased sensitivity of KAL rats to pilocarpine warrants further study on the possible effectiveness of cholinergic drugs in HD.

1 Supported by the MRC and the HD Foundation.
1 Coyle, J.T. *et al. Prog. Neuropsychopharmacol.* 1, 1977, 13-30.
2 Sanberg, P.R. *et al. Pharm.Biochem.Behav.* 10, 1979, 137-144.

259 SENSORY AND MOTOR CONCOMITANTS OF JAW MOVEMENTS IN CAT CAUDATE NEURONS. J. S. Schneider, F-C. T. Chang* and T. I. Lidsky. Dept. Psychol. SUNY, Stony Brook, N. Y. 11794.

Single neurons were recorded from the head of the caudate in partially restrained, behaving cats. Milk was presented to the animal via a tube resting against the upper lip at the midline. Milk delivery elicited rhythmic jaw movements and associated postural adjustments. Sampled cells were assessed first for involvement in ingestion-related movements and subsequently for sensory responsiveness.

Three populations of cells showed ingestion-related changes in activity. The most frequently encountered type (25% of sampled cells) showed firing rate changes that were time-locked to milk delivery. These cells possessed no obvious somatosensory or gustatory sensitivity. Response magnitude was unrelated to the topography of ingestion-related movements; indeed, unit responses persisted in the absence of any motoric response to the milk. Current work is investigating the possible involvement of these cells in attentional aspects of behavior.

The second type (15% of sampled cells) was characterized by a high degree of somatosensory responsiveness. Light tactile stimulation was optimal for effecting activity changes. Receptive field sizes were moderate (eg. front of lip) to large (eg. entire face) and always included the perioral tissue. Heterogeneity of response within receptive zones suggested encoding of stimulus location. Ingestion-related activity change in these cells was apparently secondary to facial stimulation caused by licking and jaw movements.

The third type (4% of sampled cells) showed neither somatosensory nor movement-related responsiveness in the usual sense. Jaw and postural movements alone or somatosensory stimuli alone evoked no activity changes. However, somatosensory stimulation, if presented during ongoing ingestional movement, caused increased firing in these cells. Receptive fields were contralateral, perioral, and located far posterior of the area contacted by the drinking tube. These response characteristics are compatible with the encoding of stimulus location during movement and suggest possible targeting functions.

These data underscore the involvement of the basal ganglia in oral-ingestive behavior. However, the nature of this involvement remains unclear, particularly in view of the absence of pure movement-related neuronal activity. The potent influence of trigeminal afferents upon caudate neurons emphasizes analysis of sensory processing as an advantageous approach to an understanding of basal ganglia function.

260 ENTOPEDUNCULAR NUCLEUS PROJECTIONS TO THE MESENCEPHALIC LOCOMOTOR REGION. R. Skinner, E. Garcia-Rill and S. Gilmore. Dept. Anat., Univ. Arkansas for Medical Sciences, Little Rock, AR 72201.

Recent electrophysiological studies have confirmed previous anatomical studies describing a projection from the entopeduncular nucleus (EN) to the nucleus tegmenti pedunculopontinus (NTPP). This nucleus lies embedded in the brachium conjunctivum, immediately ventral to the cuneiform nucleus (CF). From another line of evidence, stimulation in the region of the CF in the cat has been found to elicit rhythmic walking movements as long as, a) a precollicular-postmamillary brainstem transection is accomplished, and b) a moving treadmill is placed beneath the limbs while the animal's weight is supported. The present study first established that, following appropriate transection, walking could be elicited on a treadmill by stimulation of the medial edge of the CF. Best parameters were 0.05 mA, 1.5 ms pulses at 60 Hz. Secondly, it was established that single neurons recorded in EN could be antidromically driven (0.12 mA, 0.05 ms, > 300 Hz) from the medial edge of the CF. Histological verification of stimulating sites showed the sites to be indistinguishable from the walking-eliciting sites. Estimates of EN projections to NTPP range from 8% to 50% for other studies. Although we have a small sample, only 14% of EN cells appear to project to medial CF.

We have been able to elicit antidromic responses in EN cells from CF, transect and elicit rhythmic movements of the limbs from the same site in the same animal. We have been unable to generate smooth walking movements, after transection and recovery, in preparations which previously were lightly anesthetized or locally anesthetized-paralyzed during single unit recordings.

Parallel autoradiographic experiments are in progress to determine whether the motor cortex also projects to the CF region. Preliminary findings suggest that injections of H^3-leucine (total 3 μCi) into the gray matter of area 6 aβ (axial motor cortex) result in labeling in the CF region.

The present studies describe convergent projections from the EN and, at least, axial motor cortex to the mesencephalic locomotor region. Functionally, these projections may not be involved in the direct control of walking. The fact that spontaneous walking may occur in "thalamic" preparations precludes this notion. Basal ganglia and motor cortex projections to this area, rather, may be involved in the sequencing and ordering (syntax) of voluntary movements. This concept is an extension of the idea that the basal ganglia are involved in the preparation for movement.

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261 GLOBUS PALLIDUS COMPONENT IN OLFACTORY TUBERCLE: EVIDENCE BASED ON IRON DISTRIBUTION. Robert C. Switzer, III and Joanna M. Hill*. Laboratory of Brain Evolution and Behavior, National Institute of Mental Health, Bethesda, MD 20205

In Nissl-stained, parasagittal sections of rat brain the globus pallidus (GP) seems to extend rostro-ventrally in a cusp-like formation into the polymorph layer of the olfactory tubercle. Such an extension suggests a pallidal complement to the striatal cells of olfactory tubercle (OT) (see Heimer and Wilson, In: Golgi Cent. Symp. Proc., 1975; Heimer, In: Limbic Mechanisms, 1978). If sectors of the polymorph layer of OT are in fact components of GP then they should be characterized by the same endogenous molecular markers: gamma aminobutyric acid (GABA) and its synthesizing enzyme glutamate decarboxylase (GAD) (see Krieger and Heller, Soc. Neur. Sci. Abs., 1978, #1414), and ferric-iron. We treated rat brain sections with Perl's method for demonstrating ferric-iron. Macroscopically, the entire GP was highlighted by the Prussian Blue reaction product indicating the presence of ferric-iron. Proceeding rostrally from the level of the anterior commisure, a rostro-ventral cusp-shaped extension of GP is visible coinciding with the locus of neurons seen in the Nissl-stained sections. The blue zone extends above the horizontal limb of the diagonal band of Broca to include the rostral substantia innominata and joins the caudal OT in the polymorph layer. More rostrally, the iron-positive zone is divided by the cell bridges joining the ventral striatum above with the superficial layers of OT below. The elaborate relationship of the iron-positive zone with the islands of Calleja and striatal cell bridges is described elsewhere (Hill and Switzer, this volume). Microscopically, the large multipolar neurons of GP and of the rostro-ventral extension are embedded in a blue matrix of the neuropil populated by glia whose cytoplasm is stained intensely blue. Occasionally some of the fine processes of the glia are revealed by the Prussian Blue. Compared with the GP, the iron-positive glia in the caudate-putamen are sparse and the blue neuropil is absent. The boundary between the blue of pallidum and adjacent structures is distinct; even the narrow, striatal cell bridges of the OT are iron-negative in spite of the intense blue of the surrounding polymorph layer.

Taken together, the iron-positive glia and neuropil form a continuum which consolidates the telencephalic projection areas of the caudate-putamen, accumbens and the striatal cells of olfactory tubercle. Our findings complement those of Heimer and Wilson regarding the striatal character of components of OT and in light of the high GABA and GAD (Krieger & Heller) levels found in the polymorph layer, provide a strong argument for a pallidal component in OT--perhaps more correctly termed "paleo pallidum".

262 A LIGHT AND ELECTRON MICROSCOPIC STUDY OF THE DEVELOPING CAUDATE NUCLEUS IN THE PUPPY. Duke Tanaka, Jr. and Krystyna Dutkiewicz*. Dept. Anat., Coll. Med., Howard Univ., Washington, D.C. 20059 and Dept. Neurophysiol., Nencki Inst. Exp. Biol., Warsaw, Poland.

The caudate nuclei of puppies 6 hr to 30 days old were processed with the Golgi-Kopsch method or embedded in JB-4 plastic and stained with methylene blue and basic fuchsin. Sections were examined for maturational changes in somatic size, dendritic branching, and axonal arborization. Additional caudate nuclei of 3-5 and 19-20 day old puppies were processed for electron microscopic examination of the neuropil. All results were compared with data obtained from similarly prepared tissue from adult dogs. The Golgi and methylene blue-basic fuchsin stained sections revealed that significant somatic growth and dendritic maturation occurs during the first postnatal week. The somata increased in average diameter from 9μm at 4 days to 13μm at 7 days and dendritic length increased from 60-80μm at birth to 80-150μm at 7 days. In addition, fewer thick proximal dendrites, which are characteristic of immature neurons, were noted at the end of this week. Due to the immature features of these neurons, it was very difficult to classify neuronal types during the first week. However, during the second week the continued development of both axonal and dendritic processes allowed a differentiation between spiny and aspiny neurons to be made. Most spiny neurons were characterized by long thin dendrites (120-200μm long) possessing varicosities, long filopodia and protospines. A decrease in the number of filopodia and protospines occurred during the third week along with a gradual increase in the number of spines. By 22 days, the dendrites of spiny neurons ranged between 120-300μm in length and by 30 days, spine densities approached those seen in the adult. Although axons were rarely impregnated in newborn material, they could be followed for some distance in the more mature material (7 days and older) and formed part of the basis for classifying more mature neurons. Examination of the neuropil of the 3-5 day old caudate nuclei revealed the presence of immature synapses, axonal and dendritic growth cones, filopodia, and large areas of extracellular space. No myelinated axons were noted. By 19-20 days of age, the growth cones had disappeared as had most of the extracellular space while the number of mature synapses and small axonal processes had increased markedly. Thinly myelinated axons were occasionally seen in or adjacent to small fiber bundles. Synaptic density per $100\mu m^2$ in the 4 day old caudate was about 20% of that seen in the adult while the density in the 19-20 day old material had increased to about 60% of adult density.

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263 MODIFICATION OF MOTOR ACTIVITY, PASSIVE AVOIDANCE CONDITIONING AND EVOKED POTENTIALS BY MICROINJECTIONS OF PICROTOXIN IN BOTH CAUDATE NUCLEI OF CATS. Carolina Téllez*, Francisca Vázquez*, Pablo de la Mora* and Héctor Brust-Carmona. Depto. de Fisiología, Div. de Investigación, Fac. de Medicina, C.U., México 20, D.F.

Different experiments have shown modifications of Passive Avoidance Conditioning by Striatum lesions or stimulation. The inhibitory influences upon motor activity by caudate nucleus (CN) which has been very well demonstrated, could be responsible for the behavioral modification which allows the cat to learn not to pass from one compartment to the other (PAC). This inhibitory action could be blocked by local application of picrotoxin (Pi).

In 18 cats stainless steel cannulae were implanted into the anterior rostral part of the head of CN. After 5-8 days, bilateral microinjection of 5μl of NaCl (N=9) or 6μg of Pi in 5μl of NaCl (N=7) were performed. Immediately after, gross spontaneous motor activity was observed and some reflex responses were clinically tested. After 10 min, the acquisition of one trial PAC was made. Next day the latency to cross from one compartment to the other (1st test trial) was again measured. Next day a second test was performed but 10 min after bilateral microinjection of Pi or NaCl. One-two days later in the same cats in acute experiments, the CN's evoked responses (ER) produced by stimulation of NCM were recorded on FM tape. The magnitude and latency of components were analyzed using a PDP11/40 computer. After 6 samples of 10 ER, taken every 2 min; 6 or 18μg of Pi were applied in both CN. Similar samplings were obtained in the following 40-80 min. After the Pi microinjections the cats stayed quiet cleaning themselves many times and then started walking and suddenly bursts of violent motor activity appeared. They ran with the extremeties flexed, very near to the floor "flying response on the floor". Sometimes the animal crashed against the wall and kept moving in an apparent effort to continue at all costs in one direction. In the acquisition session of PAC the cats injected with Pi crossed from one side to the other in $\bar{X}$=75 sec which was very similar to the NaCl injected cats ($\bar{X}$=61.7 sec). Both groups of cats in the first test did not move in 600 sec (learning criterion). In the second test under picrotoxin or NaCl effects, the cats of the first group crossed in $\bar{X}$=401 sec which compared with 600 sec of the second group revealed a significant difference ($U=4$, $n_1=4$, $n_2=6$, $P<0.05$). In general the ER consisted of three peaks with average latency of 9, 25 and 44 msec. After the Pi microinjections the first and second peak increased significantly at the level of $P<0.05$ ($N=95$, $x=21$, $Z=5.34$ for the first peak and $N=152$, $x=38.2$, $Z=6.08$ for the second; sign test). The described results further support the suggestion that the CN usually acts in a manner which produces inhibition both electrographically and behaviorally.

264 SINGLE DORSAL RAPHE NEURONS PROJECTING AXON COLLATERALS TO BOTH THE SUBSTANTIA NIGRA AND CAUDATE-PUTAMEN IN RAT. Derek van der Kooy and Toshi Hattori. Department of Anatomy University of Toronto, Toronto, Ontario, Canada.

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

265 EPSPs RECORDED IN RAT NEOSTRIATAL NEURONS FOLLOWING STIMULATION OF THE DORSAL RAPHE NUCLEUS. C.P. VanderMaelen, A.C. Bonduki, & S.T. Kitai, Dept. Anat., Michigan State University, E. Lansing, MI 48824

Investigators utilizing extracellular recording techniques have concluded that the projection from the dorsal raphe nucleus (DRN) to the neostriatum is inhibitory. In the present study intracellular recordings were obtained in neostriatal neurons of the rat in order to investigate the postsynaptic effects of DRN stimulation. Male hooded rats were anesthetized with urethane, and some of these were paralyzed with a mixture of Flaxedil and tubocurarine, and artificially respirated. Recording microelectrodes were filled with 2 M KCl or 2 M K-citrate, or with a mixture of 4% horseradish peroxidase (HRP), 0.4 M KCl, and tris buffer (pH = 7.3). Microelectrode resistances ranged from 30-70 megOhms. Electrical stimulation of the DRN was monopolar through stainless steel insect pins insulated except for about 1.0 mm at the tip. Single stimulus pulses of 0.15 msec duration and up to 18 V intensity were delivered once every 1.5 - 2.0 sec. Stimulating electrodes were also implanted in the ipsilateral cerebral cortex, and in some rats, in the contralateral brachium conjunctivum caudal to the DRN, as a control for stimulus spread from the DRN to this structure.

Stimulation of the DRN elicited monosynaptic EPSPs in neostriatal neurons. In no case was an initial hyperpolarizing response seen. Response latencies ranged from 2.4 to 7.2 msec (mean = 4.9; S.D. = 1.32; N = 33). In most neurons the EPSP was followed by an IPSP during which action potentials were suppressed. This, in turn was often followed by "rebound excitation" with spikes. The suppression of spikes during the IPSP, as well as the "rebound excitation" occurred even when the initial EPSP failed to generate an action potential. 90% of the neurons which responded to DRN stimulation also responded to stimulation of the cerebral cortex. Intracellular injection of some recorded neurons with HRP revealed them to be "spiny-I" neurons. These results are discussed in light of previous work which has shown 1) convergence of excitatory inputs from cortex, thalamus, and substantia nigra onto spiny-I neostriatal neurons; 2) the probable source of the IPSP as intrinsic to the neostriatum; and 3) the conflicting reports of excitation and inhibition in the neostriatum in response to microiontophoretically applied serotonin. (Supported by NIH grants NS 05576-02 to A.C.B. and NS 14866 to S.T.K.)

266 TESTOSTERONE AND 17-β ESTRADIOL LEVELS IN ACAUDATE, AFRONTAL AND INTACT CATS. J.R. Villablanca, C.E. Olmstead and G.H. Stabenfeldt* Depts. Psychiat., Anat., and MRRC, UCLA, Los Angeles CA, 90024 and Dept. Reproduction, Sch. Vet. Med., Univ. Cal. Davis, CA.

We have previously defined the behavioral changes which follow extensive caudate nuclei lesions in cats and kittens. A characteristic of caudatectomized male cats is the appearance, immediately after recovery from anesthesia, of a stereotyped estrous-like behavior to stimulation of the perigenital region which includes lordosis, tail deviation, hind paw treading and, occasionally, vocalization. The present experiments were designed to test if there is an endocrinological basis for this behavior. It has been postulated that the striatum may be involved in endocrine control (Barbeau, 1970, 1973). Plasma levels of testosterone and 17-β estradiol (Schille, et.al., in press) were measured by radioimmunoassay in intact (N=7), acaudate (N=7; median removal 84%, range 35-90%), afrontal (N=4; all tissue in front of A 22 removed) and operated control (N=4; 2 with a large septal lesion and 2 with hemispherectomy). Plasma samples (3-5ml) were taken at two day intervals from restrained cats over a two week period for a total of 10 samples per subject. The data analysis was on the daily medians for each group, subject medians across samples and grand medians for daily and subject medians. Intact cat values for both testosterone (md 6.7, range 2.6-9.3 ng/ml) and estradiol (md 15.4, range 10.0-19.6 pg/ml) agreed with values from other labs. A two-way ANOVA showed a significant group effect for both median testosterone ($p<.05$) and 17-β estradiol ($p<.001$). Comparisons of the group medians (Mann-Whitney U) showed that both acaudates ($p<.05$) and afrontals ($p<.05$) had significantly lower levels of testosterone than intact animals. Comparisons for 17-β estradiol showed afrontals to be significantly higher than either intacts ($p<.001$) or acaudates ($p<.01$), while the operated controls were significantly lower ($p<.001$) than the other groups. Spearman rank correlations between magnitude of the caudate lesion and plasma levels were not significant for either testosterone ($r_s=-0.34$, $p=.50$) or 17-β estradiol ($r_s=0.0$, $p=1.0$). However, a similar correlation of lesion and estrous-like behavior was significant ($r_s=.84$, $p<.02$) and the behavior was not suppressed by castration (performed in 2 acaudates). These data suggest that decreased plasma levels of testosterone are not specific to caudate ablation. In fact, this is a case of defined behavioral change without a hormonal specificity (acaudate) and of an increase in estrogens without detectable sexual changes (afrontals). We therefore hypothesize that the estrous-like behavior seen in acaudate males is probably due to the changes in somatosensory responsiveness and in "affect" that we have reported and not to shifts in their endocrinological status. (USPHS Grants HD-05958 and HD-04612).

267 A PHARMACOHISTOCHEMICAL PROCEDURE FOR GABA-TRANSAMINASE: IMPLICATIONS FOR BASAL GANGLIA ANATOMY. S.R. Vincent, H. Kimura*, and E.G. McGeer. Division of Neurological Sciences, Dept. of Psychiatry, Univ. of British Columbia, Vancouver, Canada, V6T 1W5.

GABA is an important neurotransmitter in the basal ganglia, particularly in the descending striato-pallidal and striato-nigral pathways. However, striatal GABA neurons have not been successfully visualized histologically. GABA is catabolised by the enzyme GABA-Transaminase (GABA-T), and a histochemical method for this enzyme has been reported (Van Gelder, 1965). Results using this method indicate a neuronal localization for GABA-T in the cerebellum. Intense staining has also been observed in the striatum and substantia nigra, however, the precise location, whether in nerve cell bodies or in nerve terminals in juxtaposition was difficult to discern. This same problem has been reported for Acetylcholinesterase staining in the basal ganglia and was resolved by using the DFP pharmacohistochemical technique (Butcher, 1975). Recently, two specific and irreversable GABA-T inhibitors (EOS & Gabaculine) have become available, and we reasoned that they could be used to resolve the localization of GABA-T in the basal ganglia.

Adult Wistar rats received intraventricular injections of EOS (2mg/kg) and were sacrificed and processed with our modified GABA-T histochemical method at various survival times. Forty-five minutes after the injection of EOS, no striatal neurons staining for GABA-T could be observed. By four hours some neurons could be observed which stained for GABA-T, indicating that they had resynthesized the enzyme. Eight hours after the injection, the regeneration of GABA-T staining was almost complete. These results indicate that this pharmacohistochemical procedure for GABA-T may be a useful tool for the localization of GABA systems in the basal ganglia, and other areas of the central nervous system.

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268 EFFECTS OF PICROTOXIN UPON SINGLE UNIT ACTIVITY OF SUBSTANTIA NIGRA NEURONS. B.L. Waszczak, N. Eng*, and J.R. Walters, NIH, NINCDS, Bethesda, MD 20205

The substantia nigra (SN) is the site of termination of at least one major GABAergic projection. GABA-containing afferents to the nigra from the striatum and globus pallidus have been thought to provide a tonic inhibition of SN pars compacta dopamine (DA) neurons. However, we have recently observed that while cells of the SN pars reticulata exhibited a marked sensitivity to the inhibitory actions of the GABA agonist, muscimol (administered i.v.), the pars compacta DA neurons were paradoxically stimulated (Waszczak et al., Neurosci. Abstr. 4:436, 1978). Similarly, pars reticulata neurons were found to be much more sensitive to inhibition by iontophoretically-applied GABA and muscimol than the DA neurons. These observations suggested that a population of neurons in the SN pars reticulata have the capacity to be more affected by a nigral GABAergic input than the DA cells. To investigate this possibility further, the effects of i.v. administration of the GABA antagonist, picrotoxin, upon the extracellular, single-unit activity of both types of nigral neurons were determined in chloral hydrate-anesthetized rats. Doses of picrotoxin, administered in 1 mg/kg increments to a total cumulative dose of 7.0 mg/kg, caused gradual increases in the firing of DA cells (n=12). The average increase in activity, which was maximal between 5.0 and 7.0 mg/kg, measured approximately 30% over the baseline firing rate. In contrast, these doses of picrotoxin induced dramatic dose-related increases in the firing of pars reticulata cells (n=9). The maximum stimulatory effect achieved was an increase to 154% over the baseline rate after 7.0 mg/kg picrotoxin. These results were consistent with the idea that the reticulata cells may be more likely recipients of a major GABAergic input to the SN than the DA neurons.

To assess whether previous destruction of the striatonigral GABA pathway might attenuate the stimulatory effects of picrotoxin upon nigral neurons, the effects of i.v. picrotoxin were also determined 2-3 weeks after unilateral kainic acid (KA) (1 µg/0.5 µl) lesions of the striatum and globus pallidus. There were no changes in the responses of DA neurons to i.v. picrotoxin in lesioned animals while there appeared to be a slight but insignificant attenuation of the stimulatory effect of picrotoxin upon reticulata cells. These findings suggest that 1) picrotoxin may stimulate nigral neurons by a non-specific excitatory mechanism unrelated to GABA receptor blockade, or 2) another GABA projection to the SN, other than that destroyed by striatal KA lesions, may either indirectly or directly inhibit these cells. This latter possibility is supported by biochemical evidence that approximately 30% of the control level of GAD activity consistently remains in the SN after striatal KA lesions.

269 Electrophysiological and Biochemical Examination of the Cholinergic System in Rat Neostriatal Slices. Molly H. Weiler*, Ulrich Misgeld*, Il-Jin Bak, Donald J. Jenden. Depts. Pharmacol. & Neurol., UCLA School of Medicine, Los Angeles, CA 90024 & Max-Planck Inst. Brain Res., Frankfurt, Federal Republic Germany

Local stimulation in superfused rat neostriatal slices evokes orthodromic (latency: 2.5-4 msec) and antidromic (latency: 0.5-1.5 msec) discharges (Misgeld et al., Exp. Brain Res. 34:575,1979). Pharmacological data indicate that acetylcholine (ACh) is the transmitter of this intrinsic excitation. Nicotinic agents such as d-tubocurarine (0.7×10^{-6} M), mecamylamine (10^{-5} M), or hexamethonium (10^{-4} M) selectively blocked the orthodromic discharges, and atropine (10^{-4} M) was without effect (Misgeld, Pfl. Arch. 379: R45, 1979). When the slices were exposed to an acetylcholinesterase inhibitor (physostigmine, 10^{-7} M; paraoxon 10^{-5} M; tetram 10^{-6} M) the amplitude of the orthodromic population spike increased. The synaptic activity of the neostriatal slices was unaffected by choline (10 µM) in the perfusion medium during electrophysiological recording. With higher frequency stimulations (10-30 Hz) the orthodromic population spike was facilitated in the presence of acetylcholinesterase inhibitors and this frequency facilitation was blocked by atropine (10^{-6} M). Intracellular recordings showed that a slow depolarizing potential caused the frequency facilitation. These results suggest that, as in cholinergic systems in the superior cervical ganglion and the spinal Renshaw cells, nicotinic receptors mediate a fast and muscarinic receptors a slow excitation in the rat neostriatum. Neurochemical studies of ACh synthesis in the neostriatal slices show that the concentration of ACh reaches an apparent steady state after 1 hr incubation (7.54 ± 0.32 nmol (mg protein)$^{-1}$). When hemicholium (5 µM) was present in the medium the ACh levels were 75% lower after 1 hr incubation (1.92 ± 0.13 nmol (mg protein)$^{-1}$). There was no clear cut effect of HC-3 on the evoked field potentials at lower concentrations (5 µM), and at higher concentrations (100 µM) there was no recovery of unitary spikes following high frequencies of stimulation. Thus, the in vitro slice preparation offers a unique electrophysiological and biochemical approach to studying the intrinsic cholinergic system of rat neostrial tissue.

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270 EFFECT OF DOPAMINE AGONISTS ON ^{3}H-SPIROPERIDOL RECEPTOR BINDING. R.L. Weir*, R.E. Hruska, and F.K. Silbergeld (SPON: J.R. Walters). Howard University, Washington, DC 20060 and Experimental Therapeutics Branch, NINCDS, NIH, Bethesda, MD 20205.

Dopamine agonists of the ergot alkaloid type (such as bromocriptine) have been shown to have beneficial clinical effects in Parkinson's disease and some endocrine disorders (amenorrhea or galactorrhea). Thus, it was of interest to study the effects of an ergot drug on dopamine receptors in the caudate nucleus, and to compare it to apomorphine.

Bromocriptine (15 mg/kg) was administered subcutaneously to rats for 4 days. The rats were sacrificed 24 hr after the last injection, the brains rapidly removed, and the caudate nuclei isolated. The tissue was homogenized and carefully washed before ^{3}H-spiroperidol binding was measured. The concentration of ^{3}H-spiroperidol was maintained at low concentrations (less than 200 pM) in our assays, to assure that only dopamine receptors were labeled. From Scatchard analyses, the number of binding sites decreased about 20% relative to controls, and there was no change in the affinity of the receptor sites for ^{3}H-spiroperidol. When bromocriptine was added in vitro to normal rat caudate tissue, the ^{3}H-spiroperidol binding was again decreased with an inhibition of 50% of the specific binding occurring at 2 nM. This indicates that bromocriptine interacts potently with the dopamine receptors labeled by ^{3}H-spiroperidol.

Apomorphine (1 mg/kg) was given intraperitoneally to rats and stereotypy was noted. The rats were sacrificed 30 min after injection, the brains removed rapidly, and the caudate nuclei dissected. After careful washing, Scatchard plot analyses did not show any change in ^{3}H-spiroperidol binding compared to controls.

The acute effects of dopamine agonists are apparently due to receptor stimulation without a detectable change in the receptor. The chronic administration of these drugs causes a decrease in the number of receptor sites available for stimulation by either endogenous dopamine or administered dopamine agonists. This may contribute to problems (e.g. on-off reactions) which occur with chronic clinical usage of dopamine agonists.

271 INTRACELLULAR STUDIES OF THE CONVERGENCE OF SENSORY INPUT ON CAUDATE NEURONS. J.S. Wilson*, C.D. Hull and N.A. Buchwald. Mental Retardation Research Center, School of Medicine, UCLA, Los Angeles CA. 90024.

Anatomical studies have revealed that the striatum receives afferents from all parts of the cerebral cortex including sensory areas. Neurophysiological studies have shown that the neostriatum receives sensory input from all modalities. The purpose of this research is to elucidate the post synaptic responses of caudate neurons evoked by auditory and somatosensory stimulation. Intracellular recordings, using potassium citrate-filled micropipettes, were made in the head of the caudate nucleus of cats. Auditory stimulation was provided by a "click" generated by a speaker placed inside a hollow ear bar ipsilateral to the recording electrode. Somatosensory stimulation was produced by a subdermal, electrical shock to the right or left forepaw. The effect of ipsilateral auditory stimulation was studied in 42 caudate neurons. Twenty-six cells showed an EPSP-IPSP (E-I) response; 11 cells appeared to receive only inhibitory input; and 5 cells showed no response. Following the initial E-I response, the cells often responded with an oscillating excitatory and inhibitory wave form which lasted for a second or more. The mean latencies 1) to the onset of the E-I were 30.8 ms ± 10.2, 2) to the peak amplitude of the EPSPs 50.4 ms ± 12.6, and 3) to the peak amplitude of the IPSPs 78.8 ms ± 23.7, as measured from the first stimulus pulse. The effect of ipsilateral somatosensory stimulation was studied in 34 cells. Twenty-two cells showed an E-I response, 8 showed an IPSP response and 4 showed no response. The mean latency of the EPSP, latency to peak amplitude of the EPSP and latency to peak amplitude of the IPSP were 27.9 ms ± 6.0, 65.2 ms ± 19.4 and 184.3 ms ± 41.0, respectively. In 32 cells, the effect of contralateral somatosensory stimulation was studied. Twenty-two cells showed an E-I response, 4 cells showed an IPSP response and 6 cells showed no response. The average latency of the EPSP, latency to peak amplitude of the EPSP and latency to peak amplitude of IPSP were 23.6 ms ± 4.8, 54.6 ms ± 10.6 and 184.9 ms ± 44.2, respectively. The convergence of auditory and somatosensory stimulation was studied in 24 cells. The majority of cells were bimodal (20 cells). Four cells appeared to receive only somatosensory input. In summary, sensory stimulation most frequently evoked an E-I response in caudate neurons which was often followed by an oscillating E-I wave form. The responses were not qualitatively different for the sensory modalities studied and most cells received a convergence of sensory inputs.

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272 ACETYLCHOLINESTERASE NEURONS IN THE RAT CAUDATE-PUTAMEN COMPLEX: CHARACTERIZATION OF SOMATA TYPES AND STATISTICAL RELATIONSHIPS TO ONE ANOTHER AND TO THE TOTAL POPULATION OF STRIATAL CELL BODIES.

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Using a pharmaco-histochemical regimen for acetylcholinesterase (AChE, EC 3.1.1.7) that demonstrates the enzyme in neuronal somata and their proximal processes to a degree not possible with other protocols (Butcher & Bilezikjian, Eur. J. Pharmacol., 1975, 34, 115-125), we examined the morphologies, organization, and staining patterns of AChE-containing neurons at 9 different rostral-caudal levels of the rat caudate-putamen nucleus. At each of these levels additional analyses were performed in the dorsolateral, dorsomedial, ventromedial, and ventrolateral quadrants. As described in previous publications from this laboratory, three broad categories of AChE-containing neurons could be discerned on the basis of somata dimensions. Of these, the Type I cells (maximum soma extent = 8-12μm) were most numerous, representing 50-65% of the total population of AChE striatal neurons. The Type I cells were more numerous at rostral and caudal levels than in medial portions of the nucleus. Over 70% of Type I neurons had oval cell bodies, and 75% stained lightly for AChE. Type II neurons (maximum soma extent = 13-23μm) could be further divided into two subcategories. Type IIa cells (maximum soma extent = 13-15 μm) represented 11% of the total number of AChE neurons in the striatum; Type IIb cells (maximum soma extent = 16-23μm) represented 27%. Most Type IIa and IIb neurons were oval. The Type IIa somata stained lightly or with medium intensity, the number of cell bodies in each intensity category being essentially equal. Over 80% of Type IIb somata stained with medium intensity. Type III cells (maximum soma extent = 24-30μm or greater), representing roughly 5% of the total number of striatal AChE neurons, were primarily fusiform and stained darkly or with medium intensity. The proportion of the three types of AChE somata to one another was approximately the same at all rostral-caudal levels of the caudate-putamen complex. Type I cells appeared slightly more concentrated in ventral portions of the nucleus, however.

In brain sections processed first for AChE (pharmaco-histochemical regimen) and then counterstained with thionin, it was found that AChE-containing neurons represented only approximately 3% of the total number of neurons in the caudate-putamen nucleus. Yet these neurons presumably account for all of the AChE in a structure possessing one of the highest concentrations of acetylcholine (Butcher et al., J. Microwave Power, 1976, 11, 61-65) and choline acetyltransferase in the mammalian brain [Support: USPHS NS 10928 to L.L.B.].

BRAIN METABOLISM AND NUTRITION

273 EFFECT OF ACUTE ADMINISTRATION OF NEUTRAL AND OTHER AMINO ACIDS ON CATECHOLAMINE (CA) METABOLISM

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We have previously shown that tyrosine loading raises urinary CA and that this effect is blocked by pretreatment with a peripheral decarboxylase inhibitor, carbidopa. This finding suggests that TYR acts by increasing the saturation of the enzyme tyrosine hydroxylase, thereby enhancing CA synthesis in the sympathoadrenal system. However other mechanisms are possible, e.g.(i) a central hypotensive action via inhibition of sympathetic outflow, (ii) inhibition of CA degradation (iii) a nonspecific stress effect, causing CA release. The present study was designed to characterize the specificity of TYR effect and its biochemical consequences. Neutral amino acids (NAA) (TRP, VAL, ISOLEU, LEU, TYR) and non-neutral amino acids (GLU,ALA, LYS, ARG) were administered (at concentrations equimolar with 200 mg/kg of tyrosine) to overnight fasted rats. Urines (collected for a 3 hr. period), blood, brain, and adrenals were analyzed for CA, tyr, and MOPEG-SO4.

TYR caused 2 to 3-fold increases in tissue and blood TYR and urinary CA whereas NAA depressed these by 10-37%. GLU and ALA had no effect while LYS and ARG increased urinary CA by 10-30%. Serum TYR was highly correlated with urinary CA (r=.86). Except for TRP, coadministration of other NAA with TYR suppressed the increases in urinary CA. TRP potentiated TYR's effects on blood, brain, adrenal and urinary DA. TYR alone significantly raised brain MOPEG-SO4. In experiments with adrenalectomized rats TYR enhanced urinary DA and NE but had no effect on E. (Basal levels of DA and NE were similar in adrenalectomized and control rats.) These results show that (a) TYR is unique in causing major increases in urinary CA; its effect is not mimicked by related NAA; (b) changes in serum TYR are reflected by parallel changes in urinary CA; (c) as in the brain, TYR uptake into peripheral tissues seems to be subject to competition by NAA; however levels of NAA needed to compete are probably much higher. This study provides further evidence that availability of the amino acid precursor tyrosine affects peripheral CA synthesis and release.

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274 CHANGES IN CORTICAL OXIDATIVE METABOLISM ELECTRIC ACTIVITY WITH ALTERED BRAIN PERFUSION PRESSURE.

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Brain perfusion pressure is the difference between blood pressure and intracranial pressure. In the case of the open skull, intracranial pressure is normally close to zero and perfusion pressure equals local or diffuse arterial blood pressure. We have studied cortical oxidative metabolism in cats under N_2O/O_2 anesthetic in a 2/1 ratio, where the perfusion pressure was altered locally by - 1) bilateral common carotid occlusion; 2) unilateral common carotid occlusion; and, 3) pial artery compression. Perfusion pressure (PP) was altered diffusely by - a) intravenous Arfonad (lowered BP); b) intravenous Ephedrin (raised BP), or c) arterial bleeding (lowered BP). In general, these techniques caused a change in cortical PO_2 (bPO_2), which was proportional to the change in mean BP. The redox state of NADH and Cyt. a,a_3 were monitored by non-invasive optical techniques. Cortical electric activity was monitored with bipolar Ag-Agd$_2$ electrodes and analyzed in the frequency domain by a Fast Fourier Transform technique. Usually, decreased PP produced decreased bPO_2, and this was accompanied by reduction of NADH and Cyt. a,a_3; with the latter affected to a greater extent. Reducing the FiO_2 prior to lowering the perfusion pressure accentuated the reduction of NADH and Cyt. a,a_3, as well as prolonging the recovery period. Cortical electric activity, after 5-30 sec. of decreased PP, was initially increased in amplitude between 3 - 8 Hz. These studies correlate with the results of monitoring bPO_2 in patients undergoing microanastomosis for brain ischemia, where STA flow results in increased cortical PO_2 and oxidative metabolism. Increasing the perfusion pressure following microanastomosis of the STA to MCA in most patients showed increased STA flow and increased bPO_2.

275 THE EFFECT OF CHRONIC MIDDLE CEREBRAL ARTERY OCCLUSION ON LOCAL BLOOD VOLUME AND CYTOCHROME a,a_3 REDOX LEVELS IN RAT BRAIN.

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The resting local blood volume and reduction/oxidation (redox) level of cytochrome a,a_3 were monitored in the brain region over a chronically occluded middle cerebral artery (MCA) on the ipsilateral side and compared with the opposite side (contralateral). The vascular and metabolic responses to alterations in the fraction of inspired oxygen (FiO_2) were also monitored in both the ipsilateral and contralateral hemispheres. The rats were anesthetized using sodium pentobarbital (50 mg/kg,I.P.) and immobilized with tubocurarine chloride (0.5 mg/kg, I.V.). The animals were placed on a positive pressure rodent respirator and the skin overlying the cranium was reflected. Dual wavelength reflectance spectrophotometry was used to monitor directly through the skull simultaneous changes in local blood volume and redox levels of cytochrome a,a_3 (605-590 nm). Monitoring was performed over the lateral, superior surface just posterior to the coronal suture. Arterial blood gases and blood pressure were also monitored throughout the experiment. At rest, the contralateral side maintained stable local blood volume and cytochrome a,a_3 redox levels. Responses to changes in the fraction of oxygen in the inspired gas (FiO_2) were predictable: a substantial decrease in FiO_2 resulted in an increase in the level of reduced cytochrome a,a_3 and a compensatory increase in local blood volume; an increase in the FiO_2 caused a marked oxidation of cytochrome a,a_3 and a decrease in local blood volume. In contrast, the ipsilateral hemisphere failed to maintain stable baseline levels; local blood volume randomly ebbed and flowed with simultaneous alterations in cytochrome a,a_3 redox levels reflecting these changes. Local blood volume increases occurred coincident with increases in the relative oxidation level of cytochrome a,a_3. Responses to alterations in the FiO_2 were attenuated and superimposed on the wandering "baseline": in the ipsilateral hemisphere. We interpret these data to represent a loss of autoregulation in the relatively ischemic cortex.

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276 THERMOREGULATION AND ADRENOMEDULLARY ACTIVITY: COORDINATED ADAPTIVE RESPONSES TO NUTRIENT DEPRIVATION.

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Activation of the adrenomedullary sympathetic nervous system (AMSNS) increases hepatic glucose production. Lowering of body temperature (T_B) reduces cellular utilization of substrates. Neural pathways capable of increasing nutrient mobilization (activation of AMSNS) and lowering T_B provide a mechanism to insure adequate metabolizable substrate for tissues such as the brain during periods of nutrient deprivation. The possibility that activation of the AMSNS and lowering of T_B might be controlled by similar mechanisms in a coordinated fashion has been investigated. These studies were carried out using rats with chronic lateral ventricular cannulae and intrajugular catheters. AMSNS activity was assessed by measuring plasma epinephrine and norepinephrine. Catecholamine determinations were made using a radioenzymic method. Bombesin (100ng) given to rats intracisternally (ic) or intracerebroventricularly (icv) reversibly lowers T_B and activates the AMSNS, thus showing that a single putative neurochemical messenger can produce these two effects. Lowering of T_B is not a result of increased AMSMS activity since adrenalectomy does not prevent T_B changes following bombesin. 2-deoxyglucose (2DG, 5mg) but not D-glucose (5mg) given ic or icv activates the AMSNS and lowers T_B. Insulin (50OmU) given subcutaneously increases AMSNS activity and reduces T_B. Fasting of animals transiently increases AMSNS activity and chronically lowers T_B. To determine if the decrease in T_B following the above treatments results because of irreversible inhibition of thermoregulation, we assessed the ability of animals made hypothermic by the methods cited above to respond to a neurochemical stimulus which increases T_B. Somatostatin and various central nervous system-specific analogs of SS increase T_B and decrease AMSNS activity. A central nervous system-specific analog of somatostatin, des-AA^{1,2,4,5,12,13}-[D-Trp^8]-somatostatin, acutely reverses the hypothermia and increased AMSNS activity induced by bombesin, 2DG, insulin-induced hypoglycemia and fasting. Thus, the animals receiving these treatments exhibit no impairment of their thermoregulatory response to the hyperthermia-producing stimulus of this somatostatin analog. These results show that several neural stimuli reversibly activate both the AMSNS and lower T_B. These data support the concept that changes in T_B may potentially subserve neurally induced mechanisms to reduce nutrient utilization in states which may pharmacologically or physiologically mimic nutrient deprivation.

277 CEREBRAL REGIONAL O_2 CONSUMPTION AND SUPPLY IN CATS.
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The present study represents the first quantitative measurement of O_2 consumption and supply on a regional basis in the brain through the application of a new microspectrophotometric method. Twelve cats were tranquillized with ketamine and then anesthetized with α-chloralose. Artificial respiration was begun, and a left thoracotomy performed at the 5th intercostal space. Left atrial and femoral artery catheters were inserted. Heart rate, blood pressure, arterial and superior saggital sinus blood samples were obtained. 141-Ce microspheres (15 ± 3 μ) were injected into the left atrium and cereberal blood flow determined by the reference sample method. The cats' heads were quickly cut in 2 parts and frozen in liquid N_2. Ten different regions of the frozen brain were examined. Arterial and venous O_2 saturations were measured in each region by examination of vessels in 30 μ frozen sections with a Zeiss microspectrophotometer. O_2 consumption was determined as the product of flow and O_2 extraction. Blood pressure, heart rate, and blood gas values were within the normal range. Flow values ranged from 36 ± 9 ml/min/100g (Mean ± S.E.) in the medulla to 60 ± 20 ml/min/100g in the hippocampus. The highest O_2 extraction was noted in the thalamus (6.4 ± 1.0 ml O_2/100ml) and the lowest O_2 extraction in the anterior cortex (4.5 ± 0.5 ml O_2/100 ml). O_2 consumption ranged from 1.4 ± 0.4 ml O_2/min/100g in the medulla to 2.8 ± 0.8 in the thalamus. The lowest ratio of oxygen supply to demand was 2.76 ± .45 in the thalamus. Supply of O_2 to the brain regions studied was more than adequate to meet demand throughout the brain despite a two fold range in regional O_2 consumption. This new method for the determination of O_2 consumption and supply may be applied to many experimental conditions. Supported by grants from U.S. Public Health Service NS-13118 and HL-21172; a Grant-in-Aid from American Heart Association-NJ Affil., and by a grant from the Foundation-CMDNJ.

278 CHANGES IN DRUG INDUCED SLEEP PATTERNS DUE TO PROTEIN DEFICIENCIES.
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A major health problem of today is that of chronic malnutrition. On admission to the hospital many surgical patients are moderately or severely malnourished. Depressed immunity, increased sepsis and a high mortality rate are associated with severe malnutrition. Protein deprivation specifically is often an unavoidable concomitant of the early postoperative period and this compounds the already poor nutritional status of these individuals. Sleep behavior is a sensitive index of neurological function. Thus, evaluation of the duration of drug induced sleep may reveal important information relevant to the neurological status of protein deprived individuals. Because pentobarbital is a frequently used central nervous system depressant in current medical practice, it was selected for use in this animal study.

The purpose of this study was to determine the effect of protein-calorie malnutrition during fetal and postnatal life in Sprague-Dawley rats on the duration of pentobarbital induced sleep. Dams of experimental animals were fed isocaloric diets containing either 25% casein (well nourished) or 8% casein (malnourished) for two weeks prior to mating, and throughout gestation and lactation. Dietary regimes were continued after weaning in the young and subsequently adult male and female offspring were administered varying doses of sodium pentobarbital. Each animal was given either 20, 40 or 60 mg/kg of the drug intraperitoneally. The time which elapsed from the injection of the drug to the loss of the righting reflex (onset of sleep) and from the loss to the regaining of the righting reflex (duration of sleep) was recorded.

The well nourished animals slept longer than the malnourished under the influence of the drug except for the females receiving only 20 mg/kg. Furthermore, at all doses the control and experimental females slept longer than the respective males. Finally, only 7 out of 11 females survived the 60 mg/kg dose. None of these animals died within 4.5 hours following drug injection.

The results demonstrate the well known dose dependency of the duration of sleep following intraperitoneal injection of pentobarbital in experimental animals. The wide variation in sleep time among females and their sensitivity to the 60 mg/kg drug dosage may well be related to their four-day ovarian cycle. However, in both sexes, attention should be given to other factors relating to drug dosage, such as the nutritional status of the individual.

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279 EFFECTS OF BOVINE SERUM ALBUMIN ON ARACHIDONIC ACID-INDUCED EDEMA IN ISOLATED RAT BRAIN CORTEX.
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We have demonstrated previously that polyunsaturated fatty acids (PUFAs) induce rat brain cortical edema *in vitro* (Science 201:358, 1978). Edematous cortex is characterized by increased water content, lactate production and intracellular Na^+, and decreased extracellular space and intracellular K^+. The present study deals with brain edema induced by arachidonic acid (C20:4) and the effects thereon of bovine serum albumin (BSA). Cortical edema induced by arachidonate was dependent on the duration of incubation. Induction of brain edema approached a plateau after a 30 min incubation in 0.5 mM arachidonate which was followed by 60 min additional incubation in Krebs-Ringer control medium or in BSA (0.1 mM). Co-incubation of arachidonate (Ara) with BSA at a molar ratio of 5 (Ara/BSA) or less greatly inhibited the arachidonate-induced effects. Cortical edema increased as the molar ratio increased above 5. However, the swelling was not reversible by BSA, although the post-incubation of BSA released 46% of incorporated [^{3}H]arachidonic acid in cortical slices. [^{3}H]-arachidonic acid transport was completely abolished by 0.1 mM BSA and partially inhibited by exogenous arachidonate. We conclude that brain edema induction by arachidonate requires that free or unbound exogenous arachidonate be transported into cortical slices. It does not appear to be a surface effect. The molar ratio of Ara/BSA plays a critical role in the binding and the transport of arachidonate in isolated rat brain cortex. Supported by NIH Grant NS 14543.

280 INTRAVENTRICULAR INFUSIONS OF GLYCEROL PRODUCE BODY WEIGHT LOSS IN FEMALE RATS.
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It has been previously demonstrated by Wirtshafter and Davis (Science 198:1271, 1977) that subcutaenous injections of glycerol (40 mg/kg 4 times daily) produced body weight loss during the period of injections. Similar subcutaneous injections failed to produce an effect in female rats. However, in the present experiment injections of small amounts of glycerol into the third ventricle are shown to produce a decrease in food intake and body weight in females, as did similar injections in males previously reported by Davis and Wirtshafter (Neurosciences Abstracts, no. 525, 1978).

Body weight and food and water intake were measured twice daily in female rats before, during and after 11 days of continuous infusion of glycerol into the third ventricle of the brain. Vaginal smears were taken concurrently with above measurements to determine the stage of the estrus cycle. Unincumbered infusion of glycerol was accomplished by use of a subcutaneously implanted Alzet osmotic minipump connected to a stainless steel ventricular cannula by a section of polyethylene tubing. Glycerol was delivered at a rate of 27 μg/hr in a volume of 0.5 μl/hr.

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

281 PROTEIN SYNTHESIS IN VARIOUS BRAIN REGIONS FOLLOWING ACUTE HEMISPHERIC ISCHEMIA. G.A. Dienel*, W.A. Pulsinelli*, and T.E. Duffy (SPON: J.P. Blass). Dept. of Neurology, Cornell University Medical College, New York, N.Y., 10021 and Dementia Research Program, Burke Rehabilitation Center, White Plains, N.Y., 10605.

Inhibition of protein synthesis during cerebral ischemia and delayed or incomplete recovery of synthesis in the post-ischemic recirculation phase could contribute to irreversible cell damage after stroke. We induced hemispheric ischemia in conscious rats by occlusion of the four major cerebral arteries (Pulsinelli & Brierley, Neurology 28:379, 1978). One day prior to the experiments, both vertebral arteries were cauterized and polyethylene cuffs were placed around both common carotid arteries of all animals. Ischemia was produced by constriction of the carotid cuffs; cuffs on control rats were only manipulated. After 10 or 30 min of ischemia, the carotid cuffs were removed and [1-^{14}C]valine (7.5 mmol/kg, 40 μCi/kg) was injected i.p. at different times during the recirculation period. The rats were killed 70 min after ^{14}C-valine injection, and the trichloroacetic acid-soluble and particulate fractions of 5 brain regions were analyzed. Measurements on control (n=6) and 6-hr post-ischemic (n=6) rats established that: (i) incorporation of ^{14}C-valine into protein was linear for at least 100 min after valine injection, and (ii) although brain valine content was 35-40% higher in the 6-hr post-ischemic animals, the specific activity of brain ^{14}C-valine was similar (within 12%) in both groups. The rates of incorporation of ^{14}C-valine into protein in cerebral cortex, caudate-putamen, hippocampus, midbrain-diencephalon, and brain stem of control animals (n=7) were similar, averaging 32.2±3.0 dpm/mg protein. In rats rendered ischemic for 30 min, protein synthesis in caudate, hippocampus, and cortex was inhibited by 80% during the first hour of recirculation (n=4). The rate of recovery of protein synthesis in these regions was slow: 50-60% of control rates were measured at 6 hr of recirculation (n=6), 75-85% at 12 hr (n=5) and 24 hr (n=7), and 100% at 48 hr (n=4). Midbrain-diencephalon, which is partially perfused during the ischemic interval, showed less inhibition of protein synthesis during the first hour of recirculation (65%), and recovery was complete by 12 hr. Protein synthesis in brain stem was unaffected, in keeping with the fact that this structure is continuously perfused during 4-vessel occlusion. Following a 10 min ischemic insult, protein synthesis in cortex was 35% of control during the first hour of recirculation (n=4); it recovered to 70-80% at 3 hr (n=6), remained at this level at 6 hr (n=6), and was normal at 24 hr (n=3). Thus, the degree of inhibition of brain protein synthesis and the time to re-establish normal rates of synthesis are dependent upon both the severity and duration of the ischemic insult.

283 ^{125}I-IODOBENZENE AS A RADIOCHEMICAL MYELIN MARKER. K. Frey*, C.A. Boast, D. Wieland*, L. Brown*, B. Dudzinski* and B.W. Agranoff. University of Michigan, Ann Arbor, MI 48109.

Successful radioautographic visualization of regional brain cerebral blood flow and glucose metabolism has been reported in a variety of species. Gray matter is visualized more readily than white matter in these studies due to its greater vascularity, rate of blood flow and metabolic rate. Recent technological developments in brain scanning have demonstrated that various radiochemical tools can be applied to non-invasive imaging for clinical purposes. We report here the direct radioautographic imaging of brain white matter. Although small, metabolically inert radiolabeled lipophilic substances injected intravenously initially distribute in the brain according to blood flow (that is, more in gray matter than in white matter), after an appropriate period of time, the high lipid content of myelin leads to preferential accumulation of the substance in white matter. We selected labeled iodobenzene as a candidate molecule for these studies because of its favorable size, melting point, chemical stability and apolarity. A preliminary experiment in the rat indicated that the white/gray matter distribution ratio of ^{125}I-iodobenzene was maximal 30-60 min after an intravenous pulse. Subsequent experiments using radioautography have provided direct visualization of myelin in both the rat and rabbit brain. Forty-five min after intravenous injection of ^{125}I-iodobenzene, the brains are removed and frozen. Cryostat sections are exposed to X-ray film at -70°C. The resulting radioautographs indicate that ^{125}I-iodobenzene provides an indication of the lipid content of major brain fiber bundles such as corpus callosum, internal capsule, fornix and optic tract. Potential clinical applications of labeled agents that accumulate in brain lipid include the evaluation of the integrity of myelinated CNS pathways.

282 LOCAL CEREBRAL GLUCOSE METABOLISM AFTER PORTACAVAL (PC) SHUNT. Thomas E. Duffy and Nancy F. Cruz* (SPON: P. Tsairis). Dept. of Neurology, Cornell Univ. Medical College, NY, NY 10021.

Portacaval shunting and the associated hyperammonemia increase global cerebral glucose consumption (James et al., 1972), but whether local utilization rises uniformly and to the same degree in animals shunted acutely and chronically is unknown. We used the ^{14}C-2-deoxyglucose (2-DG) method to assess regional cerebral glucose metabolism in rats at 1, 4, 8, and 12 weeks after the construction of a PC shunt and in weight-matched controls.

All animals were anesthetized with halothane for insertion of cannulae into one femoral artery and vein. At least 3 hr later with the rats awake, normotensive, normothermic, and restrained, 0.2 mCi/kg of [1-^{14}C]2-DG was injected i.v.; arterial blood was sampled at intervals for plasma glucose and ^{14}C-2-DG specific activity measurements. After 45 min, rats were decapitated; the brains were removed, frozen, and sectioned at 20 μm for autoradiography with calibrated ^{14}C-standards (Sokoloff et al., 1977).

Arterial blood ammonia concentrations were twice the control value of 85±7 μM in rats shunted for 1 week, and were 2.5 times control in the 4-, 8-, and 12-week PC groups. Plasma glucose, on the other hand, averaged 25-35% below the control value of 10.6±0.6 mM in all shunted groups. Local cerebral glucose use (μmol/100 g/min) among major neuroanatomical structures was

STRUCTURE	CONTROL (n=9)	WEEKS AFTER PORTACAVAL SHUNTING 1(n=5)	4(n=5)	8(n=7)	12(n=5)
Parietal Cortex	124±5	92±3*	93±7*	98±6*	115±5
Frontal Cortex	120±6	86±4†	96±10	113±13	129±7
Caudate Nucleus	121±8	95±6	104±10	150±16	178±10*
Thalamus	101±4	88±7	111±11	144±16*	144±8*
Putamen	107±4	86±4	93±11	137±19	175±10*
Hippocampus	86±5	71±2	76±6	93±9	101±5
Inf. Colliculus	159±6	148±9	169±12	184±12	207±19†
Sup. Colliculus	91±6	80±3	103±11	120±13†	133±5*
Vestibular Nucleus	117±5	113±3	123±8	137±12	167±10*
Cerebellar Hemis.	57±4	49±3	55±7	76±11	84±9†
Cerebellar Nuclei	102±5	105±5	103±11	126±12	151±8*
Cerebellar White M.	30±3	26±3	33±4	42±5	53±5
Cortical White M.	42±4	21±2*	27±3†	39±5	49±5

*Different from control with p<0.001; †p<0.05.

the same or decreased up to 4 weeks after shunting, whereas at 8 weeks, many, and at 12 weeks, most areas exhibited increased glucose use; cerebral cortex and white matter were notable exceptions to this trend. Changes in local glucose consumption after shunting thus appear to be unrelated to blood ammonia concentrations, per se; chronic hyperammonemia and other factors (e.g., the degree of astrocytic pathology, which develops only after 4 weeks) may be contributory.

284 PROGRESSIVE NEUROLOGICAL DEPRESSION DURING CONSTANT HYPOGLYCEMIA IN UNANESTHETIZED RATS. J.G. Ghajar*, F. Plum, and T.E. Duffy. Dept. of Neurology, Cornell Univ. Med. Col., NY, NY 10021.

A model of low-dose, insulin-induced hypoglycemia was developed in female rats that produced a constant level of plasma glucose for 1.5 hr. Female rats, weighing 200 g and fasted overnight were anesthetized with ether for cannulation of the tail artery and vein. Rats recovered for 2-3 hr before receiving i.v. insulin (U40 Iletin). Rectal temperature was kept near 38°C.

At 20 min after 4.5 U/kg insulin, plasma glucose fell below 1.6 mM, remained constant for 1.5 hr, and slowly recovered. At 20 min, rats had active EEGs, were lethargic, and nonsupportive. At 30-40 min, rats had high-amplitude, slow-wave EEGs and increasing lethargy. At 40-60 min, rats were stuporous, progressing to coma (at approx. 80 min) with isoelectric EEGs. After the comatose period, seizures occurred; rats then either died or slowly recovered. Rats that maintained a plasma glucose above 1.6 mM became lethargic and recovered after 2 hr, but never developed coma or seizures. Blood pressure, gases, pH and hematocrit remained normal during hypoglycemic stupor and coma.

For neurochemical studies, brains were "freeze-blown" at 20, 30, 40, 60, and 80 min after insulin (n=4 each group). Values in controls (mean±SEM, n=6) were 1.37±.13 mmol/kg brain (glucose), 0.143±.006 (glucose-6-P), 2.97±.12 (glycogen), 2.36±.05 (ATP), 3.73±.10 (P-creatine), 0.063±.007 (NH_4^+), and 5.87±.37 mM (plasma glucose). Within 20 min after insulin, plasma glucose fell to 1.35±.04 mM and remained constant through 80 min. Brain glucose and glucose-6-P decreased at 20 min to 7% and 59% of control, respectively, remained at these levels through 60 min, and then fell to 3% and 34% of control at 80 min (coma). Glycogen decreased progressively to 18% of control during coma; ATP and P-creatine remained normal prior to 60 min, but declined to 65% and 60% of control respectively, during coma. Ammonia levels rose 3-fold at 20 min, and were more than 1.0 mmol/kg during coma.

Rats at 40 min of hypoglycemia (1.29±.04 mM plasma glucose) were made normoglycemic (6.02±.14 mM) within 10 sec and maintained at this level for 10 min by a programmed i.v. infusion of 5% glucose-saline. All rats (n=5) became self-supportive and alert between 7 and 10 min. Brains were freeze-blown at 1 and 10 min (n=4 each group) after the start of glucose infusion. Brain glucose rose to 34% and 78% of normal, glucose-6-P to 74% and 98% of normal, and glycogen to 31% and 40% of normal at 1 and 10 min, respectively.

In summary, neurological depression, progressing to coma, occurs at a constant plasma glucose below 1.6 mM. Normalization of neurological function following hypoglycemic depression is not instantaneous with normalization of plasma glucose but requires at least recovery of brain glucose-6-P.

285 IMPAIRED SYNTHESIS OF ACETYLCHOLINE (ACh) BY MILD HYPOXIC HYPOXIA OR NITROUS OXIDE. G.E. Gibson, T.E. Duffy & D.J. Jenden. Dementia Program, Burke Rehab. Ctr., Dept. of Neurology, Cornell Univ. Med. Col., N.Y., N.Y. 10021, & UCLA School of Medicine, L.A., CA 90024.

Earlier studies have shown that even mild histotoxic hypoxia (KCN) or anemic hypoxia ($NaNO_2$) impair the synthesis of brain ACh without altering the concentrations of lactate, ATP or cAMP (Gibson et al., 1978). We now report that mild hypoxic hypoxia impairs ACh synthesis. Since previous studies of hypoxic hypoxia on brain metabolism have often involved, as controls, animals breathing N_2O-O_2 mixtures (c.f. Siesjö, 1978), we also examined the effect of N_2O on ACh metabolism.

Adult rats (200 g) were used for all experiments. Tail artery and jugular vein cannulae were inserted the day before the experiment. During the experiment, rats were restrained in a "brain-blower", modified with a nose cone to deliver defined gas mixtures. One min before sacrifice, the rats received an iv pulse injection of [U-^{14}C]glucose (1 mCi/kg) and [$^2H_4$]choline (20 µmol/kg).

ACh synthesis was decreased in animals breathing 70% N_2:30% O_2 for 40 min, compared to rats breathing 70% N_2:30% O_2. The specific activity of the ACh synthesized from [$^2H_4$]choline ([$^2H_4$]ACh/total ACh) declined 53.5% [0.016±0.003 (n=9) to 0.007±0.001 (n=6), $P<0.05$]. The specific activity of ACh synthesized from [U-^{14}C]glucose (^{14}C-DPM in ACh/nmol of ACh) declined 54% [55.7±6.6 (n=3) to 25.7±1.4 (n=3), $P<0.01$]. ACh levels did not change significantly. Decreases in ACh specific activity were not due to decreases in the specific activities of the precursors, glucose and choline, nor to changes in blood pressure, PaO_2, $PaCO_2$, pH, or body temperature.

Thus, hypoxic hypoxia was studied in rats breathing N_2:O_2, and not N_2O:O_2, mixtures. ACh synthesis from both precursors was reduced when the inspired O_2 concentration was reduced from 30% (Pa O_2=127±3 mm Hg) to 15% (PaO_2=65±3 mm Hg) or 10% (PaO_2=46±3 mm Hg) for 15 min. The specific activity of ACh from [$^2H_4$]choline was reduced ($P<0.05$) by 49±13% (n=4), and by 63±12% (n=2) by 15% O_2 and 10% O_2, respectively. The specific activity of ACh from [U-^{14}C]glucose was reduced ($P<0.05$) by 44±8% (n=3), & by 46±6% (n=3) with 15% & 10% O_2, respectively. Neither the level of ACh nor the precursor pool specific activities changed significantly. Brain lactate increased ($P<0.05$) by 84±13% (n=4) and 292±42% by 15% O_2 and 10% O_2, respectively. $PaCO_2$, pH, and body temperature were unaffected; blood pressure fell ($P<0.05$) in the 10% O_2 group (112±3 to 96±6 mm Hg).

In conclusion, nitrous oxide anesthesia & mild hypoxic hypoxia decrease ACh synthesis in brain. Since brain lactate also rose in the hypoxic animals, additional experiments with even milder degrees of hypoxia will be required to distinguish whether decreased ACh synthesis or increased lactate concentrations occur first.

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286 ELEVATION OF PLASMA TYROSINE LEVELS IN NORMAL HUMANS AFTER ORAL ADMINISTRATION OF L-TYRSONE. Bruce S. Glaeser*, Eldad Melamed, John H. Growdon*, and Richard J. Wurtman. Laboratory of Neuroendocrine Regulation, M.I.T., Cambridge, MA 02139.

L-Tyrosine, 100 mg/kg or 150 mg/kg was administered to two groups of fasting human subjects. Plasma tyrosine levels were significantly elevated 2-8 hours after tyrosine ingestion. Mean plasma tyrosine levels were maximal at 2 hrs, rising from 69 ± 3 to 154 ± 10 nmol/ml after 100 mg/kg and to 203 ± 32 nmol/ml ($\bar{X}$ ± SEM) after the 150 mg/kg dose ($p < 0.001$ for both groups). The uptake of tyrosine into the brain is dependent on the tyrosine ratio, defined as the ratio of the plasma tyrosine level to the sum of the concentrations of the plasma neutral amino acids (i.e. valine, methionine, leucine, isoleucine, phenylalanine, and tyrptophan) that compete for the same blood-to-brain transport system. At 2 hours, the mean tyrosine ratio increased from 0.10 ± .02 to 0.28 ± .04 ($\bar{X}$ ± SEM) after the 100 mg/kg dose and to 0.35 ± .05 after the 150 mg/kg dose, indicating that oral tyrosine probably increases brain tyrosine levels in humans. In related studies, subjects took 100 mg/kg of tyrosine in 3 divided doses, along with 3 daily meals containing 115 grams of protein. The increase in plasma tyrosine was similar to that seen in the fasting subjects. The peak plasma tyrosine ratio was 0.21. In rats, administration of tyrosine elevates plasma and brain tyrosine concentrations and can accelerate the synthesis of brain dopamine and norepinephrine. If similar changes in catecholamine synthesis occur in humans, then tyrosine administration may be useful in treating certain CNS disorders characterized by inadequate catecholaminergic tone.

PLASMA TYROSINE CONCENTRATIONS AFTER TYROSINE ADM.

Tyrosine Dose	Plasma Tyrosine Concentration-nmol/ml ($\bar{X}$+SEM) 0HR	2HR	4HR	6HR	8HR
100 mg/kg	69±3	154±10	129±7	121±11	110±10
150 mg/kg	69±3	203±32	158±13	128±9	106±6

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287 SCIATIC NERVE RESPONSES OF PROTEIN MALNOURISHED RATS INDICATE PERIPHERAL ORIGIN OF HYPERREACTIVITY TO FOOT SHOCK. Robert D. Hall. Worcester Foundation for Experimental Biology, Shrewsbury, Mass. 01545.

Rats subjected to protein or protein-calorie malnutrition early in life frequently have been found to be more responsive than well-nourished rats to electric foot shock. This hyperreactivity has been interpreted by some investigators to indicate a hyperemotionality resulting from early deprivation. It is difficult to interpret the foot-shock data, however, because it is uncertain that effective shock levels were equated for rats of different sizes. It has been reported that small rats are more responsive to foot shock than larger ones. The present study was undertaken to determine if the malnourished rat's greater responsiveness to foot shock might be related to peripheral factors such as body size or thickness of the foot pads. Compound action potentials (CAPs) of the sciatic nerve were compared for protein malnourished and well-nourished rats.

Chronically malnourished rats were born to dams maintained on an 8% casein diet from 5 weeks before mating until weaning. The pups were maintained on the same low protein diet until they were studied at approximately 75 or 160 days of age. Well-nourished rats were reared by dams fed a 25% casein diet.

To record sciatic nerve responses the rats were anesthetized with pentobarbital and immobilized with tubocurarine. Monophasic current pulses were presented through stainless steel rods, one supporting the hindpaw, another supporting the ipsilateral forepaw, in an attempt to approximate the stimulus conditions of behavioral foot-shock situations. Contact between feet and rods was achieved by a silver skin contact solution. Single stimuli of increasing intensity were presented until the maximum CAP amplitude was obtained. A descending intensity series followed. The DC resistance between foot electrodes was measured before and after each stimulus series.

Absolute amplitudes of sciatic CAPs were greater over the entire intensity range in malnourished than in well-nourished rats. Because it is not clear why the maximum responses were larger in the malnourished rats, CAP amplitudes were transformed to percentages of each rat's maximum response. These functions also show the malnourished rat to be more sensitive to shock stimuli. Resistances between stimulating electrodes were significantly lower in the malnourished animals.

These findings indicate that the hyperreactivity of malnourished rats may be principally related to peripheral factors and not to altered CNS functions.

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288 THE EFFECT OF UNDERNUTRITION ON THE BRAINSTEM RETICULAR FORMATION OF THE RAT: A GOLGI STUDY. Ronald P. Hammer, Jr. Dept. Anatomy, Sch. Med., UCLA, Los Angeles, CA 90024.

Undernutrition has been shown to have a profound effect on the central nervous system of maturing experimental animals, not only on gross brain weight and content but also on cell structure and function. In this study, neonatal rats have been exposed to a moderate state of food deprivation to 20 days of age by increasing the litter size to 14 and removing the dam for 8 hours each day. Neurons of various Reticular Formation nuclei of the brainstem were then examined using modifications of Golgi stains.

Dendritic variation has been observed in experimental tissues in the form of nodularities and thickenings upon dendrites of lesser thickness and extent, and subtle increases in dendritic spine numbers. The resulting changes suggest that cell development is less advanced. The relative immaturity and abberant development of these neurons may be the morphologic substrate of behavioral variations observed in these animals.

289 AMINO ACID TRANSPORT INTO RAT BRAIN REGIONS IN VIVO.
Richard A. Hawkins, Anke M. Mans*, James J. Richter*, Barbara L. Helm* and Julien F. Biebuyck*. Depts. of Anes. and Phys., Hershey Med. Ctr., The Penn State Univ., Coll. of Med., Hershey, PA 17033

The carrier system transporting neutral amino acids from plasma into whole brain has been characterized in normal adult rats (Oldendorf (1971) Am.J.Physiol.22:1629-1639). The kinetics of this system, for which many neutral amino acids compete, changes considerably in rats with portacaval shunts or during hepatic failure, thus altering the rate of amino acid influx (Mans et al. (1979) J.Neurochem., in press). The localization of these changes to brain regions, however, has not been determined. In order to measure amino acid influx at the regional level, we developed a technique to measure the influx of amino acids into various regions of the rat brain under physiological conditions, (ie. without disturbing the normal equilibrium of plasma components or altering cerebral blood flow).

A tracer quantity of ^{14}C-labeled amino acid was infused intravenously at a rate which maintained a steady level of label in the blood for 1 to 3 mins (Bános et al., (1973) Proc.R.Soc. B 183: 59-70). Blood was collected throughout the experiment to obtain the plasma integral of the tracer concentration, and the complete amino acid profile by amino acid analysis. After decapitation, the brain was frozen, sections cut for quantitative autoradiography, and the regional dpm/g obtained by quantitative densitometry. The PS (permeability coefficient x capillary surface area) in different brain regions was calculated from the plasma integral and dpm/g brain after subtracting the background dpm in the area due to contaminating blood (Ohno et al., (1978) Am.J.Physiol. 235:H299-H307).

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

	PS ($ml \cdot min^{-1}$)	
	tyrosine	phenylalanine
Cortex	0.066	0.11
Caudate	0.058	0.076
Hippocampus	0.057	0.070
Inferior colliculus	0.087	0.15

For tyrosine the corresponding influx rates were 6.5, 5.7, 5.5 and 8.8 $nmol \cdot min^{-1} \cdot g^{-1}$ in cortex, caudate, hippocampus and inferior colliculus, respectively. Other substrates with low uptake rates can be studied similarly.

290 EFFECTS OF ENDOGENOUS MAGNESIUM ON THE DIURNAL RELATIONSHIPS AMONG DOPAMINE, NOREPINEPHRINE, SEROTONIN AND ELECTROLYTES IN THE RAT BRAIN.
Mary D. Healy*, Audelio Rivera*, and Michael Borucki* (SPON: Arvind T. Modak). Incarnate Word College, 4301 Broadway, San Antonio, TX 78209.

Physiological deprivation of Magnesium manifests itself through various metabolic dysfunctions. Neurological disturbances constitute the most prominent clinical indications of magnesium deficiency (MGD). Rats placed on a magnesium deprived diet (Mg = 4.5ppm) exhibited audiogenic seizures and a high degree of irritability to touch which were more pronounced during the dark cycle and during the light period. A forty-day study has shown a correlation between the susceptibility to seizures and the concentrations of norepinephrine (NE) and serotonin (5-HT) in the rat brain. In the present study the concentrations of dopamine (DA), NE and 5-HT, as well as the ions, Mg, Ca, Na, K and Zn have been determined hourly throughout the 24-hour period of the fifteen day of dietary mg restriction. The data collected was compared statistically with that obtained from similar determinations on brains from rats maintained on a normal diet (mg = 650ppm). Experimental and control were plotted on polar coordinate paper and the experimental elipses shown as ratios of the control amplitude and phases. (Supported by NIH Grant 1-S06-RR-08170-01).

291 DETERMINATION OF BIOGENIC AMINES IN DISCRETE BRAIN AREAS OF FOOD DEPRIVED RATS USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY.
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Levels of norepinephrine (NE), dopamine (DA), 5-hydroxytryptophan (5-HTP), 5-hydroxytryptamine (5-HT), and 5-hydroxyindole acetic acid (5-HIAA) in the lateral hypothalamus (LH), ventromedial hypothalamus (VMH), median raphe (MR) and dorsal raphe (DR) were determined in nondeprived and 48 hr. food deprived rats. Simultaneous determination of these compounds was accomplished by means of high performance liquid chromatography (HPLC) with electrochemical detection.

Tissue samples were homogenized in formic acid/acetone and the extracts washed with 0.5 ml of heptane/chloroform. The aqueous portion was then dried down and resuspended in 100 µl of the HPLC buffer. The HPLC system utilized a C-18 reverse phase column coupled with a glassy carbon detector set at a potential of 0.8 volts versus the reference electrode. The electronic controller was set at 5 na/v and the recorder at 0.1 volt full scale. The buffer was 0.1 M citrate-disodium phosphate, pH 3.5, containing 0.004% sodium octyl sulphate and 12.5% methanol by volume. The flow rate was maintained at 1.0 ml per min. Twenty microliters of each sample were injected on the HPLC. The compounds of interest (identified by retention times of standards) were quantified by determining the area under the peaks and their contents were determined from standard curves. Results of the HPLC assay procedure demonstrate that this is a simple, sensitive, and rapid method for the simultaneous determination of NE, DA, 5-HTP, 5-HT and 5-HIAA content in the same small brain sample.

When compared with controls, food deprived animals showed significant increases in 5-HT and 5-HIAA levels in the raphe nuclei, significant increases in 5-HIAA in the LH, but no changes in either 5-HT or 5-HIAA levels in the VMH. No changes in catecholamine levels were found in any of the brain areas studied. These results show that indoles in the raphe nuclei, as well as in the LH, are affected by food deprivation. The lack of change in indole levels in the VMH indicates that specific nuclei within the hypothalamus are differentially affected by food deprivation. (Supported by NIMH grant No. PHS-T01-MH10695-13, Indiana Dpt. of Mental Health Grant no. 178-679-005 and Indiana Attorney General's Fund.)

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

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

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

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

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

293 EFFECTS OF HYPERCAPNIA ON INTERMEDIARY METABOLISM OF DEVELOPING RAT BRAIN. Alexander L. Miller, David H. Corddry* and Colleen A. Kiney*. Labs. Psychiat. Res., McLean Hospital. Belmont, MA 02178.

Developing rats, 10 and 20 days post-natal, were placed in an atmosphere of 20% CO_2, 21% O_2 and 59% N_2 for periods up to 15 minutes. Brains were obtained with the freeze-blowing apparatus. Rates of brain glucose utilization were determined by measuring uptake of [2-^{14}C]glucose and [^{3}H]deoxyglucose. Metabolites were assayed by standard enzymatic methods.

In control rats glucose utilization by brain increased from 0.15-0.16 µmol/min per g at 10 days to 0.40-0.43 µmol/min per g at 20 days, about 60% of the adult rate. CO_2 treatment produced a 50% decline in brain glucose use which persisted throughout the period studied in animals in both age groups. In all conditions there was reasonable agreement between the rates calculated with each isotope.

Metabolite analysis indicated that early after the onset of hypercapnia (2 min) there was inhibition of phosphofructokinase out of proportion to other glycolytic enzymes. This inhibition became less evident over the time course, though glycolytic flux remained diminished. Concentrations of most intermediary metabolites and of glutamate progressively decreased during hypercapnia. It is hypothesized that these metabolites were oxidized in order to maintain energy production. In the case of the 20 day old animals, however, their rate of disappearance lessened with time, so that other non-glucose fuels would have been needed to maintain normal oxygen consumption at later times.

294 EFFECTS OF ADRENALECTOMY ON TRYPTOPHAN METABOLISM IN NORMAL AND PROTEIN MALNOURISHED RATS. Maravene Miller*, J. Patrick Leahy*, Oscar Resnick* and Peter J. Morgane. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

Rats born to dams fed a low protein diet (8% casein) or a normal diet (25% casein) starting 5 weeks prior to conception were bilaterally adrenalectomized (ADX) at 30 days of age. At 60 days of age the 8% and 25% ADX rats were compared to sham (S) operated and control (C) litter mates of each diet. The results (Table below) indicate that ADX caused significant decreases of 28-40% for whole brain serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) levels, and significant increases of 38-47% for tryptophan (TP) levels in both the 8% and 25% ADX rats as compared to their respective controls. Plasma constituents showed no significant differences between the ADX and S+C rats of each diet group except for corticosterone (COR) levels. The latter findings indicate that the adrenal gland plays a minimal role in producing the changes in plasma albumin (ALB) and fatty acid (NEFA) levels and the resulting alteration in TP availability seen in the 8% casein rats (Miller et al., Exp. Neur. 57: 142, 1977). However, the effects of long-term ADX in young rats of both diet groups indicate that the adrenal cortex may regulate brain TP metabolism by: (1) maintaining normal activity of TP hydroxylase, the rate limiting enzyme in 5-HT synthesis, and (2) inhibiting the transport of TP to the brain.

Effects of Adrenalectomy on 8% and 25% Casein-fed Rats

Diet Group (n per group)	8% S+C (7)	8% ADX (6)	25% S+C (8)	25% ADX (8)
Brain				
5-HT ng/g	632 ± 11	378 ± 11	383 ± 6	268 ± 7
5-HIAA ng/g	683 ± 16	423 ± 11	431 ± 6	310 ± 7
TP ng/g	4619 ± 93	6812 ± 12	3535 ± 11	4898 ± 67
Weight mg	1580 ± 63	1680 ± 59	1773 ± 34	1854 ± 29
Plasma				
T* TP ng/ml	7956 ± 689	8417 ± 309	18570 ± 1102	18659 ± 1050
F† TP ng/ml	3534 ± 335	3820 ± 283	1506 ± 124	1440 ± 73
Pro** mg/ml	76.8 ± 5.6	71.8 ± 4.0	89.9 ± 3.5	90.2 ± 4.7
ALB mg/ml	37.2 ± 2.5	44.4 ± 3.7	71.4 ± 3.6	65.7 ± 2.4
NEFA µeq/ml	1.078 ± .10	1.213 ± .14	0.710 ± .08	0.703 ± .08
COR µg/100ml	78.5 ± 10.2	3.3 ± 0.5	85.6 ± 22.3	5.3 ± 0.6
Body Wgt. g	114 ± 8	95 ± 17	284 ± 27	267 ± 21

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

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295 CEREBRAL BLOOD FLOW IN NORMOXIA AND HYPOXIA FOLLOWING LESIONS OF MEDULLARY REGIONS MEDIATING PRESSOR RESPONSES TO BRAINSTEM ISCHEMIA. M. Nakai*, M. Kumada, G.R. DiResta* and D.J. Reis. Lab. of Neurobiol., Dept. of Neurol., Cornell Univ. Med. College, New York, NY 10021.

The elevation of systemic arterial pressure (AP) elicited by cerebral ischemia or brainstem distortion (Cushing reflex) is mediated by a restricted region of the dorsal medullary reticular formation (DMRF) comprising, largely, portions of the parvocellular and gigantocellular nuclei (Kumada et al., Circ. Res., 1979, in press). Electrical stimulation of this region simulates the vasomotor responses to brainstem ischemia or systemic hypoxia (Dampney et al., Circ. Res., 1979 in press). Electrical stimulation of this region in sympathectomized animals increases focal cortical cerebral blood flow (CBF) (MacKenzie et al., in press). Since systemic hypoxia also increases CBF, we sought to establish if lesions of the DMRF would also impair the CBF response to hypoxia. A total of 32 rabbits were anesthetized with urethane and paralyzed by gallamine. Their cervical sympathetic trunks were severed bilaterally. Blood gases and pH were controlled by artificial ventilation with O_2, CO_2 and N_2. Local CBF was measured in the gray matter of the parietal cortex by H_2 clearance. Bilateral electrolytic lesions, destroying most of the gigantocellular and parvocellular nuclei in DMRF, were placed 1-3mm (A1-3), or 5-7mm (A5-7) rostral to obex. AP was maintained in the autoregulated range at about 105mmHg. Continuous i.v. infusion of norepinephrine (NE) was required to maintain AP constant, especially after placement of lesions. Therefore cerebrovascular responsivity to hypoxia was always evaluated as a function of the dose of infused NE. CBF in normoxia in the intact rabbits was 43.7 ± 10.6 ml/min/100g tissue (n=41). Reduction in PaO_2 to 29.8 ± 1.5mmHg resulted in an increase in CBF to 147 ± 25% (n=19) of control. Cerebrovascular responsivity to hypoxia in the rabbits with intact DMRF, with lesions of A1-3 and A5-7, did not differ from each other ($P > 0.05$). Such lesions, however, resulted in approximately a 50% increase in CBF ($P < 0.01$). We conclude: (a) Integrity of regions in DMRF mediating vasomotor responses to cerebral ischemia and distortion are not required for the increase in CBF elicited by systemic hypoxia. (b) Neurons located within or passing through the lower medulla may tonically constrict cerebral blood vessels.

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296 REGIONAL HISTOCHEMICAL ANALYSIS OF SORBITOL DEHYDROGENASE ACTIVITY IN THE NERVOUS SYSTEM OF THE RAT. Susan E. Orosz*, Samuel F. Townsend* and Patricia A. Tornheim* (SPON: G. Eckstein) Dept. Anat., Univ. Cincinnati College of Medicine, Cincinnati, Ohio 45267.

Sorbitol, a sugar alcohol, has been suggested to play a role in the development of diabetic neuropathy (Gabbay et al. Science 151:209, 1966) and cerebral edema associated with diabetic ketoacidosis (Clements et al. Lancet 2:384, 1968). Previous investigations concerning the presence of this polyol in the nervous system have consisted primarily of biochemical demonstration of sorbitol dehydrogenase activity in normal rat brain (Rehg and Torack J. Neurochem 28:655, 1977), normal and diabetic spinal cord, and normal and diabetic peripheral nerve (Gabbay and O'Sullivan, Diabetes 17:239, 1968). On the basis of their biochemical data, Gabbay and O'Sullivan suggested the activity of this enzyme is axonal in peripheral nerve and within neuronal cell bodies in the spinal cord. The present histochemical study was designed to provide fundamental information concerning regional localization of sorbitol dehydrogenase activity in the central and peripheral nervous systems of the normal rat.

For these histochemical studies, fresh, frozen samples were taken from brain (including cortex, corpus striatum, thalamus, and medulla), cervical spinal cord, and sciatic nerve in normal, adult male Sprague Dawley rats. Cryostat sections (15 u) were evaluated for the presence of sorbitol dehydrogenase activity according to the procedures described by Johnson (J. Histochem and Cytochem 13:583, 1965 and J. Histochem and Cytochem 15:207, 1967). These sections were incubated for 2 hrs in one of three media: (1) with sorbitol and phenozine methosulfate (PMS), (2) without PMS and (3) without sorbitol. Seminal vesicle was utilized as a tissue control.

Examination of sections through the central nervous system showed an absence of demonstrable enzyme activity in the parenchyma, but strong reactivity in the choroid plexus, ependyma and pia mater. Although enzyme activity was not visible in peripheral nerve processes, blood vessels associated with peripheral nerves were reactive. These data indicate that the greatest sorbitol dehydrogenase activity in the central and peripheral nervous systems of the rat is extraneuronal, not intraneuronal as suggested by Gabbay and O'Sullivan.

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297 EFFECT OF PROSTACYCLIN ON LOCAL CEREBRAL GLUCOSE UTILIZATION AND CEREBRAL BLOOD FLOW. H. M. Pappius*, L. S. Wolfe, and U. Goehlert*. Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada H3A 2B4.

Vascular endothelium synthesizes prostacyclin, an unstable prostaglandin (t 1/2, 2-4 min. pH 7.4) which strongly inhibits platelet aggregation, increases cAMP synthesis and vasodilates most blood vessels. The capacity for synthesis of prostacyclin from endogenous precursor by rat cerebral arteries and arterioles is 2-4 μg/g tissue/10 min incubation periods at pH 7.4. Highly purified preparations of cerebral capillaries isolated from rat cerebral hemispheres also synthesize prostacyclin in amounts of 12-15 ng/mg protein. Topical application of prostacyclin (10-20 μg) for 3 minute periods to the exposed cerebral cortex of rats anesthetized with nembutal caused an immediate striking vasodilation of the surface blood vessels. Local cerebral glucose utilization measured by the ^{14}C-deoxyglucose technique was markedly increased (40-45%) in the parietal and auditory cortex and the effect was dose-dependent. Local cerebral blood flow measured by the ^{14}C-iodoantipyrine method was also significantly increased by 20-28% in the cerebral cortical region of application. Application of prostacyclin to the intact dura dilated the dural blood vessels but had no effect on the glucose utilization or blood flow in the underlying cerebral cortex. These studies suggest that prostacyclin released locally from cerebral vascular beds can not only affect blood flow but also increase cerebral glucose metabolism.

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

Gonadal steroids are concentrated severalfold in brain relative to plasma, but brain corticosterone levels are only 40% of plasma values; these observations suggest differences may exist between the kinetics of brain sequestration of the gonadal steroids vs corticosterone. The rate of brain sequestration of the sex steroid hormones (progesterone, 17-hydroxyprogesterone, dihydrotestosterone, testosterone, estradiol) vs corticosterone was investigated with a carotid injection technique in barbituate-anesthetized adult male rats. The rate of change with time of test steroid radioactivity (T) in brain after pulse labeling via the carotid artery was a function of the rate of efflux (k_2) of steroid back to blood minus the rate of sequestration (k_3) of the steroid hormone by brain. Therefore, by employing an internal ^{14}C reference (R), e.g., butanol or antipyrine, that left the brain at the same k_2 as the ^{3}H-test hormone, the rate of change with time in the T/R ratio lead to the estimation of k_3 for the steroid hormone. Based on a two-compartment model, the parameters (T/R), k_2, and k_3 were related as follows:

$$T/R = \frac{k_3}{(k_2 + k_3)} e^{k_2 t} + \frac{k_2}{(k_2 + k_3)} e^{-k_3 t}$$

Given the k_2 value for the reference, the T/R ratios at various times after carotid injection (0.25-4.0 min) were fitted to the above equation by a non-linear regression analysis. The $k_3 \pm$ SE:

Steroid	k_3 (min^{-1})	Octanol:Ringer's
Dihydrotestosterone	0.33 ± 0.03	1340
Progesterone	0.29 ± 0.03	1870
Testosterone	0.29 ± 0.04	530
Estradiol	0.27 ± 0.04	196
17-Hydroxyprogesterone	0.08 ± 0.01	1809
Corticosterone	<0.05	66

No correlation was found between k_3 and the octanol:Ringer's partition coefficient ($r=0.18$, $p<0.40$), suggesting the steroids were not being sequestered by brain lipid. No change in corticosterone k_3 was observed in the adrenalectomized rat, indicating the lack of brain retention of ^{3}H-corticosterone was not due to high levels of endogenous hormone. Thin layer chromatographic analysis of brain ^{3}H-progesterone radioactivity at 3 min after carotid injection showed progesterone was unmetabolized. Conclusions: The turnover rate of brain binding of blood-borne sex steroids, $t_{½}$ approximately 2 min, is much higher than for corticosteroids, a finding which correlates with the much higher volume of distribution of the gonadal steroids in brain relative to corticosterone.

299 ISOLATION OF γ-GLUTAMYL TRANSPEPTIDASE FROM GLIAL CELLS E. Reyes* and L.C. Saland (Spon. L.D. Partridge) Depts. Pharmacology and Anatomy, School of Medicine, University of New Mexico, Albuquerque, N.M. 87131

Membrane-bound γ-glutamyl transpeptidase (γ-GTP), believed to be the key enzyme required for the coupling of amino acid uptake to glutathione metabolism via the γ-glutamyl cycle, has been found in cellular elements of rat cerebral cortex. Neuronal and glial localization of the enzyme indicates that the operation of the γ-glutamyl cycle and thus amino acid transport via this cycle occurs in neuronal and glial cells. Although the physiological role of the enzyme has not been established, there is evidence to suggest that it may be involved in alcoholism, epilepsy and mental retardation. Multiple forms of the enzyme have been isolated and partially purified from brain. The intent of the present study was to determine if multiple forms of the enzyme were present in a glial enriched fraction of rat cerebral cortex. Rats were decapitated and the cortex dissected and chopped into 1-2mm sections while suspended in a solution of cold 7.5% polyvinyl pyrolidone (PVP), 1% BSA and 10mM $CaCl_2$. The tissue was passed through a series of sieves (333 to 73 μpore size) and layered onto a two-step discontinuous sucrose gradient (1.0 to 1.75M) and centrifuged. The impure glial cells at the 1.0-1.75M sucrose interface were resuspended in 7.5% PVP and 5% ficoll, sieved through 73μ sieve and layered on a discontinuous ficoll gradient. After centrifugation the band collecting at the 1.2-1.65M sucrose interface was washed in 0.32M sucrose and saved for enzyme isolation. A portion of the pellet was fixed in cacodylate-buffered 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide, dehydrated in ethanol and embedded in Epon for microscopy. At the light microscopic level, the glial cell fraction consisted of some small cell fragments which had the appearance of glia, and a large amount of amorphous material. In contrast, the neuronal fraction contained larger cells and a few capillaries. Electron microscopy revealed that the amorphous material in the glial fraction was composed of membranes and ruptured organelles. The neuron fraction contained cells with obvious neuronal characteristics, including prominent nucleoli and abundant rough endoplasmic reticulum. Four different forms of the enzyme γ-GTP were isolated from the glial fraction by chromatography on concanavalin A. The isoenzymes were characterized with respect to molecular weight, Km for γ-glutamyl-p-nitroanilide, mobility on polyacrylamide gels and inhibitory effects of ethanol. Based upon the presence of γ-GTP in glia, we suggest that a possible role of glial cells is in the transport of amino acids to neurons via the γ-glutamyl cycle.

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300 CONTROL BY CARBON DIOXIDE OF GLYCOLYTIC FLUX OF NICOTINAMIDE ADENINE DINUCLEOTIDE IN NEURONS OF DORSAL ROOT GANGLION.
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Previous works have shown that changes of carbon dioxide partial pressure increases oxidation of the steady state level of reduced nicotinamide adenine dinucleotide (NADH) in neurons of frog dorsal root ganglion. In this work it was expected that this change observed in aerobic and in anaerobic conditions would be blocked differentially with metabolic inhibitors. 2-Deoxy-D-glucose (2-DG) and Amytal were used as inhibitors. The 2-DG blocks NADH formation in the glycolytic pathway and Amytal blocks the respiratory chain. Fluorometric determinations of NADH were done on in vitro preparations of dorsal root ganglion as previously reported, pH and pO_2 were measured simultaneously. CO_2/O_2 (2.5%/97.5%), CO_2/N_2 (2.5%/97.5%) replaced temporarily the O_2 and N_2 of the chamber. All gases were moistened and records were taken at a temperature of 25°C. NADH showed and inmediate and fast decrease(oxidation) after O_2 was replaced with CO_2/O_2 and also after N_2 was replaced with CO_2/N_2 (about 3% and 15% respectively). After the incubation of the preparation in 2-DG (20 mM, 30-60 min), oxidation with CO_2 mixtures were not observed. After incubation of the preparation in Amytal (5 mM, 5 min), NADH oxidation was observed after CO_2 mixtures, but NADH did not change after O_2 was replaced with N_2, and after O_2 replaced N_2, Amytal blocked the respiratory chain and reduction of NAD and oxidation of NADH were not observed. The steady state level of NADH is higher than normal.

2-DG blocks the glycolytic pathway (phosphoglucoisomerase) and NADH is formed less. Under 2-DG inhibition the oxidation of NADH is not observed, neither in O_2, nor in N_2 with CO_2 mixtures, but oxidation of NADH is observed after O_2 replaced N_2(respiration). The Amytal blocks the respiratory chain (NADH-flavoprotein) and oxido-reduction was not observed during O_2-N_2-O_2 transitions. Under Amytal action oxidation of NADH with CO_2 mixtures can be attributed to lactate formation.

These results indicate that the oxidation observed with the CO_2 mixtures comes from the NADH of glycolytic pathway either in aerobic and in anaerobic conditions.

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301 ^{14}C-2-DEOXYGLUCOSE UTILIZATION IN THE CEREBELLUM OF THE MONKEY DURING A CONDITIONED ARM MOVEMENT. Robert J. Schwartzman, Jen Yu, Guillermo M. Alexander*, Depts. of Medicine and PM&R, UTHSC, San Antonio, TX 78284, Joel Greenberg*, Martin Reivich, Dept. of Neurology, Univ. Pennsylvania School of Medicine, Philadelphia, PA 19104.

The metabolic rate for glucose was computed in the cortex and cerebellar nuclei using the quantitative ^{14}C-2-deoxyglucose (2DG) technique. The monkey was operantly conditioned to pull a one pound weight for 12 cm with its right arm while sitting in a primate chair. The animal was made to pull the weight for 10 minutes prior to injection of isotope, and throughout the 45 minute experiment. A bolus of 2DG (100 µci/kg) in 3 ml of saline was injected intravenously. Arterial blood samples (.5 cc) were drawn every 15 seconds for the first minute, at 1 minute intervals for the next nine minutes and at 5 minute intervals for the remainder of the study, in order to determine the arterial plasma ^{14}C and glucose concentrations. Forty-five minutes after 2DG injection the animal was sacrificed with an overdose of Nembutal followed by 10 cc of saturated KCl, the brain was removed and frozen in liquid freon. The brain was then cut into 20 micron sections and exposed on x-ray film, Kodak SB5. The tissue ^{14}C concentration was obtained by autoradiographic techniques with the aid of a microdensitometer (.25 mm aperture). The local metabolic rate for glucose was calculated quantitatively from the ^{14}C tissue concentration, the plasma 2DG and glucose curves and the rate and lumped constants as computed by Kennedy, et al (Ann Neurol 4:293-301, 1978). Cerebellar glucose utilization was analyzed from anterior to posterior in medial, intermediate and lateral cortex as well as fastigial, interpositus and dentate nuclei bilaterally. Glucose utilization was more active on the right side. The metabolic rate in µ moles/100g/min, ranged from 21.5 to 95.6 in the right cortical areas and 40.7 to 127.9 in the right nuclei. It ranged from 16.9 to 55.4 in the left cortical areas and 37.8 to 93.3 in the left nuclei. The bilateral difference varied from anterior to posterior according to present concepts of somatotopic localization. Our findings support present understanding of cerebellar physiology and anatomy. This study further demonstrates the value of the 2DG technique as an experimental tool in the study of functional neuroanatomical organization.

302 RELATIONSHIPS BETWEEN UNIT DISCHARGES, EXTRACELLULAR POTASSIUM ION ACTIVITY AND OXIDATIVE METABOLISM IN THE BULLFROG OPTIC TECTUM. Thomas J. Sick* and Norman R. Kreisman, Department of Physiology, Tulane University School of Medicine, New Orleans, La. 70112.

Unit activity, extracellular potassium ion activity (aK^+_o), and tissue oxygen tension (PtO_2) were monitored simultaneously in the optic tectum of the bullfrog (Rana Catesbeiana) in an effort to better understand the interactions between local electrical, ionic, and metabolic processes in brain. Local tissue oxygen tension and unit activity were measured from single polarographic microelectrodes and aK^+_o was monitored by means of liquid ion exchanger microelectrodes.

Physiological activation of units in the bullfrog optic tectum was achieved by moving a 10° black disc through the visual field. Such stimulation elicited a transient (2-4 sec) burst of multiunit activity, accompanied by a transient rise in aK^+_o of 0.5 - 1.0 mM. The potassium transients returned to baseline within 10-30 sec. with half-recovery times averaging 4.9 ± 0.29 sec (Mean ± SEM). These events were accompanied by a transient fall in local tissue oxygen tension lasting from 30 sec to as long as 3 min. Positive correlations were obtained between the intensity of the multiunit discharge and both ΔaK^+_o and ΔPtO_2.

Superfusion of the optic tectum with low concentrations of the Na-K ATPase inhibitor, ouabain ($5x10^{-6}$ to 10^{-5}M), resulted in a gradual rise in baseline aK^+_o. This rise was accompanied by a 50-100% increase in the half-recovery time of the potassium transient. Ouabain also reduced both baseline and ΔPtO_2 and diminished the intensity of the multiunit burst. Greater doses of ouabain induced episodes of spreading depression.

Ventilation of the bullfrog with 100% N_2 for 5-10 min produced a rise in baseline aK^+_o as well as a 50-100% prolongation of the half-recovery time of the potassium transient. The expected fall in baseline PtO_2 initially was accompanied by hyperexcitability followed by depression of multiunit activity in the optic tectum.

These results illustrate that a tight coupling exists between local electrical, ionic and metabolic events in the bullfrog central nervous system. They also demonstrate that active ion transport participates in the clearance of excess K^+_o. Energy for K^+ clearance is apparently derived, at least in part, from oxidative metabolism. The component of K^+ clearance which is mediated by oxidative metabolism is necessary for the maintenance of normal neuronal function.

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303 THE UTILIZATION OF L-3-HYDROXYBUTYRATE IN RAT BRAIN DURING DEVELOPMENT. Kenneth Swiatek*, George Dombrowski Jr.*, Kuen-Lan Chao* and Hsiang Chao* (SPON: R. Karrer). Inst. Study Dev. Disabilities, Chicago, IL 60608.

The in vivo and in vitro metabolism of L-(3-^{14}C)-hydroxybutyrate in rat brain was studied during development. Rat pups at birth, 6, 15, and 23 days of age were injected with either the D- or the L-isomer of (3-^{14}C)-hydroxybutyrate (10 µCi/100 g body wt). After 1 hr the animals were killed and the incorporation into cerebral proteins, lipids, and amino acids examined. The total incorporation as well as the incorporation into the protein, amino acid, and lipid fractions from either isomer increased from birth thru 15 days of age. Although the pups injected with the L-isomer contained significantly more label per gram brain than the controls at ages 6, 15, and 23 days, a greater proportion of the label was found in the non-metabolized (3-^{14}C)-hydroxybutyrate fraction. D-3-hydroxybutyrate labelled brain protein (1.6 X) and amino acids (1.9 X) more effectively than the L-isomer in newborn rats, and was a better precursor of brain protein at 6 and 15 days of age. Both isomers distributed similarly into four brain amino acids at 15 days of age, glutamate, glutamine, aspartate and GABA. Glutamate accounted for 51 % of the ^{14}C incorporated into brain amino acids for pups injected with either isomer. In newborn rats and at age 6 days greater incorporation into brain lipid was observed with the D-isomer. However at 15 and 23 days of age incorporation from the L-(3-^{14}C)-hydroxybutyrate into brain lipids was 2 X that found for the D-isomer. At 15 days of age 56 % of the labelled carbon of brain lipids from animals injected with the L-isomer was found in the sterol fraction, whereas only 41 % of the labelled carbon was found in the sterol fraction when the D-isomer was injected.

The production of CO_2 from both isomers was measured in vivo throughout the neonatal period as well as in a series of brain slice experiments from animals at 15 days of age. At 6 and 15 days of age the in vivo $^{14}CO_2$ production from the L-isomer was 50 % of that observed for D-3-hydroxybutyrate. In brain slices from animals 15 days of age the total CO_2 released by the oxidation of the D-isomer was 15-fold that produced by the L-isomer in 1 hr. ATP and Coenzyme A were found to stimulate the production of CO_2 from the L-isomer (2 X). Methylmalonate, a 3-hydroxybutyrate dehydrogenase inhibitor, decreased CO_2 production from the D-isomer, but was without effect on the oxidation of the L-isomer.

In conclusion it has been shown that L-3-hydroxybutyrate is incorporated throughout development into brain protein, amino acids and lipid and that its utilization is stimulated by ATP and Coenzyme A.

304 RELATION BETWEEN GLYCOGEN AND GLUCOSE LEVELS OF BRAIN STRUCTURES AND LACTIC ACID ACCUMULATION DURING CIRCULATORY ARREST. Kenneth R. Wagner and Ronald E. Myers. Lab. Perinatal Physiol., NIH, Bethesda, MD 20205

Certain nuclei in the brain stem accumulate lactic acid at high concentrations during circulatory arrest and this accounts for their special vulnerability to injury in food-deprived animals (Wagner & Myers, Neurol. 29: 546, 1979). Because circulatory arrest is associated with a metabolic locked-in state, extent of lactic acid accumulation, and, thus, vulnerability to injury is determined by glycogen and glucose levels. Vulnerable structures must contain one or both substrates at high concentrations. The present study examines this question by measuring glycogen and glucose in normal goat brain. Brain composition was preserved by in situ freezing (Welsh & Rieder, J. Neurochem. 31: 299, 1978). Tissue samples were assayed for glycogen, glucose, lactate, and ATP using enzymatic fluorometric procedures. The vulnerable brain stem nuclei contain glycogen at high concentrations (> 6.0 µmoles/g-glucosyl units). In contrast, cortex, hippocampus, caudate nucleus, thalamus, cerebellum, and hemispheral white matter all of low vulnerability exhibited lower glycogen contents (3-5 µmoles/g). Glucose varied and showed no definite pattern of distribution though it tended to be higher in cortex (range 3-5 µmoles/g) and lower in thalamus (2-3 µmoles/g) and brain stem (2.5-4 µmoles/g). Gray and white matter exhibited similar glucose levels. Lactic acid (1-3 µmoles/g) and ATP (1.75-2.5 µmoles/g) all fell within ranges described by others. These results show: (1) In situ freezing preserves normative values for metabolic intermediates throughout brain in large animals. (2) Vulnerable brain stem nuclei that accumulate lactic acid to a marked degree during circulatory arrest normally contain glycogen at the highest concentrations. (3) Glucose is distributed with no outstanding differences. Conclusion: In food-deprived animals exposed to circulatory arrest (anoxia), it is the glycogen concentration that most critically determines lactic acid accumulation and therefore the distribution of brain pathology.

305 REGIONAL VARIATIONS OF pH IN ISCHEMIC BRAIN: CORRELATION WITH TISSUE LEVELS OF ATP, LACTATE, AND NADH. Frank A. Welsh* (SPON: T.W. Langfitt). Division of Neurosurgery, University of Pennsylvania, Philadelphia, Pa. 19104.

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

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

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306 A LASTING EFFECT OF POSTNATAL UNDERNUTRITION ON THE RAT ELECTRORETINOGRAM. Wiggins, R.C., Fuller, G.N. and Dafny, N. (SPON: P. Gildenberg, M.D.). Univ. Texas Medical School at Houston, Dept. Neurobiology & Anatomy, Houston, TX 77025.

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

CEREBELLUM

307 DEVELOPMENT OF CEREBELLAR REGULATION OF FORELIMB TACTILE PLACING MOVEMENTS IN KITTENS. Vahe E. Amassian, Alan Rudell and Larry Eberle* Dept. of Physiology, SUNY Downstate Medical Center, Brooklyn, N.Y. 11203.

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

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

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

308 INTERACTION BETWEEN SIGNALS FROM THE VESTIBULAR AND FORELIMB RECEPTORS AS RECORDED FROM PURKINJE CELLS OF THE FROG VESTIBULOCEREBELLUM. J. Amat* and R. Llinás (SPON: H. Vanegas). Dept. of Physiol. & Biophys., New York Univ. Med. Ctr., 550 First Ave., New York 10016.

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

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

The present results suggest that the climbing fiber input to the vestibulo-cerebellum is involved in error signalling, given that its response only occurs in the absence of the appropriate compensatory limb movement. Thus, when the body-forelimb movement of the paradigm mimicked the compensatory movement of an unrestrained animal tilting sideways, no climbing fiber response was elicited. (Supported by USPHS grant NS-13742 from NINCDS and by the CONICIT Program of Graduate Fellowships)

309 SHORT LATENCY INHIBITORY ACTION OF A PARALLEL FIBER VOLLEY UPON ACTIVATION OF PURKINJE CELLS IN THE RAT CEREBELLUM: INDIRECT EVIDENCE FOR A FIELD EFFECT INHIBITION. Herbert Axelrad* and Henri Korn. INSERM U3. CHU Pitié-Salpêtrière. Paris 13°. France.

The influence of parallel fiber volleys set up by a surface (Loc) stimulation on antidromic spike potentials of Purkinje cells (PC) evoked by a juxta fastigial (JF) stimulation was studied on the cerebellum of lightly nembutalized albino rats. Data obtained at the level of the PC layer were computed with respect to the time of arrival of the superficially recorded presynaptic volley. The test/control curve obtained by progressively delaying the intervals between stimuli by steps of no more than 200 µsec revealed that the classical phase of PC chemical inhibition attributed to the action of cortical interneurons by ECCLES, LLINAS and SASAKI (Exp. Br. Res., 1 : 17, 1966) can be preceded by an earlier phase of inhibition. This first peak, not previously reported, starts about 0.5 msec after the presynaptic volley and lasts 0.5-1.2 msec, that is until PC activation; it brings about a slight reduction of the PC antidromic field potential of 5 to 20%. Its time course, brief duration and persistence after near complete chronic deafferentation (i.e. following section of the inferior and medial cerebellar peduncles) suggest that it is generated by a mechanism other than those already described in the cerebellum. Single unit analysis revealed that part of this peak is due to collision of the antidromic spikes with those evoked by direct activation of mossy fibers collaterals. However, in the absence of collision, a weak inhibition is still present during this period. It is indicated by a delay or a block of the PC antidromic spike under different experimental conditions: occasionally when the antidromic spike is evoked by single JF stimulations, or more consistently when it occurs during the relative refractory period of PC antidromic invasion, that is either after a spontaneous discharge of the cell, or after the second shock of adequately timed paired JF stimulations. Since this inhibition a) starts with a latency which is too short to be accounted for by two synaptic delays required for a chemical inhibition mediated through cortical interneurons b) indeed occurs well before intracellularly recorded IPSPs c) is resistant to picrotoxin unlike the chemical inhibition d) and since there is a peculiar mode of termination of basket cells upon PC (SOTELO and LLINAS, J. Cell Biol., 53 : 271, 1972) this early Loc evoked inhibitory effect is most likely the consequence of a field effect exerted by basket cells on the PC initial segment in a manner similar to that described for the Teleost Mauthner cell.

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

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

In this report we describe anatomical and electrophysiological evidence that the parafloculus (Pf) receives information from the auditory cortex (AC) via relays in the pontine grey (PG). The techniques of 1) electrical stimulation of discrete regions within the AC and unit recording of parafloccular Purkinje cells and 2) orthograde and retrograde transport of labeled amino acids and horseradish peroxidase (HRP), respectively, were employed to demonstrate the existence of an AC input to the Pf.

From unit recordings of 52 identified parafloccular Purkinje cells in halothane anesthetized rats, post-stimulus time histogram (PSTH) analysis of 10 cells (19.2%) showed evidence of either mixed, excitatory-inhibitory (4 cells, 40%) or pure inhibitory (6, 60%) mossy fiber input following electrical stimulation of the AC (double pulses, 0.2 ms duration, -0.05 - -0.6 mA, 1-10 Hz). The mean latencies to the onset of excitation and inhibition were 9.5 ± 3 and 14 ± 2.4 ms, respectively. Climbing fiber input responses were not elicited with cortical stimulation.

Following hydraulic injections (0.1-0.2 ul) of tritiated leucine (77 uCi/ul) in the AC , autoradiographic labeling representing terminal fields were observed as a column of silver grains oriented dorsoventrally under the cerebral peduncle and extending through the lateral region of the middle pons. Placements of HRP in the Pf resulted in labeled cells in the lateral area of the PG, corresponding to the lateral region of AC afferents. This anatomical finding further supports the concept of a cerebro-ponto-cerebellar pathway as defined electrophysiologically.

Our previous studies have described a visual input to the Pf. The results of the present study also demonstrate an input from the AC, which indicates that the integration of auditory and visual information by the cerebellum is carried out at least by two areas- the well known classical posterior vermal region and the Pf. (Supported by NSF BNS 77-01174 and a grant from the Biological Humanics Foundation to DJW)

311 EXCITABILITY CHANGES OF NEURONS IN THE CEREBELLAR NUCLEI FOLLOWING CEREBELLAR SURFACE STIMULATION. Heinrich Bantli, Earl Wagor and Carl R. Hansen, Jr.* Dept. Neurosurg., Univ. Minnesota, Minneapolis, MN 55455

Electrical stimuli applied to the cerebellar surface have been proposed by some investigators to activate Purkinje cells resulting in the inhibition of neuronal activity in the cerebellar nuclei. The present experiments were undertaken to evaluate this hypothesis by recording the activity of neurons in the dentate and interposed nuclei in decerebrate cats before, during and after stimulation of the cerebellar surface. Several criteria were established to assess the level of excitability of neurons before starting the stimulation paradigm. If no decrease in excitability was observed in the PSTH when either the inferior olive, face or forelimb were stimulated, then no records were obtained from the particular cell. If several consecutive cells did not demonstrate any excitability decrease, then the experiment was terminated. The cerebellum was stimulated with capacitively coupled pulses applied with bipolar platinum plates (7.6 mm^2) placed on the surface of Crus I and II. The pulse width was 0.1-0.3 msec. and the amplitudes varied from 2-12 ma and the frequency from 10, 50, 100, 150 to 200/sec. The stimulus paradigm consisted of sequential 10 minute recording and stimulation periods for as long as the cell was isolated. Each 10 minute sequence was divided into 10 recording intervals of one minute duration. The neural spike train of each interval was stored as interval histograms on the PDP 11/34. The data was obtained from 63 neurons recorded from 56 cats representing a total of 256 stimulation and 320 recording periods. The analysis included a statistical evaluation of the interval histograms and changes in the mean firing rate between stimulation and control sequences. Based on the analysis the following conclusions were possible: 1) Many cells showed significant increases or decreases in excitability. 2) These excitability changes may be frequency or amplitude dependent for a particular cell but no correlation was observed when the entire cell population was considered. 3) All cells were modulated by the cerebellar surface stimuli as determined by inspection of PSTH although the statistical tests might not have shown significant changes between stimulation and control sequences. 4) Rebound phenomena were often observed. (Supported by NIH Contract NO1-NS-4-2332.)

312 RAPHE STIMULATION MODULATES EXCITATORY AND INHIBITORY PROCESSES IN THE CEREBELLUM. Charles D. Barnes, Jean C. Strahlendorf, and Howard K. Strahlendorf. Dept. Physiology, Texas Tech Univ. Sch. of Med., Lubbock, TX 79430.

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

Glass micropipettes were used for extracellular recordings of Purkinje cells in α-chloralose anesthetized, flaxedilized, and artificially ventilated cats. Stimulation of the ipsilateral superficial radial nerve and contralateral sensorimotor cortex elicited mossy and climbing fiber discharges, respectively. "Off beam" inhibition was induced by stimulation of the superficial layers of the cerebellar cortex adjacent to the recording electrode. Evoked simple spike activity decreased after R conditioning from 3.8 to 0.6 spikes/stimulus representing an 81% change (average of 20 Purkinje cells) in comparison to a 62% decrease in spontaneous activity for a similar time period. With reference to evoked activity elicited via sensorimotor cortex stimulation, complex spike activity fell from 3.7 to 1.3 spikes/stimulus, representing a 77% change (average of 9 Purkinje cells) in comparison to a 67% decrease in spontaneous activity following R stimulation. Conditioning shocks to the R produced a marked augmentation of "off beam" inhibition at stimulus strengths (< 50μa) which failed to alter spontaneous firing patterns of Purkinje cells. Six cells sampled showed a 47% increase in the duration of "off beam" inhibition when central inferior raphe was stimulated; whereas, the maximal change in spontaneous firing failed to exceed 18%. These findings correlate with the potentiating effect of diazepam (0.1-1.0 mg/kg, i.v.) on R-induced inhibition of Purkinje cells. Diazepam enhanced the inhibition observed on Purkinje cells after R stimulation by 41% (N=4). Accordingly, bicuculline (0.01-0.1 mg/kg, i.v.) reversed the diazepam augmentation and given alone, diminished R elicited inhibition of Purkinje cells by 21% (N=6). The findings reveal a preferential inhibitory influence of the raphe nuclei on evoked simple and complex activity rather than spontaneous firing of Purkinje cells. Furthermore, the R appears to augment GABAergic "off beam" inhibition of Purkinje cells. (Supported in part by NIH Grant HL 7289).

313 DIFFERENT RECEPTORS FOR GABA AND MUSCIMOL IN THE RAT CEREBELLUM. G. Biggio*, M.G. Corda*, G. De Montis*, and G.L. Gessa* (SPON: G. Toffano). Institute of Pharmacology, University of Cagliari, Italy.

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

a) kainic acid (4 μg), microinjected into the cerebellar cortex, within 4 days decreased by 70% the number of specific binding sites for muscimol. On the other hand kainic acid markedly increased the receptor affinity for ^{3}H-GABA; this effect was associated with the almost complete disappearance of the endogenous inhibitor of GABA binding.

b) Differences in the number of specific binding sites for muscimol and GABA were present in the cerebellar cortical membranes obtained from naive rats and from rats habituated (for 15 days) to the handling maneuvers preceeding the sacrifice. Naive rats showed higher binding for ^{3}H-muscimol but lower binding for ^{3}H-GABA than habituated rats.

c) The acute administration of haloperidol (4mg kg^{-1}) decreased ^{3}H-GABA binding but increased that of ^{3}H-muscimol.

The results suggest that:

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

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

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

314 EMG AND COMPLEX SPIKE CHANGES DURING COOLING OF THE INFERIOR OLIVE[1]. V.B. Brooks, P.R. Kennedy[2], and H.-G. Ross[3], Dept. Physiol., Univ. of Western Ontario, London, Canada N6A 5C1.

Flexible cooling probe sheaths were chronically implanted just above the rostral end of the inferior olive (IO) with X-ray assisted stereotaxy (4) in 2 Fascicularis monkeys. Postoperative neurological signs receded in intensity after a few days, and were referable to tissue damage in the path of the implant. These were: ataxia of trunk and head (but never the limbs); neck deviation to the implanted side; ipsilateral facial palsy; vert. and horiz. nystagmus of both eyes; and ipsilateral ptosis and miosis. Cooling of IO for a few min, to a sheath tip temperature of 10°C or below, reduced firing frequency of spontaneous complex spikes (CS) of Purkinje cells in contralat. intermed. cerebellar cortex, as tested in one monkey (F2). Cooling IO occasionally reduced the number of repetitive CS discharges (wavelets) without diminution of amplitude of the initial spike, suggesting that wavelets are of olivary origin. The 20°C isotherm line delineated an area 5 mm in diameter and extended 2 mm forward from the tip at 10°C, and 3 mm forward at 5°C tip temp. Cooling accentuated nystagmus and ptosis, and caused fast tremor of the jaw and tongue. Accuracy of free reaching and grasping seemed unimpaired, as might be anticipated from results of partial rostral olivary lesions (5). The main finding, below, is more likely to be due to cooling IO rather than, e.g., the nearby n. reticularis gigantocellularis which would be expected to produce the opposite effect (6). During IO-cooling at tip temps tested (15°-5°C) biceps EMG responses to stretch increased while triceps EMG decreased or remained unchanged. This applied to tonic and phasic EMG in the anesthetized monkey (F2: Valium or Ketamine); but only to a lesser degree when awake and performing the Gilbert-Thach paradigm (7) at fixed load levels. The EMG increases seem to have been imposed by IO-cooling. In the awake monkey they coincided with disturbed task performance, consisting of fewer successes and less accurate return and holding of the handle with regard to the target.

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[2]Fellow of the Muscular Dystrophy Association of Canada
[3]Fellow of the Deutsche Forschungsgemeinschaft
[4]Kennedy, P. R., Ross, H.-G. and Brooks, V. B. Soc. Neurosci. Abstr. 4: 298, 1978
[5]Oriolo, F. L. and Mettler, F. A. J. Comp. Neurol. 106: 319-338, 1956
[6]Jeneskog, T. Acta Physiol. Scand. 92: 66-83, 1974
[7]Gilbert, P. F. C. and Thach, W. T. Brain Res. 128: 309-328, 1977

315 THE PARAFLOCCULUS: A POSSIBLE ROLE IN HEAD-EYE ORIENTATION. Richard A. Burne and Donald J. Woodward. Dept. of Cell Bio., Univ. Tx. Health Sci. Ctr., Dallas, Tx., 75235.

Previously we presented anatomical and electrophysiological evidence demonstrating that the paraflocculus is a cerebellar target zone for visual cortical and tectal inputs. As part of the general aim of determining the functional significance of the parafloccular visual input, this study was undertaken to further clarify the diverse anatomical connections of the paraflocculus. In this report we describe anatomical evidence on 1) the sites of origin of additional afferent inputs to the paraflocculus and 2) the terminal field distributions of the efferent projection from the parafloccular lobule of the rat cerebellum.

Following electrophoretic injections (1 μA, 20 min) of a 4% HRP-Tris buffer solution (pH 8.6) into the parafloccular lobule and reaction with tetramethyl benzidine, HRP-labeled afferent neurons were observed 1) bilaterally in the lateral cuneate nucleus, prepositus hypoglossal nucleus, locus coeruleus (ipsilateral preponderance), pontine reticular tegmental nucleus and the basilar pontine grey (contralateral predominance), 2) in the contralateral medial accessory and principal olives, and 3) in the ipsilateral spinal nucleus of V and the cerebellar dentate nucleus. No HRP-positive cells were localized in the vestibular ganglion or nuclei. Of these sources, the largest afferent projection to the paraflocculus originates from the lateral pontine grey; the same pontine region that receives efferents from the visual and auditory cortices and superior colliculus.

Hydraulic injections (0.15-0.25 μl) of ^{3}H-leucine (77 μCi/μl) into the paraflocculus resulted in efferent terminal labeling over specific regions of the ipsilateral dentate and interpositus nuclei of the cerebellum. Autoradiographic silver grains were primarily localized over the ventral-caudal region of the large cell subdivision in the lateral nucleus, and over the small and large cell subdivisions of the ventral-caudal interpositus nucleus; regions previously implicated in head-eye movement control. No grain accumulations were seen in the vestibular or other brainstem nuclei.

These results support ongoing electrophysiological studies that demonstrate Purkinje cell responses in the paraflocculus to visual stimuli with specificity to orientation, position and velocity of movement.

The afferent-efferent connections of the paraflocculus, as described here, suggest a possible role in the mechanisms underlying head-eye orientation in visual space rather than involvement with vestibulo-motor integrations.

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316 THE ACTIVITY OF DENTATE NUCLEUS AND Y-GROUP NEURONS IS RELATED TO HEAD AND EYE VELOCITY. M. Carroll Chubb* and Albert F.Fuchs Dept. Physiology and Biophysics, University of Washington,Seattle WA 98195.

The dentate nucleus of the cerebellum and the y-group of the vestibular nuclear complex have been shown to project to vertical eye movement nuclei in the midbrain, including parts of the oculomotor nuclei innervating vertical eye muscles. To investigate the role of the dentate and the y-group in vertical eye movements, neuron activity was recorded from these areas in alert rhesus monkeys trained to track a smoothly moving visual target while undergoing sinusoidal whole body rotation. The relation between unit activity and either head or eye position was determined by averaging 10 cycles of unit activity, fitting the average with the least square sine curve, and calculating its amplitude and phase relative to head and eye position. In the rostral dentate nucleus, the y-group and the adjacent areas just rostral to the dentate nucleus, the activity of some neurons was related to angular velocity of the head and eyes. When the stationary monkey tracked a target moving sinusoidally in the saggital plane these neurons fired approximately in phase with upward eye velocity. The activity of 15 units thus far analyzed during a peak to peak target excursion of 24 degrees at 0.5 Hz lagged upward target velocity by 6.7 $\pm$ 10.5 deg. When the monkey was rotated sinusoidally in the saggital plane while he tracked a spot moving with him so that his eyes did not move in his head, these units fired approximately in phase with upward head velocity. The activity of 16 units thus far analyzed during a peak to peak head movement of 20 degrees at 0.5 Hz lagged upward head velocity by 15.5 $\pm$ 4.3 deg. During smooth pursuit eye movement and during head rotation in the absence of eye movements, the sensitivity of these neurons to head and eye velocity (ie. the ratio of the amplitude of the modulation in unit firing rate to amplitude of the sinusoidal variation in head or eye velocity) remained constant over a range of frequencies from 0.3 to 1.0 Hz. Therefore, both the phase and sensitivity reflect a relationship of unit activity to head and eye velocity. For most units the sensitivities to head and eye velocity were nearly the same so that when the head and eyes moved the same amount in opposite directions, as when the rotating animal fixated a target stationary in space, the unit activity showed little or no modulation. During saccadic eye movements in all directions most of these cells fired a burst of action potentials. Therefore, the discharges of these units in the dentate nucleus and y-group are similar to those of flocculus Purkinje cells (Lisberger and Fuchs, 1979) and may have a role in modifying the vertical vestibulo-ocular reflex.

317 TOPOGRAPHY OF THE OLIVO-CEREBELLAR PROJECTION STUDIED BY A RADIOACTIVE TRACER METHOD. Jacques Courville and F. Faraco-Cantin. Centre de recherche en sciences neurologiques, Université de Montréal, Montréal.

Injections of ^{3}H-L-leucine (vols: 0.2-0.5 μl; conc.: 75-100 μC/μl) in different regions of the inferior olive in the cat were used to demonstrate the topography of olivo-cerebellar climbing fibers. Taking advantage of the complete crossing of this projection, bilateral injections were utilized. It was thus demonstrated in the same animals that complementary injections of the olive correspond to projections to entirely distinct cerebellar regions. This organization observed throughout the material indicates that there is no convergence to any cerebellar cortical region from different olivary origins. On the other hand, small localized injections of the olive demonstrate extensive projection areas distributed at right angles to the longitudinal axes of the folia. This indicates a great deal of divergence in the olivo-cerebellar distribution. A general organization of the olivo-cerebellar projection was recognized. The caudal third of the olive corresponds to vermal regions and to the flocculus. The middle third of the olive only projects to the intermediate and lateral parts of the first four lobules and to the paramedian lobule. The intermediate and lateral portions of lobules V and VI and most of the crura are projected upon from the rostral two thirds of the olive. Lateralmost portions of the crura receive projections from the rostral third, only. More specifically, the caudal medial accessory olive (MAO) projects to a sagittal strip of cortex located next to the midline, in all ten lobules. In the anterior lobe, from medial to lateral, four additional sagittal projections are found: 1) the dorsal accessory olive (DAO); 2) the MAO; 3) the DAO again and 4) the principal olive (PO). In the posterior lobe lobules VIII and IX, additional sagittal projections from the DAO and rostral MAO were seen next to the midline MAO distribution. No clear data were obtained for lobule VII. In the paramedian lobule, there are three sagittally arranged strips of projection. From medial to lateral, they come from the DAO, MAO and PO. The crura receive projections from the MAO and DAO medially and from the PO, laterally. The paraflocculus receives its projections from the MAO and PO.

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318 A COMPARISON OF THE RESPONSES OF MOSSY FIBERS AND PURKINJE CELLS TO TIME-VARIANT CUTANEOUS INPUTS. Timothy J. Ebner*, Teresa McMullen*,and James R. Bloedel (SPON: F. Torres). Dept. Neurosurg.,Univ. Minn., Minneapolis, MN 55455.

Experiments were performed in decerebrate unanesthetized cats to examine and compare the gain (sensitivity) and band-pass characteristics of the responses of mossy fiber afferents and Purkinje cells to natural cutaneous stimuli. The input signal consisted of a sinusoidal indentation of the skin in the animal's paw or forearm. The activity of Purkinje cells, identified by the presence of spontaneous climbing fiber activity, was recorded extracellularly from the ipsilateral anterior lobe of the cerebellar cortex. The responses of mossy fiber afferents were recorded in the same folia. Cycle histograms were constructed from the activity of both types of units to a wide range of input amplitudes and frequencies. The findings were twofold. First, the mossy fiber afferents were very sensitive to the cutaneous stimuli, being responsive to indentation from 75μ to 200μ peak to peak. In contrast, the activity of Purkinje cells proved to be very difficult to modulate by a continuously applied cutaneous input. In fact, the cycle histograms of most Purkinje cells did not exhibit any modulation. For units which did respond, their sensitivity to the stimulus was much less than that of mossy fibers. Second, the band-pass of the mossy fiber afferents to the modulated indentation of the skin was very wide, ranging from at least 1-100 Hz. In contrast, the responses of Purkinje cells which were modulated by the stimulus had a considerably narrower band-pass. Most cells were only modulated over a range of approximately 5 Hz. This range was usually centered around frequencies of 10-15 Hz. In conclusion, these studies demonstrate that the cerebellar cortex receives information through the mossy fiber afferents about the amplitude and frequency of time-variant, deterministic cutaneous input. However, the responses of Purkinje cells displayed considerable attenuation and filtering of the input signal. This filtering resulted in responses only over a very narrow range of frequencies. It is suggested that the processing of cutaneous information in the cerebellar cortex does not involve the modulation of Purkinje cell impulse activity over the same wide range of frequencies to which the input of this structure is modulated. (Supported by NIH Grant # RO501-NS-09447).

319 ALTERATIONS IN CORTICAL LAYERING SURROUNDING CEREBELLAR FISSURA PRIMA. M. A. E. Eriksson, W. S. T. Griffin, N. Stampfer*, M. del Cerro and D. J. Woodward. The University of Texas Health Science Center at Dallas, Dallas, Texas 75235 and The University of Rochester School of Medicine and Denistry, Rochester, New York 14623.

The three-layered appearance of the adult rat cerebellar cortex as it surrounds each lobule is well documented from pial surface to arbor vitae as pial cell layer, molecular layer, Purkinje cell layer and internal granular layer. However, we have regularly observed alterations in this three-layered arrangement of cortex surrounding fissura prima in adult rats, as well as in neonatal rats where the external granular layer is still present, adding a fourth layer. In 80 of 100 rat brains analyzed, the cortical layers surrounding fissura prima were found to be altered from the four (neonatal) or three-layered (adult) arrangement found in the majority of the remainder of cerebellar cortex. This common alteration extends long distances on either side of the midline causing fissura prima to become more and more shallow from its lateral to medial extent. In sagittal sections at the lateral edge of the alteration in neonatal cerebellum, the external granular layer and pial cells disappear leaving an expanse of molecular layer lying between internal granular layer cells of lobules V and VI. Proceeding medially toward the midsagittal section, the cells of the internal granular layer of lobules V and VI are situated closer together and often merge. In such sections Purkinje cells do not usually remain in a monolayer, but are displaced: sometimes clusters of these cells are found within the arbor vitae. Various complex configurations of internal granular layer, molecular layer, and Purkinje cell groups surrounding fissura prima were common place in regions of cerebellum as far lateral as 320 microns on either side of midsagittal. The greatest dorsoventral extent of such alterations measured was 400 microns. Although this work was an analysis of cerebella from unstressed control animals, we have regularly observed altered layering patterns in cerebella from animals with induced environmental stresses applied during cerebellar development. Therefore, analyses of structural changes in cerebellar cortical layers resulting from applied stresses should take into account the normally occurring alteration in layering patterns reported here.

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320 GRANULE CELL DEGENERATION IN THE CEREBELLUM OF THE PURKINJE CELL DEGENERATION (pcd) MUTANT MOUSE. B. Ghetti, C. J. Alyea*, J. Muller*. Dept. Pathology, Div. Neuropath., Indiana University School of Medicine, Indianapolis, IN. 46223, USA.

The pcd mutation in mice is autosomal recessive. Homozygotes will at first show normal cerebellar development; only in adolescence do the Purkinje cells start to degenerate rather abruptly. Due to the severe neurological impairment, the mortality of mutants is high after the 1st year of age; only few animals survive beyond 16 months.

A longitudinal histological and ultrastructural investigation was conducted to analyze the degeneration of the Purkinje cells and the events following their disappearance. 36 affected and 25 normal littermates were used, 17 to 591 days old.

Before the onset of degeneration, the Purkinje cells show fewer cisterns of rough endoplasmic reticulum and a basal polysomal mass. By the 17th day of age many Purkinje cell dendrites contain a floccular material and osmiophilic bodies. Between 17 and 23 days numerous Purkinje cell axons have lost their organelles and the axon terminals contain dilated tubular cisterns and clusters of synaptic vesicles of uneven size. The Purkinje cells and their processes become dark, fragmented and are removed by glia over a period of several months.

In three month old mutants scattered degenerating granule cells are seen. At age six months, although the molecular layer is reduced to half the original thickness due to the loss of Purkinje cell dendrites, the parallel fibers do not seem to be reduced in number. The majority of the endings of both the parallel and the climbing fibers have lost their post-synaptic dendrites and are apposed to glial profiles. In mutants aged between 6 and 12 months numerous granule cells have an osmiophilic, pyknotic nucleus, dense homogeneous cytoplasm and have lost their cytoplasmic organelles. Between 12 and 19 months the granule cell layer becomes progressively more depleted of nerve cells and architecturally disorganized; the molecular layer is reduced to one third due to the sequential loss of Purkinje cells and parallel fibers. Whorls of glial processes replace the degenerated elements of the neuropil. In the disorganized granule cell layer, mossy endings remain, but many are deprived of their post-synaptic dendrites. Numerous mossy fibers have fewer synaptic vesicles and these are uneven in size.

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321 QUANTITATIVE ANALYSIS OF STAGGERER ↔ WILD-TYPE CHIMERAS: FURTHER IMPLICATIONS FOR GENE ACTION AND NORMAL CEREBELLAR DEVELOPMENT. Karl Herrup and Richard J. Mullen*. Dept. Human Genetics, Yale Med. Sch., New Haven, CT and Dept. Neuroscience Children's Hosp. Med. Center, Boston, MA.

Staggerer (sg) is one of more than a dozen neurological mutants in mice which affects cerebellar structure and development. The cerebellum is smaller and less foliated than the wild-type. There is a virtually complete loss of granule cells, an absence of almost 75% of the medium-to-large cortical neurons (MLNs) and there are cytological aberrations in those MLNs which remain. Examination of staggerer ↔ wild-type chimeras revealed that all of the aberrations of the sg/sg MLNs visible in the light microscope were intrinsic to the MLN and not caused by external agents or factors (Herrup and Mullen, Brain Res., in press). These defects included small size, ectopic location and regional variation in cytology. While qualitatively it appeared as though the phenotype of reduced numbers was also expressed in the chimera, we undertook the current study to quantitate this impression. Counts were made on selected sections from 2 serially sectioned chimeric half cerebella stained for β-glucuronidase to allow distinction of sg/sg genotype cells independently of their phenotype (Ibid). The number of staggerer MLNs in the chimera (sgχ MLNs) was less than the number of MLNs in a staggerer homozygote (sg/sg MLNs) and the number of wild-type MLNs in the chimera (+χ MLNs) was less than the number of MLNs in the homozygous wild-type (+/+ MLNs). The relative numbers were such that if the number of sgχ MLNs was N% of the number of sg/sg MLNs, the number of +χ MLNs was (100-N)% of the number of +/+ MLNs. Expressed differently:

$$\frac{\text{sg}\chi \text{ MLNs}}{\text{sg/sg MLNs}} + \frac{+\chi \text{ MLNs}}{+/+ \text{ MLNs}} = 1.$$

For example, in one chimera, there were approximately 46,000 sgχ MLNs and 77,000 +χ MLNs. This compares with 66,000 MLNs in sg/sg and 243,000 MLNs in +/+ homozygotes. Thus, in terms of strict percentages, about 63% of the MLNs in the chimera were +/+. However, in terms of what we might call the original MLN pool, it appears that only 32% of the cells were +/+. This finding has two facets, both of which have important developmental implications. First, despite the presence of a relatively normal physiological and cytoarchitectonic environment sgχ MLNs were still reduced in number compared to wild-type. Second, despite the absence of many of their neighbors, the +χ MLNs were seemingly unable to compensate significantly by increasing their own numbers. The results will be discussed in terms of the timing of the action of the sg gene product. Supported by Nat. Fndn-March of Dimes, Basil O'Connor Grant and NIH Grant #HD 12213-01.

322 DETERMINATION OF POSTSYNAPTIC MEMBRANE STRUCTURE IS INTRINSIC TO THE PURKINJE CELL. Dean E. Hillman and Suzanne Chen*. Dept. of Physiol. & Biophys., New York Univ. Med. Ctr., New York 10016.

A fundamental question in understanding the developmental organization of the nervous system is whether the extent of postsynaptic specialization is determined by afferent connections or intrinsically determined by the postsynaptic cell. A reduction in granule cell number follows administration of malnourished diets during postnatal development (Winick & Noble, J. Nutrition 89: 300, 1966). Here we show that in the malnourished female the number of synaptic contacts on each Purkinje cell is also reduced; however, the total synaptic contact area remained constant for each cell. An 8% protein diet with calories equal to a 25% control diet was fed to rat dams. Sixty-day-old female offspring had an average total reduction in number of granule cells in excess of 26% while granule cells of males were decreased by only 13% as compared to their respective controls. At the same time, total Purkinje cell numbers remained unchanged in both sexes. Quantitation of molecular layer volume and synaptic density indicated that total number of spine synapses in cerebellum of males was unchanged while in the female it was reduced as much as 18%. Thus, the male Purkinje cell-parallel fiber synapses were completely compensated by an increase in synaptic sites on parallel fibers of granule cells. The females also compensated but had a residual deficit of about 18% fewer spine synaptic contacts than controls or malnourished males. Severely affected females displayed giant spines and attained a synaptic deficit greater than 30%. Electron microscopical quantitation of the spine-synaptic contact length for controls, experimental males, and experimental females with and without giant spines showed that the area of postsynaptic thickening on spine processes increased inversely with the number of Purkinje cell-parallel fiber synapses. The average synaptic contact area in males was unchanged while the females had an area increase of 17%. In severely affected females which displayed giant spines, this average area was over 30%. We conclude that (1) the total postsynaptic specialization area on each Purkinje cell remains constant following various degrees of afferent deficit, (2) postsynaptic specialization-macromolecules distribute to available parallel fiber synaptic sites, and (3) Purkinje cell spines can synaptically compensate in number up to 13% when afferents are reduced. The constancy of total spine postsynaptic area on each Purkinje cell indicates that postsynaptic junctional macromolecules must be predetermined by the Purkinje cell (presumably through the genome) and thus serve as one of the parameters that establish fundamental neuronal organization in the cerebellum. (Supported by USPHS grant HD-10934 from NICHD.)

323 TOPOGRAPHY OF PROJECTIONS FROM SENSORIMOTOR CORTEX, RED NUCLEUS, CEREBELLUM AND SPINAL CORD TO THE LATERAL RETICULAR NUCLEUS IN THE CAT. Alan W. Hrycyshyn* and Brian A. Flumerfelt. Department of Anatomy, University of Western Ontario, London, Canada.

Using autoradiographic, silver impregnation and horseradish peroxidase (HRP) tracing methods, the topographic organization of the projections to the lateral reticular nucleus (LRN) was investigated in the cat. Initially, the origins of the afferent projections were studied following injection of 20% HRP into the LRN and subsequently reacting the tissue with the chromagen benzidine dihydrochloride. Serial sections throughout the brain and spinal cord were examined for the presence of HRP-labelled neurons. The results were then mapped using an X - Y plotter. Labelled cells were found mainly within Rexed's laminae IV through VIII of the spinal cord, the anterior sigmoid and coronal gyri of the sensorimotor cortex, the caudal three quarters of the red nucleus and the ventral aspect of the fastigial nucleus. The topography of these projections within the LRN was next investigated using anterograde methods. Injections of a mixture of [^{3}H] - leucine and [^{3}H] - proline, or [^{3}H] - leucine alone were placed within pericruciate cortex, red nucleus and fastigial nucleus. After survival times of 3 - 7 days, the animals were perfused, and then frozen sections through the LRN and the injection site were processed for autoradiography. In a corresponding series of animals, electrolytic lesions were placed selectively into the above sources of reticular afferents, and degeneration within the LRN was demonstrated with the method of Fink and Heimer. An extensive input from the spinal cord was found to terminate predominantly on the ipsilateral side throughout the entire rostro-caudal extent of the LRN, except for a small rostromedial area in the magnocellular portion. The cortical projection terminated diffusely within the rostral aspect of the contralateral magnocellular part of the nucleus. The projection from the red nucleus terminated extensively within the contralateral subtrigeminal portion and the dorsolateral region of the rostral magnocellular portion of the LRN. The fastigial nucleus was found to give rise to a sparse contralateral projection, mainly to the medial aspect of the rostral extent of the magnocellular portion, with less to the parvocellular and subtrigeminal portions.

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

(Supported by the Medical Research Council of Canada.)

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

The spatial organization of the projections of the superior colliculus to the contralateral cerebellar hemisphere and posterior vermis was examined using micromapping techniques in barbiturate-anesthetized rats. Using two independent, ball-tip, tungsten microelectrodes, multiple unit clusters were recorded from tactile areas in the stratum griseum intermediale of the superior colliculus (SC) and the granule cell (GC) layer of cerebellar cortex. The receptive fields of both areas were defined by threshold mechanical stimulation of cutaneous tissues. Electrical stimulation (monopolar, biphasic pulses; 10-40 μa; 2/sec) through the SC electrode evoked responses in limited regions of the contralateral GC layer of cerebellar cortex. In most experiments, the SC stimulating electrode remained stationary while the cerebellar electrode was used to map the region of the GC layer activated by SC stimulation. In a few experiments, the cerebellar recording electrode remained fixed while the SC electrode mapped the area of effective stimulation. Puncture densities up to 75/mm^2 were necessary to fully determine the spatial pattern of these projections.

Major results are: (1) Cerebellar responses to SC stimulation are found in the GC layer of contralateral Crus I and the paramedian lobule; no responses have been seen in the uvula. (2) Peripheral receptive fields of the interconnected SC and GC loci include primarily facial structures (especially vibrissae, crown and eyelid). (3) SC sources project only within the confines of GC patches (defined by Shambes et al., BBE 15: 94-140, 1978) with homologous receptive fields. (4) Latencies of GC layer activation following SC stimulation are typically 3.0 - 3.5 msec.

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Withdrawn by Author

326 *THE ONTOGENY OF THE INFERIOR OLIVARY COMPLEX IN THE OPOSSUM.* *J.S. King, B.E. Maley and G.F. Martin: Dept. of Anatomy, Ohio State University, Columbus, Ohio 43210*

The inferior olivary complex of the opossum is first recognizable in Nissl preparations on pouch day three (15-16 days after conception). However, the nuclear subdivisions are not distinct until pouch day twenty-one (33-34 days after conception). By this latter time olivary axons from the spinal cord, midbrain and deep cerebellar nuclei have reached their respective nuclear targets. These olivopetal systems have been identified by the Fink-Schneider method subsequent to either midthoracic transections or metencephalic hemisections. The latter lesion interrupts both the midbrain-olivary and cerebello-olivary afferents on the side of the lesion and the cerebello-olivary axons on the contralateral side. Examination of the olivary complex in unoperated animals with the electron microscope at the same age reveals that many fine structural features characteristic of the adult are not present. For example the spiny appendages localized within synaptic clusters, (glomeruli) which are the primary targets of midbrain and cerebellar axons, are not evident. Golgi impregnations confirm the lack of spiny appendages at this age. The developing neuropil is characterized by small diameter neurites which contain microtubules, beaded dendrites and immature axodendritic synaptic profiles. Synaptic junctions are present, but the presynaptic profiles are only sparsely populated with synaptic vesicles.

It is not until pouch day 60 (72-73 days after conception) that electron micrographs show synaptic clusters with features comparable to those seen in the adult. Gap junctions also are evident in animals of this age. These results suggest that immature axons reach their nuclear targets prior to the development of their postsynaptic excresences which are characterestic of the adult animal. When compared to the rat (Altman and Bayer '78, J.C.N. 179:49), the time frame (pouch day 3 to day 60) for olivary differentiation in the opossum appears to be much longer.

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327 SYNAPTIC ALTERATIONS IN THE CEREBELLAR NUCLEI OF QUAKING MICE. Donna L. Koniecki* and Victor L. Friedrich, Jr. (SPON: J. S. Cowen). Dept. of Biobehavioral Sciences, University of Connecticut, Storrs, Ct. 06268.

In addition to the widespread deficit of myelin in quaking mice, axons in the cerebellar white matter exhibit focal swellings up to 10 µm in diameter (Suzuki & Zagoren). Here we report abnormalities in the ultrastructure of synaptic terminals in the cerebellar nuclei of 40 to 330d old quaking mice.

Synaptic terminals in control mice resemble those in the rat (Chan-Palay). Synapses in quaking mice share essential features with those of the control mice; however, many boutons show dystrophic alterations --- including enlargement of the terminals; increased content of mitochondria, also often enlarged; membrane whorls; and excessive numbers of synaptic vesicles.

In addition to the apparently normal synaptic vesicles, many endings contain dense clumps of very small (150-250 Å) vesicles which may be abnormal synaptic vesicles. These are seen both in profiles which are otherwise unremarkable and in profiles with the dystrophic changes described above. The affected boutons maintain their synaptic adhesions with the normal range of postsynaptic sites --- i.e., somata and dendrites.

To assess the possible loss of synapses, we measured the fraction of surface of neuronal somata in the medial cerebellar nucleus apposed to synaptic boutons; this was not reduced in quaking mice. Thus, net loss of synapses does not occur and degeneration of terminals (if it occurs at all) is followed by replacement.

We believe that the majority and perhaps all of the dystrophic terminals and those with vesicle clumps belong to Purkinje cells. Many altered profiles closely resemble Purkinje cell boutons of normal mice (moderately dense ground substance, characteristic cisternae of ER, and characteristic vesicle shapes). In addition we find the vesicle clumps and other changes in the superior and lateral vestibular nuclei and in the infra-ganglionic plexus of cerebellar cortex, where Purkinje cell axons are known to terminate. Finally, mossy and climbing fibers in the cerebellar cortex were not seen to be affected.

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

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

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328 SUBSTANCE P-LIKE IMMUNOREACTIVITY IN CEREBELLAR MOSSY FIBERS AND TERMINALS IN THE TURTLE. Gary E. Korte*, Antonj Reiner , and Harvey Karten. Dept. of Psychiatry and Behavioral Sciences ,Health Sciences Center , SUNY,Stony Brook,N.Y. 11794

Using immunohistochemical methods , we have identified a population of mossy fibers and terminals which contain substance P-like immunoreactive material (SP in the cerebellar cortex of the red-eared turtle , Chrysemys scripta elegans. The identification of the SP+ profiles is based on their exclusive location in the granule cell layer and their similarity to golgi impregnated mossy fibers and terminals. The SP+ profiles are not found in control sections treated with antisera blocked by addition of synthetic substance or in sections stained for enkephalin-like immunoreactivity.

The greatest number of SP+ mossy fibers and terminals are found in the rostral cerebellum , at levels where the cerebellum is attached to the brainstem. In this region , the lateral parts of the cerebellar cortex contain the greatest number of SP+ mossy fibers and terminals. Their numbers taper off sharply in more caudal sections. Although the cells of origin were not readily apparent , the axons of the SP+ mossy fibers could be traced into the cerebellum from a distinct fascicle of fibers located on the dorso-lateral surface of the medulla. Some fibers also enter from the periventricular region of the fourth ventricle. In the cerebellum , they course sagittally through the granule cell layer as parallel arrays.

This observation provides further evidence that cerebellar mossy fibers are histochemically heterogenous (eg. Hunt and Schmidt,1978) . More broadly , it supports suggestions that there are histochemical differences among the members of the several morphologically similar classes of neurons and afferents which make up the cerebellar cortex(Sar et-al,1978).

329 AN AUTORADIOGRAPHIC ULTRASTRUCTURAL IDENTIFICATION OF TERMINALS OF CEREBELLAR NUCLEOCORTICAL FIBERS IN THE CAT. K. Kultas-Ilinsky, D.L. Tolbert, and I.A. Ilinsky. Coates EM and Murphy Neuroanat. Res. Labs., Depts. Anat. and Surg., Sch. Med., St. Louis U., St. Louis, MO 63104.

The cerebellar nucleocortical projection has been identified in a number of different laboratory animals. From ^{3}H-leucine injections in the cat and monkey cerebellar nuclei, orthogradely labeled nucleocortical axons could be followed into the granular layer of the cortex where they appeared at the light microscopic level to terminate within cerebellar glomeruli (Tolbert et al., Neurosci. 1:205, 1976; Exp. Brain Res. 30:425, 1977). To confirm these light microscopic observations EM autoradiographic experiments were undertaken following injections of 3'4'5-tritiated leucine into the cat dentate, interposed, and fastigial nuclei. Following survival periods of 2 to 7 days these animals were sacrificed and samples of the cerebellar cortex were processed for EM autoradiography. Only the structures with 2 or more developed silver grains overlying them were considered to be orthogradely labeled. Numerous labeled large myelinated axons were observed entering the cortex at the base of the granular layer, but only labeled unmyelinated axons were found in the granular layer itself. The most common type of labeled terminal occurred at an expansion of a large unmyelinated fiber and was characterized by numerous mitochondria, very dense packing of synaptic vesicles, and a central core of neurofilaments. The majority of the synaptic vesicles within these terminals were of a round and clear type, with some dense core vesicles also being present. In most cases developed silver grains were localized over mitochondria and the synaptic contact. Labeled axonal expansions formed the central core of a glomerulus and established asymmetrical-type synaptic contacts with surrounding granule cell dendrites. Occasionally en passant synapses were observed between labeled fibers and granule cell dendrites. In several instances labeled fibers synapsed on a granule cell dendrite which was also postsynaptic to terminals forming symmetrical type contacts which were presumably Golgi cell axons. Silver grains were never observed to be above background levels in the Purkinje cell or molecular layers. This ultrastructural study confirms previous light microscopic data that the cerebellar nulceocortical projection has a mossy fiber-type termination within the granular layer.

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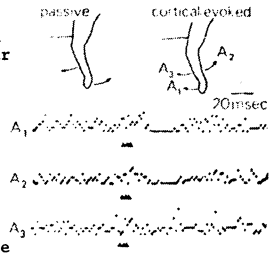
330 GLIAL CELL ANTIGENS DETECTED BY MONOCLONAL ANTIBODIES Carl Lagenaur*, Ilse Sommer* and Melitta Schachner. Institute of Neurobiology, University of Heidelberg, Im Neuenheimer Feld 347, 6900 Heidelberg, G.F.R.

Monoclonal antibodies that distinguish glial subpopulations in mouse cerebellum were detected in froze thin sections by indirect immunohistology. Mice immunized with crude mouse cerebellar membrane preparatio or with bovine corpus callosum provided spleenocytes that were fused with NS 1 mouse myeloma by the technique of Lemke, et al. (Nature 271:249, 1973) to yiel antibody producing hybridomas. Antibody M-1, arising from crude cerebellar membrane immunizations, stained fibrous astrocytes in the white matter, but did not stain other cerebellar glial cell types. Although Bergmann glial cells in wild type cerebella were M-1 negative, this cell type was M-1 positive in homozygous weaver mutant cerebella.

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

331 MOTOR CORTICAL MODULATION OF CEREBELLAR AND RUBRAL OUTPUT. Kenneth D. Larsen and Haruhide Yumiya*. The Rockefeller University, N.Y., N.Y., 10021.



The purpose of these experiments was to determine the manner in which the motor cortex modulates cerebellar and rubral output. An array of microelectrodes was implanted in the motor cortex, 12-pulse stimulus trains were delivered through each electrode to evoke movements, and then neurons in the intermediate and lateral cerebellar nuclei of cats and the red nucleus of cats and monkeys were isolated with recording electrodes. The neurons were identified by their somatosensory receptive field, determined with natural stimulation, and the averaged response to three-pulse cortical stimulation was seen in peri-stimulus time histograms. A receptive field was identified in 143 of 196 cerebellar neurons, 83 of which were driven by passive movement of one (59) or two (24) joints in one direction. When 46 of these 83 were tested, 33 had response pattern A in which they were driven by passive movement of a joint in one direction and were suppressed by stimulation of the cortical site from which movement was evoked in the opposite direction (figure). Increasing the duration of the cortical train increased the duration of the suppression. Ten other cells had response pattern B in which their discharge was suppressed by stimulation of a cortical site which evoked movement at an adjacent joint or at the same joint in a different plane. Red nucleus neurons had the same response patterns as cerebellar neurons although a short latency facilitation, presumably mediated by the direct corticorubral projection, occurred either preceding or without the response presumably mediated by the cerebellum. Not all components of the response (i.e. the corticorubral facilitation and the cerebellar-mediated suppression) were present in every cell, but in cats pattern A was present in 23 of 40 neurons which were tested, and 7 of 20 responded with pattern B. The same response patterns which were found in cat rubral neurons were also found in monkeys in rubral neurons with an input from proximal joints as well as those with an input from digits. Of 41 neurons, 17 responded with pattern A, while 12 of 25 had pattern B. In summary, the predominant pattern found in the cerebellar nuclei and mediated to the red nucleus was that neurons driven by passive movement of a joint in one direction were suppressed by stimulation of the cortical site from which movement was evoked in the opposite direction.

332 GRANULE CELL MIGRATION IN DEVELOPING CEREBELLAR CORTEX STUDIED WITH ALTERED THYROID STATES. Jean M. Lauder. Dept. Anatomy, Univ. North Carolina School of Med., Chapel Hill, N.C. 27514.

^{3}H-thymidine autoradiography with multiple survivals was used to determine the transit time and rate of movement of labelled cells from the proliferative zone (PZ) of the external granular layer (EGL) to the subproliferative zone (SPZ); from the EGL(SPZ) into the molecular layer (ML); and from the EGL(SPZ) to the internal granular layer (IGL) in 10-day-old control rats and in 10-day old rats made hypo- or hyperthyroid from birth.

Hyperthyroidism accelerated movement of labelled cells from the EGL to the IGL (granule cell migration), and increased the rate of entry of these cells into the ML from the EGL. The magnitude of the effect on granule cell migration rate was directly proportional to the previously reported effect on rate of parallel fiber (PF) growth (Lauder 1978, Br. Res. 142:25-39). Hypothyroidism, on the contrary, decelerated both granule cell migration and PF development. The effects of hyperthyroidism lend support to the hypothesis that the granule cell body is translocated within the descending portion of the elongating PF whose growth provides the motive force for granule cell migration. The alternative hypothesis that the amoeboid-like movement of the cell body provides the main impetus for migration seems less likely since accelerated PF growth would probably not result in faster migration in this model, even though PF growth would still be a requisite condition for perikaryal movement towards the IGL.

Granule cell acquisition in the IGL was decelerated in both thyroid states apparently as a consequence of combined effects on cell production in the EGL and movement of cells to the IGL. In hyperthyroidism, a reduced proportion of proliferating precursor cells in the EGL and slower movement of postmitotic cells from the PZ to SPZ resulted in slower granule cell acquisition in the IGL which could not be compensated for by the accelerated rate of migration. In hypothyroidism, the slower rate of granule cell migration was also coupled with a reduced proportion of proliferating cells in the EGL causing an even slower rate of cell acquisition in the IGL. The net result of hyperthyroidism was to produce a deficit in the number of labelled cells reaching the IGL, whereas hypothyroidism led to a transient deficit which was eventually compensated for by a prolonged migration period.

These results demonstrate the usefulness of such hormonal manipulations for the study of normal neurogenic events and provide evidence for an intimate link between parallel fiber growth and granule cell migration in the developing cerebellar cortex of the rat. (Supported by NIH grant NS-13481).

333 SOMATOTOPIC ORGANIZATION OF CLIMBING FIBER PROJECTIONS FROM CUTANEOUS AFFERENTS TO LOBULE V VERMAL CORTEX OF THE CAT CEREBELLUM. Kenneth D. Laxer*, Lee T. Robertson, and Ann Mason*. Neuro. Sci. Inst., Good Sam. Hosp. & Med. Ctr., Portland, OR 97209.

Previously we have demonstrated the fine detail of climbing fiber projections to the pars intermedia of lobule V in the cat cerebellum in response to low threshold natural cutaneous stimulation. These projections form a complex mediolateral organization of patches that were elongated in the anteroposterior direction. The cortex received input from the entire ipsilateral, anterior quadrant of the body; the face was represented laterally and the distal forelimb medially. The present study investigates the cutaneous climbing fiber projections to the vermis of the lobule V in the cat cerebellum. Extracellular single unit Purkinje cell responses to natural stimulation were recorded in 20 cats anesthetized with pentobarbital. A computer-controlled punctate stimulus was used to delineate those areas which could activate climbing fiber responses (CFR). Peri-stimulus histograms were computed for CFR's; the latency and thresholds were determined.

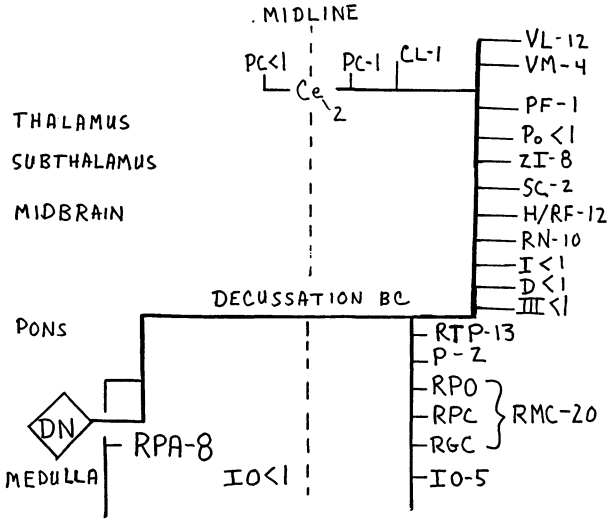
In contrast to the organization of the pars intermedia, the CFR in the vermal cortex showed no distinct topographic distribution of cutaneous input. Although small clusters of cells with similar receptive fields were occasionally identified, the vermal cortex typically consisted of a mosaic of cells that responded to low to high threshold stimulation of the forelimb, hindlimbs, and back. In the most medial area (midline to 1.0 mm), the majority of cells (82%) could not be activated by any cutaneous stimulation, although interspersed throughout this zone were a few CFR's that responded to stimulation of the upper dorsal trunk. The more lateral vermal cortex (1.0-2.5 mm) consisted of a heterogeneous mixture of responsive (80%) and unresponsive (20%) Purkinje cells. The responsive cells were predominately activated by stimulation of the ipsilateral forelimb, which included the paw and wrist (59%), wrist (13%), or forearm (4%); whereas 22% of the units were sensitive to stimulation of the hindlimb. Unlike the findings in the pars intermedia, no cells were identified that were responsive to stimulation of the face or to the contralateral limbs. The distribution of CFR's in the lobule V vermal cortex activated by natural stimulation was similar to that described by Eccles but was contradictory to that produced by electrical stimulation of the peripheral nerves as reported by Oscarsson.

334 LONG TERM EXCITABILITY CHANGES IN MAMMALIAN INFERIOR OLIVE NEURONS IN VITRO. R. Llinás and Y. Yarom* (SPON: E.Simon). Dept. Physiol. Biophys., New York Univ. Med. Ctr., New York 10016.

Electrical activity of inferior olive neurons was analyzed intracellularly in guinea pig brain stem slices and in vitro whole brain stem. As reported in the accompanying communication (Yarom & Llinás, 1979), inferior olive neurons demonstrated two calcium conductance changes to both depolarization (dendritic) and hyperpolarization (somatic) membrane changes, the latter as an unmasking of a calcium conductance inactivated at resting potential. Following D.C. depolarization or hyperpolarization from resting level, both calcium currents required a certain minimum time for their appearance and once uncovered, the membrane retained this new conductance for a certain period of time (tens of seconds). More specifically, starting from a resting potential of -70 mV, an abrupt D.C. depolarization caused a large conductance change (demonstrable by test current pulses). During this time, dendritic calcium spikes were difficult to generate, probably due to the short λ. If depolarization was continued, the membrane conductance decreased and there was a small shift of the membrane level. This was accompanied by the generation of local responses and finally full calcium action potentials by the test current pulse. If the membrane potential was returned quickly to resting level for a few seconds and then returned to previous value, dendritic calcium spikes were obtained without delay and the input resistance remained high. A similar finding could be seen when the membrane was hyperpolarized from resting; i.e. there was initially a large conductance change which subsided and allowed somatic inactivating calcium spikes to occur during test pulses. While the long term excitability change generated by membrane depolarization can be ascribed to the inactivation of the voltage-dependent potassium conductance change which had a time course of tens of seconds, the mechanism for the membrane conductance change to hyperpolarization is as yet unclear. Preliminary data suggest that it may involve the activation of a potassium current generated by inward calcium current due to the increased E_{Ca} during hyperpolarization. The above results taken together with the electrotonic coupling suggest that the inferior olive is capable of complex and long term excitability changes. In addition, bath application of harmaline (known to produce oscillatory behavior in inferior olive) hyperpolarized the neuron and thus unmasked the calcium-dependent somatic spike. The actual mechanism for harmaline action is unclear; since it was not accompanied by large conductance change, one must assume either activation of an electrogenic pump or its acting as a transmitter on a probable catecholaminergic receptor. (Supported by USPHS grant NS-13742 from NINCDS)

335 CEREBELLAR DENTATE NUCLEAR OUTPUT - A QUANTIFICATION OF TERMINAL FIELDS. Eric Lothman*, Terry Der* and Robert C. Collins. (Spon. Sven G. Eliasson) Dept. of Neurol., Wash. Univ. Med. Sch.; St. Louis, MO 63110

Although descriptions of cerebellar efferents exist, quantitative data of the relative density of the terminal fields is lacking. A quantitative autoradiographic method (Collins and Der - these abstracts) was applied to the rat dentate nucleus (DN). 14C-proline (0.03 to 0.18µl, 1µCi/µl) was stereotactically injected into DN. After 24 hours brains were perfused/fixed and triplicate 20µm sections were cut every 300µm to obtain autoradiographs of the entire brain. These sections were thionin stained later. Rostrally, DN projects almost entirely to contralateral fields. Caudally there are ipsi- and contralateral projections. Terminal fields were calculated from optical densities x volume, measured planimetrically, and expressed below as percent of total (terminology of Faull, JCN 178: 495, '78).



336 KAINIC ACID NEUROTOXICITY IN THE MOUSE CEREBELLUM. Kathryn L. Lovell and Margaret Z. Jones. Dept. Path., Michigan State Univ., East Lansing, MI 48824.

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

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

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337 PURKINJE CELL ACTIVITY IN THE VERMAL CEREBELLUM OF THE CAT DURING TRAINED SACCADIC EYE MOVEMENTS AND FIXATIONS. James G. McElligott Depart. of Pharm., Temple Univ. Medical School, Phila.,Pa.19140.

Purkinje cell activity from the posterior vermis (lobes VI and VII) of the cerebellum was recorded in awake,head restrained cats. These animals were trained to maintain fixations or to make precise saccadic eye movements to visual target lights (green light emitting diodes). These were mounted in a square matrix array (10 x 10) placed at 5 degree intervals in horizontal and vertical orientations on a tangent stimulus board. Chronic unit recording was carried out, while the horizontal and vertical components of the eye movements were recorded via an ocular electromagnetic search coil technique. The position of the fixation points as well as the position, amplitude and direction of the saccades were dictated by training the animal to look at sequences of illuminated target lights. The experiment was controlled by a digital computer which randomized light presentations and determined if the animals made proper fixations and saccadic eye movements. Successful completion of a sequence of fixations and saccades was followed by a milk reward. Previous results from our lab indicated that feline Purkinje cells were directionally sensitive and modulated their firing rates (increases, decreases or biphasic combinations), before as well as after the initiation of a saccade. Fifty percent of the Purkinje cells manifested short phasic responses related to saccadic onset. Twenty-five percent of these phasic cells also produced different tonic firing rates related to eye position. Typically, for these cells a tonic response would be recorded for movements along one primary axis (e.g. vertical) and phasic responses recorded along the other axis (e.g. horizontal). For most of the multi-directional cells (80%), there was a change in the temporal relationship between the neural response and saccade initiation that depended on the direction of the movement. The majority (60%) of the eye movement related cells also produced neural responses that were dependent on the absolute position of the saccade. Comparisons between saccade amplitude and the neural response, showed that 50% of the cases tested produced equal neural responses to saccades of different amplitudes. The other 50% of the cases were divided evenly into 2 groups that produced either direct or an inverse relationship between the amplitude and the magnitude of the neural response. Thus, it appears that direction and absolute eye position before and during saccades are 2 of the important parameters for determining the response characteristics of the Purkinje neural response.

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338 EFFECTS OF BULBOSPINAL SYSTEMS ACTIVATED FROM THE DENTATE NUCLEUS ON THE STRETCH REFLEX. Teresa McMullen* James R. Bloedel .Dept. Neurosurg., Univ. of Mn., Minneapolis, MN 55455.

Experiments were performed on decerebrate cats which were either unanesthetized or anesthetized with halothane. The tendon of the gastrocnemius muscle was mounted on a muscle puller, and a fine wire was inserted into the belly of the muscle to record multiple unit EMG activity. Responses to muscle stretch were recorded in the presence and absence of dentate stimulation at frequencies ranging from 5-100 Hz. To ensure that the activation of rubrospinal and reticulospinal pathways was not accompanied by the activation of spinocerebellar fibers, the responses evoked in the cervical spinal cord by the dentate stimulus were monitored. The electrode was positioned so that only descending projections were activated. When these conditions were satisfied, dentate stimulation produced one or both of the following changes in reflex activity: (a) reduction or total elimination of the response of one or more motor units; (b) an decrease in the phase lead of the response of individual motor units. These effects were more pronounced and occurred more frequently among motor units producing large spikes than those producing small spikes. In addition, when several motor units were affected, those with the largest spikes were always affected first after the onset of dentate stimulation. This observation was made at several recording sites within the same muscle. These data indicate that descending projections from the brain stem which are activated by the dentate nucleus produce characteristic changes in the excitability of stretch reflexes and suggest the possibility that motor units are differentially affected by these descending systems according to their size. (Supported by NIH Grant # RO501-NS-09447 and NIH contract NS 42332).

339 AN ELECTRON MICROSCOPIC AND HRP STUDY PROVIDING EVIDENCE THAT BOTH THE CORTICOPONTINE AND CEREBELLOPONTINE SYSTEMS ARE EACH COMPOSED OF TWO SEPARATE NEURONAL POPULATIONS. Gregory A. Mihailoff and Carl B. Watt* , Dept. Cell Biology, Univ. Texas Health Science Center at Dallas, Dallas, Tx.

As part of ongoing studies concerning the synaptic organization of the rat basilar pontine nuclei (BPN), an attempt has been made to 1)identify axonal boutons of the corticopontine and cerebellopontine systems using routine electron microscopic degeneration techniques and 2)to demonstrate the cells of origin of these two systems by injecting horseradish peroxidase (HRP) into the BPN. Our results indicate that following unilateral decortication, two separate populations of boutons in the BPN appeared to undergo degeneration. Most numerous were small boutons (less than 1 um) exhibiting the typical dark type of degeneration and observed to contact small dendrites and spines. Also apparent (but in smaller numbers) were larger boutons (1-3 um) terminating on proximal portions of pontine neurons and exhibiting a filamentous reaction prior to becoming electron dense. When lesions were restricted to sensorimotor cortex, the same mixture of dark and filamentous boutons were noted, however, when lesions involved visual cortex, essentially only filamentous boutons were observed. Similar results were obtained when the brachium conjunctivum was unilaterally transected thereby interrupting cerebellar nuclear axons projecting to the BPN. Numerous large boutons contacting a characteristic cluster of dendritic protrusions underwent filamentous degeneration while at the same time a population of small boutons having a different postsynaptic locus exhibited electron dense degeneration. Taken together, these results suggest that both the corticopontine and cerebellopontine systems might each be composed of two neuronal populations, one type providing filamentous degenerating boutons, the other dark degenerating boutons. To test this notion, HRP was injected into the BPN using a ventral approach. Labelled neurons in sensorimotor cortex were distributed throughout layer Vb while in the cerebellar nuclei, numerous large spherical neurons were labelled along with a lesser number of smaller spindle-shaped somata. These findings, when correlated with the degeneration studies suggest that the sensorimotor cortical input to the BPN consists of 1)corticospinal axon collaterals (dark boutons, soma in deep layer Vb) and 2)corticobulbar or direct corticopontine axonal terminals (filamentous boutons, soma in superficial layer Vb). Similarly the cerebellopontine system consists of two components, large neurons which provide collaterals (filamentous boutons) to the BPN as they project rostrally to the red nucleus or thalamus and collaterals (dark boutons) of smaller neurons which project caudally to the inferior olive.
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340 CELLULAR LOCALIZATION OF β-1 AND β-2 ADRENERGIC RECEPTORS IN RAT CEREBELLUM. K.P. Minneman*, R.N. Pittman*, P.B. Molinoff and D. J. Woodward (SPON: R. Lasher). Dept. of Pharmacol., Univ. of Colo. Med. Ctr., Denver, CO and Dept. of Cell Biol., Univ. of Texas Health Sci. Ctr., Dallas, TX.

The rat cerebellum contains both β-1 and β-2 adrenergic receptors. Although β-1 adrenergic receptors comprise 18% of the total number of receptors in the cerebellum in 2 week old rats, by 3 months of age β-1 receptors comprise only 2% of total β-adrenergic receptors in the cerebellum. Since we have previously shown that in the rat cerebral cortex the β-1 adrenergic receptors are involved in neural transmission, it was of interest to examine the cellular localization of the β-1 and β-2 adrenergic receptors in the cerebellum.

Rats were subjected to X-irradiation on days 1, 4, 6, 8, 10, 12 and 14 of life. Conventional histological techniques were used to confirm the nearly total absence of the late-maturing granule, basket and stellate cells. The early-maturing Purkinje cells were spared, however, and both histological and electrophysiological studies suggest that these cells are relatively "normal" after X-irradiation.

Rats were killed after 2 or 6 weeks of life and β-1 and β-2 adrenergic receptors were measured in homogenates of the cerebellum and cerebral cortex. The density and total number of each receptor subtype was compared to that in control animals subjected to sham X-irradiation. No differences were observed in the concentration of β-receptor subtypes in the cerebral cortex of X-irradiated animals as compared to controls. Large changes were seen, however, in the cerebella of X-irradiated animals. At both ages a large (60-75%) decrease in the weight of the cerebellum was observed. There was, however, a 62-70% increase in the density of β-1 adrenergic receptors in the X-irradiated cerebella, indicating that remaining cellular components were enriched with respect to β-1 receptors. Conversely, the specific activity of β-2 adrenergic receptors was unchanged at 2 weeks of age but decreased by 53% at 6 weeks of age in the X-irradiated cerebella. This indicates that the cellular components destroyed by X-irradiation are relatively enriched with respect to β-2 adrenergic receptors. Calculation of the total number of receptors in the cerebellum at six weeks of age showed that the number of β-2 adrenergic receptors per cerebellum had decreased by 83% in the X-irradiated animals, but that the number of β-1 adrenergic receptors per cerebellum had decreased by only 33%. These data are consistent with the hypothesis that there are β-1 adrenergic receptors on Purkinje cells of rat cerebellum. (USPHS NS 13289 and 09199).

341 EYE VELOCITY AND POSITION SIGNALS IN FLOCCULAR PURKINJE CELL ACTIVITY DURING SMOOTH PURSUIT EYE MOVEMENTS. Hiroharu Noda, Tateo Warabi* and Naohiro Ishii*. Brain Research Institute, Depts. Physiol. and Anat., Sch. Med., UCLA, Los Angeles, CA 90024.

When a monkey moved its eyes, pursuing a sinusoidally oscillating visual target, simple spike discharges of Purkinje cells of the flocculus showed a cyclic modulation. The modulation frequently occurred in phase with the eye velocity curve, exhibiting a ½ π radians phase shift from the horizontal EOG. However, in the majority of Purkinje cells, the peak activity did not occur in phase with the maximum eye velocity. A wide range of phase shifts was observed between the activity and the EOG. The activity in these cells included a non-velocity component in addition to the eye velocity component. The velocity component could be demonstrated by changing the frequency of sinusoidal excursions of a constant magnitude. The peak firing rates were found to increase linearly with higher frequencies which were associated with higher peak velocities. In Purkinje cells showing a phase shift from the eye velocity curve, a source of the non-velocity component was the activity representing eye position. The eye position component was demonstrated in the following ways: (1) These cells exhibited tonic activity which was proportional to eye position during steady fixation of a stationary target. (2) During a zig-zag eye movement, tracking ramp target movements of a constant velocity, the firing rate of these cells increased or decreased depending on the direction of the eye movement. (3) When sinusoidal eye movements were executed, pursuing a target oscillating in the central, right, or left visual fields, the level of the cyclic modulation in activity varied markedly depending on the field of eye movement. (4) Eye velocity and position components of unit activity were dissociated during eye movements involved with pursuing a complex target motion driven by a composite of sinusoids of different frequencies. In such complex tracking eye movements, the peak eye velocity as well as the peak eye position were different in every cycle and the potential predictability of a pure sine wave was eliminated. Instantaneous discharge rates during such eye movements were continuously correlated with the eye position curve (EOG) or eye velocity curve (the derivative of the EOG). The activity during smooth pursuit eye movements included both eye velocity and eye position signals, although their proportions differed from unit to unit. (Supported by NIH Grant EY 01051).

342 CEREBELLAR COORDINATION : COVARIANT ANALYSIS AND CONTRAVARIANT SYNTHESIS VIA METRIC TENSOR. A TENSORIAL APPROACH TO THE GEOMETRY OF BRAIN FUNCTION. A. Pellionisz and R. Llinás. Dept. Physiol. & Biophys., New York Univ. Med. Ctr. 550 First Ave, New York 10016

A wealth of experimental data indicates brain function (e.g. motor coordination by cerebellar networks) to be a distributed and parallel property. It is imperative, however, to develop formal treatments capable of allowing precise conceptual descriptions and quantitative formulations of such global properties. Thus, a tensorial approach was introduced (Pellionisz & Llinás: *Neurosci. Abst.* 4,1978; *Neuroscience* 4:323,1979) which considered brains in terms of abstract geometry. Here, tensor network theory is utilized in the analysis of the coordination of limb movements and vestibulo-ocular responses.

Movements emerge from collective vectorial actions of the many segments of a limb, or of the extraocular muscles. The present treatment is based on the view that movements are reference frame invariant vectors (i.e. tensorial entities). Since movement vectors occur both in the 3-dimensional space and in the multi-dimensional space of the CNS, the fundamental problem of motor coordination is: how can an intended movement (which refers to the 3-space) be executed by a high dimensional motor system? This leads directly to the inherent properties of the spaces that contain the vectors : *a) Is the CNS hyperspace metric?* Then, if the coordination problem is regarded as an embedding of the 3-dimensional space into the CNS hyperspace, the question is: *b) How can the decomposition be unique despite the overcompleteness of the hyperspace?*

We assume that the CNS space *is* endowed with an inherent geometry: which in the case of motor system is given by the prewired matrix of the cerebellar metric tensor: Θ. Assuming a local homomorphism of the two overlapping spaces (3-dimensional and CNS hyperspace) leads to a two-step scheme of coordination. *(1) A decomposition of the intended vector into an overcomplete number of covariant components, using the geometry of the three-space, (2) A transformation of the covariant components into contravariant components by a CNS metric tensor.*

As shown by computer modeling, the covariant vector components can be established, even for an overcomplete number of coordinates. However, when used directly to generate the intended movement, the covariant components yield an ataxic, dysmetric movement, but when transformed to contravariant components, the intended movement is generated in a coordinated unique style.

Beyond providing a formal scheme of coordinated motor action by the known cerebellar neuronal network, some general conclusions are reached regarding the properties of CNS space and generalizing the covariant analysis and contravariant synthesis to sensory systems.(Supported by USPHS grant NS13742 from NINCDS)

343 VESTIBULO-CEREBELLAR AFFERENTS FROM THE INFERIOR OLIVE AND THE VESTIBULAR NUCLEAR COMPLEX. Kevin D. Phelan* and William R. Mehler. (SPON: J. deGroot) Ames Res. Ctr., Moffett Field, CA 94035 and Dept. Anat. Univ. Cal. San Francisco, CA 94143.

Single horseradish peroxidase (HRP) injections (.5-2.0 ul, 50%, Sigma VI) were placed into the nodulus, uvula or lingula by a horizontal stereotaxic approach via the posterior vermis of the cerebellum of 10 adult cats (42-72 hr survival, 0.5% para - 1.5% glut, DAB or BDHC substrate). These experiments showed cell labelling chiefly in the contralateral olive while cells in other brainstem nuclei, such as the vestibular nuclei, labelled bilaterally.

Afferents to the nodulus were shown to arise mainly from the dorsal cap of Kooy (dc), the ventrolateral outgrowth of the dc (vlo) and some cells in the rostral part of the medial accessory olive (MAO). Labelled cells consistently appeared in dc and vlo only when the spread of HRP involved the nodulus. These findings are similar to those cell patterns reported in the rabbit (Alley, et al., Brain Res. 98:582-589; Hoddevik and Brodal, J. Comp. Neur. 176:269-280, 1977). In injections involving mainly the uvula without apparent nodular spread, heavy cell labelling was found throughout nucleus B and the dorsal medial cell column (dmcc) and in the rostral parts of the MAO, as has been previously reported in cats (Brodal, J. Comp. Neur. 166:417-426, 1976). In some nodular injection cases varying amounts of leakage occured in passage through the folia of the uvula and in all available cases the HRP spreads into the overlying ventral uvula, part of which belongs to the vestibulo-cerebellum. Thus, in all nodular cases, labelling of B and dmcc cells occured in varying degrees, increasing in number of HRP positive cells as the spread into the uvula increases. These experiments suggest that there are possible B and dmcc projections onto the nodulus, but further small injection experiments utilizing various angular approaches to the nodulus are needed to settle this question. The HRP spread in the presently available experiments are too large to confirm the topographical projections of B and rostral MAO onto the nodulus as suggested by recent autoradiographic studies in the cat (Groenwegen, Voogd and Freedman, J. Comp. Neur. 183:551-602, 1979).

Anterior lobe injections using this horizontal approach into the lingula and some parts of centralis strongly label cells only in the caudal ventrolateral MAO, the caudal ventrolateral DAO and B, results similar to those reported by Brodal and Walberg (J. Comp. Neur. 172:85-108, 1977) using a more dorsal approach.

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

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344 TOPOGRAPHY OF CLIMBING FIBER PROJECTIONS TO THE INTERMEDIATE CEREBELLAR CORTEX OF THE MONKEY. Lee T. Robertson and Kenneth D. Laxer*. Neuro. Sci. Inst., Good Samaritan Hosp. & Med. Ctr., Portland, OR 97209.

The organization of climbing fibers to the cerebellum has been extensively studied in the cat, but almost no similar data are available for the monkey, which has a larger cortical area and a different repertoire of movements. The present work describes the somatotopic organization of climbing fibers in the intermediate zone of lobule V of the Macaca fascicularis that were responsive to natural stimulation. In monkeys anesthetized with pentobarbital, extracellular recordings of single Purkinje cells with climbing fiber responses (CFR's) were evaluated by gentle taps to the body and by computer controlled punctate stimuli. Peristimulus histograms were computed for the CFR's; latency and force thresholds were measured. The stereotaxic position of each unit was determined; electrode location was confirmed histologically.

The climbing fiber projections consisted of a mediolateral (3-9 mm) organization of patches that were elongated parasagittally. (1) Hindlimb--a 1.0 mm wide cell population that responded to low threshold stimulation of the ipsilateral dorsal foot and leg; a few cells responded to stimulation of the hip or tail. (2) Nonresponsive--a 0.5-1.0 mm strip of cells that did not respond to any of our cutaneous stimuli. (3) Forelimb--a 1.0-1.5 mm wide area where the majority of cells were activated by low threshold stimulation of the dorsal hand or to 2-3 fingers; a few cells received input from just the thumb or a finger or from the proximal areas of the limb. (4) Face--a 2 mm wide region that could be subdivided into a medial portion that primarily received input from the ophthalmic and maxillary divisions of the trigeminal nerve and a lateral portion where cells were activated by stimulation of areas supplied by the mandibular division. (5) Nonresponsive--a 1 mm zone where no cells responded to cutaneous stimulation.

The general somatotopic organization of intermediate cortex of lobule V in monkey is similar to the pattern described for the cat, but the proportion of cells responding to a particular receptive field is very different between the species. In the monkey only a few cells were activated by stimulating the ventral hand; a large proportion of cells were associated with the fingers, lower jaw, teeth, and tongue. It is proposed that the different somatotopic arrangement reflects diverse spatially organized skilled movements.

345 SURFACE PROTEINS OF CULTURED MOUSE CEREBELLAR CELLS. H.Rohrer+ and M.Schachner, Institute of Neurobiology, Heidelberg University, Heidelberg, GFR (Sponsor: V.Braitenberg)

Surface proteins of cultured monolayer cells from embryonic and early postnatal C57BL/6J mouse cerebella were identified by a lactoperoxidase catalysed ^{131}iodine labeling technique. Iodinated proteins have molecular weights of approximately 200, 145, 120, 85, 65, 50 and $30x10^3$ daltons (P200, P145 ...) as estimated by SDS-polyacrylamide gel electrophoresis in gradients of 4-15%. Membrane glycoproteins of apparent molecular weights of 200, 145, 100, 85 and $50x10^3$ daltons are detectable by biosynthetic labeling with ^{3}H-fucose. The two major iodinated proteins are the glycoproteins P200 and P145. P145 is released from the surface into the medium.

No changes in the patterns of labeled cerebellar cell surface proteins are detectable between embryonic day 12 and postnatal day 10. A pattern similar to the one seen with cerebellum is obtained with embryonic cerebral cortex, whereas early postnatal retina displays a distinctly different pattern, with P145 not being a major iodinated component.

An antiserum to 4-day-old cerebellar membrane fraction (anti-NS-4) reacts with P200 and P145 from solubilized plasma membranes of embryonic cerebral, and postnatal cerebellar and retinal cells. No structural similarities between these polypeptides is detectable by one-dimensional finger print mapping of peptides from Staphylococcus aureus V8 protease digests.

P200 and P145 are found most prominently in granule cell enriched fractions obtained by a bovine serum albumine step gradient separation method. The glial cell enriched fraction shows increased P100, P85 and P50. These three proteins are also increased in cultures of staggerer mutant mice.

346 SPONTANEOUS AND EVOKED RHYTHMIC, SYNCHRONOUS NEURAL ACTIVITY IN INFERIOR OLIVE UNDERLIES HARMALINE-INDUCED TREMOR. Jesse Schulman* and Floyd Bloom. (SPON: E.F. Battenberg) A.V. Davis Ctr. for Behav. Neurobiology, Salk Inst., La Jolla, CA 92037.

Much indirect evidence suggests that cells of IOC tend to fire rhythmically and that neighboring IOC cells tend to fire synchronously. This evidence comes primarily from studies demonstrating synchronous occurrence of climbing fiber-mediated excitation in neighboring Purkinje cells (Bell and Kawasaki, J. Neurophysiol. 35, p.155, 1972), and from studies of the drug harmaline, which elicits a 8-12 Hz tremor by causing rhythmic and synchronous activity in IOC (deMontigny and Lamarre, Br. Res. 53, p. 81, 1973; Headley et al., Br. Res. 101, p. 461, 1976). However, there is no report directly demonstrating the normal occurrence of such activity in IOC itself and the suggestion that harmaline accentuates rather than generates such an occurrence is unproven. Using extracellular metal microelectrodes, which are particularly useful for recording aggregate activity of groups of neurons, we find episodes of spontaneously occurring rhythmic and synchronous activity in IOC, and find that cutaneous stimulation can evoke episodes of such activity lasting .5 seconds or more. Responses were recorded throughout IOC, but were most consistently found in caudal dorsal accessory nucleus or in medial accessory nucleus near the center of its rostro-caudal extent. Moreover, recording from IOC before and after administration of harmaline shows directly that spontaneous episodes are increased in amplitude and duration by the drug. By filtering the signal from the metal microelectrode in different ways, we found it possible to study simultaneously single-unit, multi-unit, and aggregate neuronal activity. We find that the waves of both evoked and spontaneous aggregate activity, and repetitive evoked single-unit responses, all express the same underlying rhythm, whose period is identical to the most common interspike interval of the spontaneously firing unit. These findings constitute the first direct demonstration that a tendency to rhythmic and synchronous activity underlies spontaneous, evoked, and harmaline-induced activity of IOC. Implications of these findings regarding transmission in the olivo-cerebellar pathway will be discussed. Further, there exists a normal physiological 8-12 Hz tremor (see Joyce and Rack, J. Physiol. 240, p. 375, 1974). Since harmaline produces a similar but more severe tremor by exaggerating normal IOC activity, this physiological tremor may be a consequence of spontaneous IOC activity.

347 ERRONEOUS ZONES OF THE CEREBELLAR FLOCCULUS. John I. Simpson. Dept. Physiol. & Biophys., New York Univ. Med. Ctr., 550 First Ave., New York, N.Y. 10016

The climbing fiber (CF) projection from the inferior olive to the cerebellar cortex is organized into a number of zones oriented normal to the long axis of the folia. By taking an eclectic approach focused on the flocculus, a proposal on the nature of CF zones may be offered in specific behavioral terms. Groenewegen and Voogd (1977) have shown anatomically in the cat that the CF projection from the dorsal cap of the inferior olive to the rostral flocculus delineates three zones — a central zone which receives from the caudal dorsal cap and two bordering zones which receive from the rostral dorsal cap. In this laboratory we have shown that dorsal cap CFs in rabbit are visually responsive in a direction and speed selective manner. The CF signals constitute error signals (retinal image slip) indicating deviation from optimal motor performance. In addition, we have also shown that three preferred directions in visual space are defined by the visual CFs. Two of the preferred directions (horizontal, up with a posterior component) are signaled in the flocculus from the ipsilateral eye. The third direction (down with a posterior component) is signaled from the contralateral eye. Recordings from the dorsal cap have shown that the horizontal direction is represented in the caudal part of the dorsal cap while the off-vertical directions are represented more rostrally. Taken together, the anatomy and physiology indicate that the central zone of the rostral flocculus is related to horizontal eye movements while the two bordering zones are related to off-vertical eye movements. These predictions are borne out by the finding (Ito, et al, 1978) that with electrical stimulation of the rabbit flocculus three zones can be distinguished on the basis of the directions of the evoked eye movements. Horizontal movements are evoked from a central zone and off-vertical movements are evoked from the bordering zones. The three directions of the evoked eye movements have an internally consistent relation to the three directions of visual space established by the visual CFs. Thus, each of the three visual CF zones in the flocculus is associated with one of three specific directions, which may be viewed as coordinates of sensory-motor space for compensatory eye movements. For the flocculus, the coordinates represented by the CF zones can be readily referred to the external world geometry, but for most other cerebellar regions interpretation of CF zones in terms of coordinates of sensory-motor space will not likely be so directly referable to the external space. Supported by USPHS Grant NS-13742 from NINCDS.

348 ACTIVITY OF NEURONS IN THE CEREBELLAR CORTEX RELATED TO PARAMETERS OF A CONTROLLED ISOMETRIC PREHENSION TASK PERFORMED BY MONKEYS. Allan M. Smith and Daniel Bourbonnais*(SPON: H.H. Jasper). Centre de Recherche en Sciences Neurologiques, Université de Montréal, Montréal, Québec.

Recent studies of the cerebellum have emphasized the role of this structure in the initiation of quick movements. In contrast the present study was designed to examine the discharge of cerebellar cortical neurons during highly controlled finger pressures Monkeys were trained to exert and maintain precise forces on a hand held strain gauge for fruit juice reward. An exhaustive analysis of 26 forearm and intrinsic muscles of the hand indicated that the task is accomplished by co-contraction of antagonist muscles. To date recordings have been made from 136 neurons in the paramedian border area between the anterior and posterior lobe (lobules V and VI). Sixty-six units had receptive fields on the forearm, wrist and fingers and discharged in relation to the task. In general, neurons which did not demonstrate a climbing fiber discharge increased activity during prehension. In contrast, most, but not all, Purkinje cells identified by the presence of complex spikes were inhibited during some phase of the task. Complex spike activity did not appear related in any way to the task performance. Several of the unidentified neurons demonstrated significant correlations between discharge frequency and either rate of force change or force or both these parameters. These preliminary findings suggest that the cerebellum plays at least as important a role in slow controlled motor function as it does in fast movements. Furthermore, the discharge pattern of Purkinje cells indicates that the majority of these neurons are inhibited during the co-contraction of antagonist muscles.

(This research was supported by the Medical Research Council of Canada)

349 A NON-INACTIVATING VOLTAGE-DEPENDENT SODIUM CONDUCTANCE IN MAMMALIAN PURKINJE CELL SOMATA, STUDIED IN VITRO. M. Sugimori* and R. Llinás (SPON: M. E. Hatten). Dept. of Physiol. Biophys., New York Univ. Med. Ctr., New York 10016.

An in vitro slice preparation of guinea pig cerebellum has allowed investigation of the voltage-dependent electroresponsiveness of Purkinje cells at somatic and at different dendritic levels. While it was previously reported that, in these neurons the somatic action currents are mainly carried by sodium, and the slower dendritic action potentials by calcium current (Llinás & Sugimori, Soc. Neurosci. Abst. 4, 1978), a new type of voltage-dependent spike was recently observed. Thus, following blockage of the slow calcium current (by adding either manganese, cobalt or cadmium salts to the superfusion fluid, or by simply exchanging calcium with magnesium), direct stimulation of the soma generated, besides simple spikes, a slowly rising and prolonged all-or-none response. This response had a lower threshold and thus served to trigger simple action potentials during its rising phase. At its plateau level approximately 40 mV from the resting potential (generally 65 mV), there was a complete inactivation of the fast spikes. This plateau was maintained as long as the depolarizing current was applied and its duration outlasted the stimulus. The duration of the plateau after termination of the stimulus may range from a few milliseconds to several hundred milliseconds, depending on the stimulus amplitude. A test pulse applied during the plateau indicated a fourfold increase in membrane conductance during this period.

Application of 10^{-5} tetrodotoxin to the bath reversibly blocked both the action potentials as well as the plateau response. Similar findings could be obtained if sodium was replaced in the superfusion fluid by tris or by choline chloride.

Results similar to those obtained at soma may also be seen at dendritic level. However, in these cases the amplitude of the response was much smaller and its threshold higher, indicating that this non-inactivating sodium current is most prominent at somatic level.

It is thus concluded that at the soma Purkinje cells are capable of demonstrating a non-inactivating (or very slowly inactivating) sodium current which is distinct from that generating the fast action potentials. The plateau level of this all-or-none response seems to be produced by an equilibrium state between voltage-dependent sodium (g_{Na}) and potassium (g_K) ionic conductances since if tetraethylammonium was injected intracellularly, the plateau level of this sodium response was shifted towards E_{Na}. The possible role of this slow sodium current in Purkinje cell integration will be discussed in light of the repetitive firing properties of this cell. (Supported by USPHS grant NS-13742 from NINCDS)

350 VISUAL AND PURSUIT EYE MOVEMENT SIGNALS IN THE MONKEY VERMIS. David A. Suzuki*, Manabu Kase* and Hiroharu Noda (SPON: S. Hagiwara) Brain Research Institute, Depts. Physiol. Anat., Sch. Med., UCLA, Los Angeles, CA 90024

The cerebellar vermis has been known to be involved with the processing of various sensory information and recently with the control of smooth pursuit eye movements. To further elucidate the relationship between motor and sensory signals in the vermis, three paradigms were employed whereby a monkey was trained to 1) track a moving small red spot, 2) fixate a stationary red spot during movement of a random dot background or 3) during movement of a small, white test spot not associated with a reward.

Modulations in Purkinje cell and mossy fiber activities recorded from lobules VI and VII were observed in association with the various paradigms employed. All units in our population exhibited discharge modulations which reflected the velocity of smooth pursuit eye movements. In addition to having this eye velocity component, some units were also found to be responsive to retinal image motion. When eye movements were minimal or absent, the movement of the background pattern was associated with activity changes reflecting background movement velocity or with a direction dependent increase in firing whereby movement per se, rather than velocity, was the determining factor behind peak firing rate.

Modulation in unit activity was observed in some units in conjunction with the movements of the test spot occurring while the monkey fixated the stationary red spot. As the velocity of test spot movement increased, the peak firing rate exhibited by these units also increased, within limits, in a fairly linear manner. For sinusoidal test spot movements, unit activity was modulated in phase with the changes in the retinal slip velocity associated with the test spot movement. The encoding within the same unit of retinal slip and eye velocity signals implicates the presence of neural correlates of target velocity signals.

Among the units whose activities reflected both eye and retinal slip velocities, some were also responsive to slippage of the entire visual field across the retina. In these units, it was again the velocity or the direction of movement which were the parameters reflected in unit discharges. Visual signals within the vermis, therefore, appear to convey 1) velocity information with respect to moving visual stimuli in general or 2) velocity information concerning discrete targets (spots) and directional information concerning movement of non-discrete images (background) across the retina. The target velocity signals would be of specific import with respect to the role that lobules VI and VII of the vermis play in the control of oculomotor function. (Supported by NIH Grant EY01051).

351 VISION STABILIZES FASTIGIAL NYSTAGMUS. T. Vilis and J. Hore, Dept. of Ophthalmology and Dept. of Physiology, University of Western Ontario, London, Canada.

The role of the fastigial n. in the maintenance of a stationary eye position was examined in 7 Cebus monkeys. Eye position was measured by EOG's or by the magnetic search coil technique. Reversible lesions of the fastigial were produced by means of a stereotaxically implanted cryoprobe.

In complete darkness, cooling the fastigial produced, in all monkeys, a pronounced nystagmus with horizontal drift (vel. approx. 100 deg/sec) of the eye towards the side of the lesion. The rate of drift was independent of eye position. Vision stabilized this drift in two phases; a fast phase in which the velocity dropped by 20% in less than 1 sec and a slow phase in which the velocity dropped exponentially by 63% of the remaining velocity in approximately 6 sec. A return to darkness usually reestablished the drift with a linear time course (63% increase in 8 to 25 s) and with the drift reaching a smaller maximum velocity. After multiple periods of light and dark, the drift tended to stabilize or occasionally reversed in direction. In this latter case a subsequent period of light and dark frequently reestablished a high velocity drift in the original ipsilateral direction.

The degree of cooling and thus the size of the reversible lesion did not affect the drift velocity in a graded fashion. In 3 monkeys in which this was studied, temperatures above a critical value produced no drift or drift that quickly stabilized, while temperatures below this value produced maximum drift.

If during cooling in the dark the monkey was rotated in the horizontal plane, the drift velocity was modulated above and below the mean value obtained without rotation. For sinusoidal rotations of .5 to 1 Hz, the gain of the VOR in the dark increased from .8, prior to cooling, to 1.0 during cooling.

These results suggest that lesions of the fastigial n. produce a destabilizing effect on the tonic balance between the vestibular n. which normally provide an internal estimate of head velocity. Vision corrects this velocity error by providing 1) a short term contribution (probably through a combination of the pursuit and optokinetic systems) which masks the velocity error as long as vision is present and 2) a long term homeostatic contribution which attempts to rebalance velocity in subsequent darkness.

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352 EFFECT OF RETINAL IMAGE MOTION UPON FLOCCULAR PURKINJE CELL ACTIVITY DURING SMOOTH PURSUIT EYE MOVEMENTS. Tateo Warabi*, Hiroharu Noda and Naohiro Ishii* (SPON: H. W. Magoun). Brain Research Institute, Depts. Physiol. and Anat., Sch. Med., UCLA, Los Angeles, CA 90024.

During smooth pursuit eye movements, simple spike activity of floccular Purkinje cells of monkeys exhibited cyclic modulations. The modulation occurred in darkness where only a red spot was visible. As retinal image movement was negligible, the modulation reflected signals arising in the oculomotor system. In some Purkinje cells, the discharge pattern was markedly modified when eye movements occurred in light, implicating an interaction between oculomotor and visual signals.

Five paradigms were employed in order to dissociate these signals: (1) tracking of a red spot in darkness, (2) tracking of the target in the presence of a stationary random-dot background, (3) fixation of the stationary target while the background was moved sinusoidally, (4) tracking, with target and background moving together in the same direction, and (5) tracking, with target and background moving in opposite directions.

In the first group of Purkinje cells, the modulation of activity appeared only in association with eye movements, and merely moving the background (paradigm 3) failed to produce any consistent modulation. The firing patterns observed during smooth pursuit in the presence (paradigm 2) or absence of the background (paradigm 1) were almost identical in these cells and primarily reflected eye velocity. In the second group of Purkinje cells, the presence of visual background (paradigm 2) markedly modified the activity changes associated with pursuit eye movements in the dark. The movement of the image of the background was the primary cause of the modification, since the background and target movement in opposite directions (paradigm 5) produced the most dramatic effect. The activity pattern associated with movement of the target and background in the same direction (paradigm 4) was the same as seen in darkness (paradigm 1). In some Purkinje cells, the secondary modification was due to an inhibitory effect of the visual inputs. In these cells, when the background was moved sinusoidally, during fixation of the stationary target (paradigm 3), discharges decreased in relation to the peaks of the velocity curve of the background movement. Purkinje cells of the flocculus appear to encode both eye and retinal image motion information. To different degrees, therefore, both oculomotor and visual information aid the flocculus in the control of smooth pursuit eye movements.(Supported by NIH Grant EY 01051).

353 THE CEREBELLOPONTINE SYSTEM IN THE RAT; AN AUTORADIOGRAPHIC AND HRP STUDY. Carl B. Watt* and Gregory A. Mihailoff. (SPON: E.D. Ross). Cell Biology, Univ. Texas Hlth. Sci. Ctr., Dallas, Texas.

In Long-Evans rats the autoradiographic technique was utilized to investigate the pattern of organization in the cerebellopontine projection system. Tritiated leucine was deposited in the deep cerebellar nuclei utilizing either a pressure or an electrophoretic injection system. After a 24 hour survival period, frozen sections were processed routinely for light microscopic autoradiography and exposed for four weeks. The results of the investigation were threefold. First, pontine afferents appeared to originate from each each of the major nuclear subdivisions of the deep cerebellar nuclei. Projections arising from the lateral cerebellar nucleus terminated extensively throughout the rostrocaudal extent of the contralateral pontine nuclei. Furthermore, terminal zones of the lateral nucleus were found within each major subdivision of the pontine nuclei including the dorsal peduncular nucleus. The interpositus nucleus was observed to project less extensively to the contralateral pontine gray with most of its terminal zones lying within the same pontine regions receiving input from the lateral cerebellar nucleus. The medial nucleus appeared to give rise to the least extensive number of cerebellopontine axons which terminated contralaterally within a small restricted portion of the dorsomedial pontine region throughout the caudal 3/5 of the pontine nuclei. Unlike the projection zones of the lateral and interpositus nuclei, the projection zones of the medial cerebellar nucleus did not consist of continuous, longitudinally oriented columns but rather formed an interrupted series of relatively small bursts of terminal labelling in the dorsomedial pontine region. Second, comparison of the various cerebellopontine terminal zones with the projection zones of other pontine afferents revealed that, although certain pontine regions were reserved for cerebellar nuclear input, there was significant overlap with projections from sensorimotor cerebral cortex as well as the superior colliculus. In addition, it was noted in several cases that the pontine projection from the lateral and interpositus nuclei gave rise to a small, but readily distinguishable ipsilateral component. Third, injections of horseradish peroxidase within the deep cerebellar nuclei demonstrated the existence of a ponto-deep nuclear projection system arising in part from each of the major subdivisions of the pontine nuclei. Furthermore, it appeared that many of the HRP-labelled somata were located within the same pontine regions which received an input from the cerebellar nuclei as determined in the previous autoradiographic studies. Such observations support the notion of reciprocal connectivity that has been reported to exist between the cerebellar nuclei and the pontine gray.

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354 BRANCHING OF TRIGEMINAL MOSSY FIBER AXONS AND THE PRECISION OF BODY SURFACE REPRESENTATION IN THE CEREBELLUM. D.C. Woolston*, J.J. Kassel, J.M. Gibson, and W.I. Welker. Dept. Neurophysiology U. Wisconsin, Madison WI 53706.

Micromapping studies of tactile inputs to the rat cerebellar granule cell layer have revealed a precise mode of body surface representation termed "fractured somatotopy" (Shambes et al., BBE 15: 94,1978). It is characterized by the existence of "patches": precisely organized somatotopic representations of body parts, arranged into a non-somatotopic mosaic. Regions of the head and mouth are typically represented on several folia, and more than once on a single folium. Anatomical studies reveal extensive inter- and intra-folial branching of mossy fiber inputs, and have been interpreted as precluding precise spatial patterning of cerebellar afferents. A hypothesis compatible with both findings is that trigeminal mossy fiber branching occurs, but within the framework of precise, fractured somatotopy. Experiments with barbiturate anesthetized rats involving alternate stimulation and recording with tungsten microelectrodes in homologous patches on separate folia supported the hypothesis. Monopolar stimulation in crus IIA, crusIIB, and the paramedian lobule yielded short latency (0.75-1.0ms) responses which were confined to homologous patches in the uvula, lobulus simplex B, and crus IIB, respectively. To determine that these responses were mediated by trigeminal mossy fiber collaterals, experiments were conducted with two cerebellar stimulating electrodes (monopolar stimulation, 20-100μs biphasic pulses, 3-100μA) and a third electrode for recording antidromically evoked responses in the homolateral spinal nucleus of V, pars interpolaris. The three electrodes were positioned until similar receptive fields were obtained; in such cases antidromic spikes were frequently evoked from both stimulating loci. Collision of the responses at short intervals between stimulation at the separate loci was sought as evidence for axon branching. Such collisions were obtained with granule cell layer stimulating loci in the following pairs of folia: crus IIA & crus IIB; simplex B & crus IIB; crus IA & crus IC; crus IIA & the uvula; crus IIA & crus IIA contralateral; and simplex B & crus IIA; branching was also demonstrated between two like patches on the same folium. The probability of collision increased with the degree of receptive field overlap between the stimulating loci.

We conclude that the characteristic collateralization of mossy fibers is compatible with the finding of precise, fractured somatotopy, since the branching of trigeminal mossy fibers appears to occur in a highly organized manner within and among homologous body part patches. (Supported by NIH grants NS14748 and NS07026, and NSF grant BNS 77-16230.)

355 ELECTROPHYSIOLOGICAL PROPERTIES OF MAMMALIAN INFERIOR OLIVE NEURON IN IN VITRO BRAIN STEM SLICES AND IN VITRO WHOLE BRAIN STEM. Y. Yarom* and R. Llinas. Dept. of Physiol. & Biophys., New York Univ. Med. Ctr., New York 10016.

Two in vitro brain stem preparations have been developed. First, using our cerebellar slice technique (Llinás & Sugimori, Soc. Neurosci. Abst. 4, 1978), sagittal slices were prepared from guinea pig brain stem, which allowed direct recording from identified neurons in the inferior olive and in several reticular and motor nuclei. The second preparation consisted of whole brain stem comprising the levels from the C_1-bulbar junction to the inferior colliculus. Brain stems were perfused with modified Ringer's solution through the basilar artery following ligature of both vertebral arteries. While the slice preparation could survive for >48 hrs after isolation, the brain stem survived for close to 7 hrs. Both preparations permitted the study of electrical properties of inferior olive cells. These neurons were seen to generate, through antidromic or direct stimulation, a fast action potential followed by a prolonged calcium-dependent spike. This spike, most probably of dendritic origin, triggered a potassium conductance change which generated a large (10-15 mV) after-hyperpolarization lasting for 200-250 msec. The initial fast spike could be blocked by 10^{-5} tetrodotoxin or by removal of external sodium from the perfusion medium. The calcium dendritic spikes were not blocked by those procedures but could be prevented either by application of cadmium, cobalt, or manganese salts, or by removal of calcium from the superfusion fluid. In addition to the above, hyperpolarization of 5-10 mV beyond resting level (70 mV) revealed a second calcium current of probable somatic origin. This voltage-dependent calcium conductance, which was inactivated at the normal resting potential, was capable of generating a calcium spike at a lower threshold than that required for generating sodium-dependent action potentials. However, it was not blocked by tetrodotoxin or removal of sodium, but was blocked by cadmium, cobalt or manganese. In addition, and as opposed to the dendritic calcium spikes, it showed a marked refractoriness indicating that these two g_{Ca} differ in their properties and location. Thus, during the normal firing, sodium-dependent action potentials activated dendritic calcium action potentials. In turn, the large calcium-dependent potassium conductance generated the prolonged after-hyperpolarization, and as a rebound from this hyperpolarization a calcium-dependent somatic spike, which restarts the whole cycle. The inferior olive cell is therefore capable of behaving as a single cell oscillator if modulated to the proper membrane potential levels. Finally, simultaneous penetration of a pair of inferior olive cells allowed direct demonstration of electrotonic coupling between these neurons. (Supported by USPHS grant NS-13742)

CEREBRAL CORTEX

356 BOTH OBJECT AND LOCATION REVERSAL ARE IMPAIRED AFTER FRONTAL OR HIPPOCAMPAL SYSTEM DAMAGE IN RATS. J.T. Becker*, D.S. Olton, C.A. Anderson, & R.S. Margolies, Dept. of Psych., The Johns Hopkins University, Baltimore, MD, 21218, U.S.A.

This experiment investigated the effects of lesions of the Medial Frontal Cortex (MF) and the Fornix (FX) on the retention and reversal of a non-spatial Object Discrimination and a spatial Location Discrimination.

Acquisition training, prior to surgery, resulted in each rat learning one of the two discriminations. The Object discrimination was designed so that the rats learned to discriminate between two objects based on characteristics of the objects themselves. Neither the location of the objects in the test arena nor the turns required to approach them predicted the correct choice. The Location Discrimination was designed so that rats learned to discriminate between two objects based on their location within the test arena. Neither the objects themselves nor the turns required to approach them predicted the correct choice. All rats learned a discrimination and performed well on various transfer tests designed to be sure they had used the appropriate choice strategy.

Retention testing began three weeks after the rats had received either 1) a lesion of the MF, 2) a lesion of the FX, or 3) a control procedure. Rats with lesions of the MF were unimpaired in the retention of both the Object and Location Discriminations. Rats with lesions of the FX, however, were impaired in the retention of the Location but not the Object Discrimination.

Reversal training was begun after the rats had completed retention testing. Each rat was given three successive discrimination reversal problems. Rats with lesions of the MF were impaired in both reversal tasks, as were rats with lesions of the FX. Rats with FX lesions, however, were significantly more impaired in reversal than rats with MF lesions.

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

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

357 ASSOCIATION AND COMMISSURAL PROJECTIONS FROM AREA 18 AND THE MEDIAL BORDER OF AREA 17 IN THE ALBINO RAT. Helen Benzinger, Leo C. Massopust and Paul A. Young. Francis and Doris Murphy Neuroanat. Res. Lab., Dept. Anat., Sch. Med., St. Louis University, St. Louis, MO 63104.

The purpose of this investigation was to examine by a modern hodological method the connections of area 18 and the medial border of area 17 as defined by Krieg in the albino rat. Injections of tritiated leucine were made in these cortical areas and the locations of the injections were determined by means of coronal sections made in the same plane as those in the atlas of Konig and Klippel (1963). Brain sections were processed using the autoradiographic technique of Cowan et al. (1972). Fibers from the injected areas crossed in the splenium of the corpus callosum and terminated in a columnar configuration in the contralateral homologous cortical area, with slightly heavier labelling in layers I, IV and inner V. Immediately adjacent to the injection site a spray-like projection reached Krieg's areas 29b and 29c of the cingulate cortex with the heaviest concentration of terminal silver grains in layers I and IV. In sagittal sections two columnar configurations were seen in ipsilateral area 18a. Since the axis of these configurations was in the sagittal plane, they could not be discerned in coronal sections. A light projection to cortical layer I in the superior lip of the rhinal sulcus was also observed. In area 17 contiguous to the injection site a density of silver grains was found with the heaviest concentration in layers I, II, III, and V. Thus, area 18 and the medial border of 17 have commissural connections to the contralateral cortical homologue, whereas ipsilaterally these areas connect with the contiguous area 17 and area 18a. There also appear to be ipsilateral connections with the limbic system, specifically through the cingulate cortex and the cortex in the superior bank of the rhinal sulcus.

358 CORTICAL LOCATION OF PYRAMIDAL TRACT NEURONS ESTABLISHED WITH HRP. M.A. Biedenbach and J.L. De Vito. Dept. Physiol., U.T. Health Science Ctr., San Antonio, TX 78284 and Reg. Prim. Res. Ctr., U. of Wash., Seattle, WA 98195.

Past evoked potential and lesion studies attempted to determine the location of neurons whose axons project in the pyramidal tract (PT). Results indicated that all PT-neurons reside in the rostral third of the cerebral cortex, with maximum concentration in motor and somatosensory cortex. However, both methods yield only very approximate surface maps of underlying "PT-cortex" and do not identify the exact location of PT-cells.

We used the horseradish peroxidase (HRP) technique to determine quantitatively the distribution in cortex of the total population of PT-cells and to study cell morphology. In anesthetized cats, one pyramidal tract was microdissected off the ventral surface of the medulla. A complete cross-sectional cut was made and HRP applied to the proximal PT-stump. Thirty hours later, the cats were perfused and the brains removed. Whole-brain frozen sections were cut (frontal and sagittal planes), reacted with DAB and stained with cresyl-violet. Using dark-field microscopy and an x-y plotter (controlled by the microscope stage), the brain sections were outlined on paper and each labelled neuron in the section plotted as a dot. The total cortical region containing PT-cells was divided into eight subregions and the percent of PT-cells determined in each. Subregions 1, 3 and 8 are surface cortex: #1 (containing 6-12% of PT-cells) extends rostral to the cruciate sulcus, #3 (15-20%) extends caudal to cruciate sulcus up to the lateral sulcus, #8 (7-8%) covers cortex laterally adjacent to #3. Subregions 2, 4, 5, 6, and 7 represent sulcal cortex: #2 (containing 8-10% of PT-cells) is the lateral bank of presylvian sulcus, #4 (16-22%) the ventral bank of the cruciate sulcus (Brodmann's area 6), #5 (30-35%) the dorsal bank of the cruciate sulcus (Brodmann's area 4), #6 (2-5%) the dorsal bank and #7 (1-4%) the ventral bank of the coronal sulcus. Thus, the HRP-technique revealed that only 30-40% of PT-cells reside in surface cortex, but the majority in sulcal cortex. However, projection of all labelled PT-cells to overlying surface cortex yields an area of "PT-cortex" in rough agreement with that of the older studies. PT-cells were also found in a new cortical area, the lateral bank of the presylvian sulcus, thus far not considered part of "PT-cortex". The cruciate sulcus extends for a considerable distance (10 mm) caudally, below surface cortex. The hidden banks of this sulcus contained greatest concentration of PT-cells.

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

The pattern of thalamocortical projections was studied in 49 adult mice. In each animal a lesion was made in either the anterior, lateral, metathalamic, or posterior (but not in the medial or intralaminar) nuclear groups; after 4 days the animals were sacrificed and their brains stained with the Fink-Heimer method. Sections from unoperated brains stained with cresyl violet or the Fink-Schneider silver stain for normal fibers were used to establish cytoarchitectonic parcellations of the thalamus and neocortex.

Thalamocortical projections terminate in three distinct tiers superficial, intermediate, and deep in the neocortex. There are two types of thalamocortical axons: Class I - intermediate and large diameter axons whose telodendria are concentrated in the middle tier (layer IV and/or layer III) although they may send collaterals to the inner or outer tiers; Class II - small axons whose telodendria are not concentrated in the middle tier and which may terminate in one or more tiers. All thalamic nuclei projecting to the neocortex have class II projections; many also have class I connections. With one possible exception, a single cytoarchitectonic area of the neocortex appears to receive class I projections from only one thalamic nucleus, although it may receive the class II projections of multiple nuclei.

Small thalamic lesions produce intracortical terminal degeneration with a restricted tangential distribution. Class I projections produce in the tangential cortical plane a topologically continuous representation of two dimensions of their nucleus of origin. This representation appears to be topologically continuous with that of at least one adjacent nucleus (lesions straddling part of the internuclear border produce degeneration straddling the border of the corresponding cortical projection areas) such that the ensemble of class I connections produces a topologically continuous cortical representation of the ensemble of their nuclei of origin.

Variations of lesion position parallel to the third nuclear axis (not represented in the cortical tangential plane) produce systematic changes in the overall density of intracortical degeneration without altering the relative densities in the three tiers or the tangential distribution of degeneration. From this it is inferred that i) thalamic relay neurons are organized along "lines of projection", neurons in the same line projecting to the same tangentially restricted cortical region and ii) the neurons of origin of class I and class II axons are intermixed along the lines of projection.

360 AUDITORY "ASSOCIATION" CORTEX AND DELAYED VISUAL MATCHING. John A. Costalupes, James H. Dewson, III, and Richard C. Placone, Jr*. Auditory Neurobiology Laboratories, Hearing and Speech Sci., Stanford Univ School of Medicine, Stanford, CA 94305.

Five monkeys (M. fascicularis), previously evaluated on a delayed cross-modal symbolic matching task, have been trained to perform a delayed visual identity-matching task (red and green) in which intratrial delays are varied from trial to trial by titration. The group includes three animals with lesions of the left superior temporal gyrus (so-called auditory association cortex), one with a unilateral lesion of the primary auditory cortex, and one unoperated control. In a previous report (Dewson, et al., J ACOUST SOC AMER., 58:S66, 1975), the operated animals were required to pair, after unfilled delays, an acoustic sample (a tone or a noise) with a visual match (red or green). Animals in which the left superior temporal gyrus had been removed could not achieve the delay durations they had achieved preoperatively on this task. In the present experiment, as would be expected, delays achieved for strictly visual matching are longer than those attained on the cross-modal task. Ablation of the auditory "association" cortex does not, therefore, appear to affect performance on a delayed visual identity-matching task.

In addition, the monkeys are being tested on a delayed visual symbolic matching task in which a cross and a triangle are matched with the colors red and green, respectively. This experiment will demonstrate whether ablation of the left superior temporal gyrus affects symbolic matching in the visual modality. Deficits in performance on the symbolic visual task attributable to prior removal of the auditory "association" cortex would show that the observed effect cannot be limited specifically to the auditory modality and would further suggest that the deficit is due to a disruption of a second-order association of the match with the sample.

361 DEVELOPMENTAL DIFFERENCES IN AFFERENTS OF MEDIAL PREFRONTAL CORTEX IN THE GOLDEN HAMSTER. James E. Crandall and Christiana M. Leonard. Dept. Neuroscience, Col. Med., Univ. of Fla., Gainesville, FL 32610.

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

Small volumes (.01-.1 µl) of 30% HRP were injected unilaterally through glass micropipettes (tips=30-60 µm) into the MPFC of hamster pups. The animals were sacrificed after 24 hours and their brains processed with the TMB procedure of Mesulam (1976). We describe here preliminary results from 8 brains (2 each at 10, 20, 25 and 33 days of age) with relatively large injections involving areas 8 and 24 from the frontal pole to the level of the genu.

Heavily labeled neurons were located at all ages in subcortical structures including substantia innominata, lateral preoptic area, globus pallidus, lateral nucleus of amygdala, claustrum, numerous thalamic nuclei (mediodorsal, anteromedial, ventromedial, midline and intralaminar groups), ventral tegmental area, raphe nuclei, and mesencephalic reticular formation. After injections at Day 10, glia were heavily labeled bilaterally in the corpus callosum, anterior commissure and internal capsule. Few labeled cortical neurons could be found outside of the injection site. Although callosal projections were heavily labeled by Day 20, ipsilateral cortical projections were sparse with only a few labeled neurons present in caudal suprarhinal cortex. Widespread labeling of ipsilateral cortical neurons was first seen after MPFC injections in Day 25 and 33 animals in layers V and VI of area 35 just dorsal to the rhinal fissure and extending dorsal into areas 40 and 41 (parietal and temporal association cortex). Whether the difference in cortical labeling seen after injections at different ages reflects a difference in uptake or transport mechanisms or a true difference in neuronal connectivity is currently being investigated.

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362 EFFECT OF VISUAL CORTEX LESIONS ON RADIAL MAZE PERFORMANCE IN RATS. Robert H. I. Dale* and Melvin A. Goodale* (SPON: M. L. Wolbarsht). Dept. Psych., Univ. West. Ont., London, Ont., Canada N6A 5C2.

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

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

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

363 CORRELATION OF METABOLISM, BLOOD FLOW AND BLOOD VOLUME WITHIN SUPERFICIAL MICROAREAS OF THE CAT BRAIN CORTEX BY MICROFLUORO-REFLECTOMETRY. András Eke* and Arisztid G. B. Kovách* (SPON: Vernon Pegram). Exp. Res. Dept., Semmelweis Med. Univ., Budapest

Simultaneous, optical assessment of tissue NADH-level, blood flow and blood volume in superficial microareas of the brain cortex with dimension of the order of 1 mm^3 is presented. Focusing on the exposed brain cortex through a glass window implanted into the parietal bone of anesthetized cats, the intensity of the diffusely scattered light and that emitted by the NADH molecules was continuously measured at 366 nm and 450 nm respectively. The tissue NADH-level was monitored by measuring the NADH-fluorescence at 450 nm (according to Chance et al.(Science 137:499-508,1962)) and it was corrected for the hemodynamic artifact following the method of Harbig et al.(J. Appl. Physiol. 41:480-488,1976). The absolute values of blood flow and blood volume were determined at the same microarea by the microreflectometric indicator dilution method of Eke et al.(Am. J. Physiol. 236(5):H759-768,1979), which analyzes the optical density of the tissue at 366 nm during induced tissue hemodilution. A computer controlled microfluororeflectometric system measures all of these parameters automatically, providing a value for the corrected NADH-level, blood flow and blood volume every 30 second. In this way an accurate correlation of these parameters with respect to time can be made, even during extremely rapid transients of tissue metabolism and blood supply (see shaded area in the figure). As the neuronal tissue and its supplying microvessels are in contact only at the microcirculatory level, and since we are monitoring events at this level, our method can provide needed data on the interdependence and interrelated control of tissue metabolism and blood supply in the brain cortex. The non-invasive feature of the methodology seemes essential in such a study.

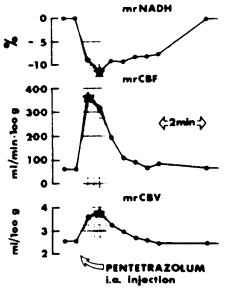

Figure: Cerebrocortical microregional corrected NADH-level (mrNADH), blood flow (mrCBF) and blood volume (mrCBV) before, during and after an induced epileptic seizure indicated by burst activity in the fronto-parietal ECoG (shaded area). After this time the ECoG activity returned to normal. Pentetrazolum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

364 CATECHOLAMINES LIBERATION IN THE BRAIN CORTEX DETECTED BY A VOLTAMMETRIC TECHNIQUE. Juan García Ramos. Dept. Physiol. Escuela Médico Militar, Mexico.

At the peak of a triangular pulse applied to two similar circuits consisting each of a graphite anode and a large silver plate as cathode, the extra-currents due to the electro-oxidizable substances near the graphite electrode tip were recorded on a Grass Model 79 Polygraph, as potential differences with opposite polarities, through resistances of equal values. The voltage reached its peak of 0.28-0.32 volts in 200 milliseconds. The pulses were applied every five seconds.

In one of these twin circuits the graphite anode was placed over the cortical surface of a curarized cat. In the other the graphite anode was over the skull bone, on a piece of cotton wet with the cerebrospinal fluid of the same animal. In this type of circuits the extra-current is due to the electro-oxidizable substances at the anode. At the voltage employed these substances can be the catecholamines or their metabolites. The difference in the extra-current occurring in both sircuits can reasonable be attributed to changes in the concentration of an oxidizable substance in the brain cortex. Unfortunately, with this technique it is not possible to differentiate electrochemically catecholamines from ascorbate, for example. Previous studies*, however, indicate that the experimental results may be attributed mainly to changes in concentration of noradrenaline.

The measured currents increased when the animal cortex changed from the sleeping to the waking condition. There were also increases after stimulation of several afferent nerves, as well as after an intravenous administration of adrenaline, or after a short period of asphyxia. Conditions all that were already reported to be associated with noradrenaline liberation into the cortical brain layers.

* García Ramos, J., E. de la Cerda, B. H. Ibarra, C. Orozco B.: on the role of catecholamines in the humoral modulation of the electrocortical activity in the cat. Acta physiol. latinoam., 25: 433-445, 1975.

365 BICUCULLINE AND MOTOR CORTEX. J.D. Glass, G.H. Fromm, and A.S. Chattha*, U. of Pittsburgh School of Medicine, Pgh. PA 15261

We previously described in detail the visually evoked response from the motor cortex of cat. These studies reported the postnatal development of the response in normal[1] and visually deprived cats[2,3] and the responses interaction with arousal levels[3,4]. We now report the effects of bicuculline applied topically to the motor cortex upon the slow-wave and unit response.

Full-field patterned stimuli were presented to chloralose anesthetized cats. Slow-wave and unit activity were recorded from the forearm region of motor cortex. A microliter syringe was used to apply to the recording area microliter quantities of bicuculline (10^{-3}, 10^{-4} M) dissolved in saline.

The bicuculline, in a dose dependent fashion, decreased the positive component and increased the negative component of the slow-wave response. Prior to the application of the bicuculline, single units were not spontaneously active and discharged only in association with the positive component of the evoked potential. Following the application of the bicuculline, a record with an isolated unit was changed to a multi-unit record with intense spontaneous activity and an increased response to the stimulus. The larger amplitude spikes continued to occur only during the positive wave while the smaller amplitude spikes occurred during both positive and negative waves. There was an intense suppression of unit activity following the bicuculline enhanced negative wave.

Our results show, as reported by others, that bicuculline has a convulsant effect upon unit activity. In addition, we found bicuculline to enhance a slow-wave component associated with a suppression of unit activity. This suggests that bicuculline induces heightened activity levels in cortical neurons whose collateral axons synapse upon inhibitory interneurons. The increased interneuron activity then generates an enhancement of inhibition.

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2. Glass, J.D., Exp. Neurol., 34: 123-129, 1973.
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366 THALAMIC CONNECTIONS OF PARALIMBIC CORTEX IN THE TEMPORAL POLE OF THE MACAQUE. E. C. Gower* and M.-M. Mesulam. Neurological Unit, Beth Israel Hospital, Boston, MA 02215

Temporo-polar cortex is generally known as TG, but it is cytoarchitectonically heterogeneous and contains at least two distinct subareas. The anterior, lateral and inferior faces of the pole are isocortical in type,but with a poorly developed ivth layer. On the other hand,the medial face is similar to the perirhinal regions with which it is caudally continuous,and is characterized by clustered large, chromophilic neurons in lamina ii. We will refer to both of these subdivisions as examples of paralimbic cortex.

Each subdivision of TG has an individual pattern of thalamic connectivity,and these can be demonstrated both by autoradiography and by HRP neurohistochemistry. When a ^{3}H AA(amino acid)injection is restricted to lateral TG,silver grains were observed only over nuclei of the posterior thalamus: the medial pulvinar, the densocellular division of the medial dorsal nucleus(MD) and the nucleus limitans. When lateral TG injections of ^{3}H AA extended more caudally,additional grain appeared over other posterior nuclei: the medial geniculate complex,the suprageniculate nucleus and the parafascicular nucleus. In three cases with HRP injections in lateral TG,retrogradely and anterogradely transported label was seen in the same set of thalamic nuclei. In a fourth case,the injection was predominantly in medial TG, and the medial pulvinar, the medial dorsal and the limitans nuclei were labeled as before. However, a significant proportion of HRP-containing perikarya were found in midline structures: namely, the paratenial,the paraventricular, the reuniens and the subfascicular nuclei, and the central superior, intermediate and densocellular nuclei. In two cases, all HRP-containing perikarya in the thalamus were counted in a series of coronal sections separated by 400 μ. With a lateral TG injection, the thalamus contained 2585 labeled neurons; 52% of these occupied the medial pulvinar and adjoining caudal MD, and 36% appeared in the medial geniculate complex. Midline structures contained less than 1% of the total. When both lateral and medial TG were injected,the medial pulvinar nucleus together with MD still contained 53% of the labeled perikarya. However, in contrast to the previous case,24% were located in midline thalamus and only 7% in the medial geniculate complex. Intralaminar neurons accounted for the residual in both cases.

These results support two hypotheses concerning the thalamic connectivity of the temporal pole. (1) The paralimbic regions of TG are reciprocally associated with a sector of the posterior thalamus that includes the medial pulvinar and adjacent subdivisions of the medial dorsal nucleus. (2) Lateral TG is clearly innervated by the posterior thalamus. While medial TG may share these connections, it is also prominently associated with midline nuclei of the thalamus. (Supported by NIH grant NS-09211).

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

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

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

Evidence suggesting that some commissurally projecting neurons receive monosynaptic thalamocortical inputs will be presented.

368 THALAMIC PROJECTIONS TO LAYER I OF RAT NEOCORTEX. Miles Herkenham. Lab. Neurophysiol., NIMH, Bethesda, MD 20205.

In previous autoradiographic studies of thalamocortical connections, I have shown that the ventromedial nucleus (VM) projects to layer I of widespread neocortical regions (JCN, 1979, 183, 487), whereas intralaminar nuclei project to deep cortical layers of a similarly wide cortical extent (Anat. Rec., 1978, 190, 420). Analysis of over 100 cases of small iontophoretic injections of tritiated amino acids placed into most of the thalamic nuclei has revealed a region of thalamus, located for the most part laterally adjacent to the intralaminar nuclei, that projects primarily to layer I. The region comprises the ventral anterolateral (VAL), posterior (PO), and magnocellular medial geniculate (MGm) nuclei. The projections from this region differ in two respects from those of VM: they are directed to more restricted cortical areas, and they have secondary layers of termination. The dorsal and medial parts of VAL project far beyond the limits of the traditionally accepted terminal zones in the motor areas, into layers I and VI of parietal and occipital areas. Termination in the superficial half of layer I of visual cortex is remarkably dense. PO projects to layers I and V of parietal areas; the heavy layer I band occupies a deeper location, in Ib, whereas the secondary band is situated in the cell poor zone, Va. MGm projects to the outer two-thirds of layer I, and to a lesser extent to deep layers in the regions of auditory cortex and more rostral levels. Depth profiles indicate that there are no sharp boundaries of terminal layering, even in layer I, after the MGm injections.

Still other thalamic nuclei project to layer I, but not as heavily as to deeper layers. These include the mediodorsal, lateral dorsal and lateral posterior (LP) nuclei, whose primary layer of termination is found in layers III and IV. Interestingly, several thalamic nuclei have terminations whose laminar patterns are area-dependent. The VAL nucleus has a tri-laminar distribution in motor cortex, but the aforementioned I/VI distribution in most other areas. The PO nuclear group projects to Ib and Va of SmI cortex but to I and IV of the region of SmII cortex and to I and III of motor cortex. Another striking instance of area-dependent lamination is seen after posterior LP injections; both cortical areas 17 and 18 are labeled, but whereas the laver I band is continuous across the cortical boundary, the intermediate band shifts from layer IV to Va in passing from 18 to 17. Taken together, the results show a large area of thalamus laterally adjacent to the intralaminar nuclei that sends widespread projections to layer I and, in addition, to deeper layers whose precise locations are area-dependent.

369 THE DISTRIBUTION OF THALAMOCORTICAL SYNAPSES ON THE APICAL DENDRITES OF LAYER IV,V, AND VI PYRAMIDS. Steven M. Hersch* and Edward L. White. Dept. of Anatomy, Boston Univ. Sch. of Med., Boston, MA. 02118

Every cortical neuron which has dendrites within layer IV and lower layer III has been found to receive thalamic afferents (White,'78; Peters et al, in press). Quantitative data on the thalamocortical afferents in mouse primary somatosensory cortex has suggested that, for a given length of dendrite, nonspiny stellate and bipolar cells receive more input from the thalamus than spiny neurons, and that spiny stellate cells receive thalamic afferents with the next greatest frequency, followed by the basal dendrites of layer III pyramids and, finally, by apical dendrites of superficial layer V pyramids which receive only few thalamic synapses. Other types of deep pyramids, in layers IV-VI, were not examined and there is evidence that, in rat visual cortex, pyramids with cell bodies deep in layer V receive greater numbers of thalamocortical synapses than those superficial in layer V (Ryugo et al., '75). In the present study, layer IV, V, and VI pyramids, located in the postero-medial barrel subfield of the mouse (CD/1), are being examined in order to characterize the distribution of thalamic synapses on the layer IV and lower layer III portions of their apical dendrites. Electrolytic lesions have been placed in the ventro-posterior thalamus, pars lateralis, and in the posterior thalamic nucleus to induce degenerative changes in thalamic terminals. Pyramidal cells postsynaptic to the degenerating terminals have been identified with the Golgi/EM technique of Fairen et al. ('77). A single deep layer V pyramid, whose apical dendrite bifurcates at the lower border of layer IV into two main branches which later terminate in layer III, has so far been examined in serial thin sections. The density of thalamo-cortical synapses per unit length of its dendrite is comparable to that of layer IV spiny stellate cell dendrites. Most of these synapses are axospinous and the remaining few are with small dendritic protrusions which might be considered sessile spines. Quantitative data on the thalamocortical relations of layer IV, V, and VI pyramids will be presented and their functional implications discussed.

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370 HOPPING LOCOMOTION IN RATS IRRADIATED IN UTERO. Samuel P. Hicks and Constance J. D'Amato. Department of Pathology, University of Michigan, Ann Arbor, Michigan 48109.

A spectrum of highly reproducible malformations can be induced in rats with pre- or early postnatal x-irradiation, and much is known about their morphogenesis (Hicks and D'Amato, 1978). 150 R on the 13, 14, or 15th day yielded a large subcortical ectopia, a thin cortex, a malformed spinal cord, and a hopping gait. The malformed cord and deficiencies of forebrain commissures have been suggested as causes of the hopping. Our studies point to abnormalities of the "spinal locomotor generator" as a major part of the problem, but they also indicate that the cortex and other supraspinal mechanisms adapted their controls to the abnormal cord. The cord abnormality was a deficiency of neurons and distortion of the architecture of the gray matter, especially the posterior horns, with midline fusion of the central gray. H_3 thymidine labeling showed that most large ventrolateral motor neurons were proliferated early in the 12-16 day period followed by neurons of ventromedial, central and posterior horn, and contributions to Rexed laminas I, II last. Posterior horns were the most affected after 13-15th day irradiation, because of the balance between cell destruction and regulative capacity for repair peculiar to that period. Retrograde HRP labeling showed a diminished complement of corticospinal neurons, some appearing in the ectopia; corticospinal tracts were formed. From birth, fore- and hindlimb pairs moved synchronously rather than in the normal alternating mode. Some animals gradually switched to a partially or nearly normal gait of forelimbs at about three to five weeks, but only rarely did hindlimbs switch. Most animals jumped accurately from one level platform to another; they swam as they walked. They righted when dropped from a supine position, but limb movements were not recorded. Tactual placing by one forelimb could be elicited, even in rats that hopped with forelimbs, but scratching by one hindlimb was accompanied by mirror movement of the other. Hours after transection of the mid-thoracic cord, firm pinching of one hind foot elicited an exaggerated, repeated crossed extension reflex (mass reflex), and simultaneous pinprick of the dorsa of hindfeet resulted in withdrawal of both feet, in both normal and irradiated rats. As the animals became stabilized, normal rats usually responded to bilateral pinprick with crossed extension, and irradiated rats showed withdrawal of both feet.

(USPHS NS 10531).

371 LAMINAR DISTRIBUTION OF CHOLINERGIC INNERVATION IN RAT NEOCORTEX: LESIONS OF EXTRINSIC AND INTRINSIC COMPONENTS. Michael V. Johnston and Joseph T. Coyle. Dept. Pharmacology, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Cholinergic neurotransmission plays an important role in cortical function. Lack of a highly specific neuroanatomical method for visualizing cholinergic neurons has prevented a precise description of the organization of this innervation. Our previous experiments indicated that half of the cholinergic innervation to rat fronto-lateral neocortex projects from neuronal perikarya in the basal nucleus (BN) (Trans. ASN 10:73, 1979); and up to 40% may reside in intrinsic cholinergic neurons (Brain Res., in press).

In the present experiments, the laminar distribution of cholinergic innervation was examined quantitatively by measuring the activities of choline acetyltransferase (CAT) and acetylcholinesterase (ACHE) in microdissected rat sensorimotor cortex. Fresh slabs of rat lateral neocortex were frozen onto a microtome stage and five reproducible laminar samples of 350μ thickness were obtained by shaving with the blade at progressively deeper layers. Small portions of each layer were left attached to the remaining specimen which was then examined histologically to correlate biochemical and cytoarchitectonic composition. In the unlesioned rats (N=8), CAT activity was higher in cortical laminae I (116 $\pm$ 2% of mean cortical specific activity) and laminae IV and V (105 $\pm$ 3%) than in laminae II-III (90 $\pm$ 2%) and lamina VI (85 $\pm$ 4%). The distribution of ACHE activity was similar to that of CAT. One week following injection of 3.5 nmol of kainic acid into BN, CAT activity in ipsilateral cortex was lowered 43 $\pm$ 3% (N=5, $p < .01$) and ACHE was reduced 52 $\pm$ 3% ($p<.01$). These reductions were reflected equally in all cortical laminae.

The laminar distribution of these cholinergic markers was also examined in the neocortex from adult rats that had been treated at 15 days gestation with methylazoxymethanol acetate (MAM) which deletes neurons in laminae II-IV. CAT activity in cortex following this lesion is almost entirely related to subcortical afferents since electrolytic NB lesions in MAM treated rats reduced total CAT by 90%. In MAM treated rats, CAT activity was highest in the outer layers and decreased progressively in lower layers so that the deepest zone was 58% lower than the subpial zone.

Data from these experiments are consistent with the view that rat neocortex contains subcortical cholinergic afferents projecting to all layers and a population of cholinergic intrinsic neurons localized to the superficial layers which may project to deeper cortical layers. Supported by USPHS grants MH-26654, NS-13584, RSDA II MH-00125 and Fellowship NS-06054.

372 A (^{14}C)DEOXYGLUCOSE STUDY OF SOMATOSENSORY AND ASSOCIATED CORTICAL AREAS IN THE MONKEY. S. Juliano*, P. Hand, B. Whitsel, C. Goochee*, P. Karp*, and R. Bajcsy*. Department of Animal Biology School of Veterinary Medicine, School of Allied Medical Professions; and Moore School of Electrical Engineering, University of Pennsylvania, Philadelphia, PA 19104; Department of Physiology, School of Medicine, University of North Carolina, Chapel Hill, NC 27514; and Laboratory of Cerebral Metabolism, NIMH, Bethesda, MD 20014.

In a previous report, we described cortical columnar (modular) organization, using the (^{14}C)-2-deoxyglucose (2DG) technique, in primary and secondary somatosensory cortices (SI,SII) of an unanesthetized, paralyzed Cynomolgous monkey. This animal was stimulated on volar thumb, thenar eminence and index finger with servomotor controlled brush strokes. (Anat. Rec., 193; 260-1, 1979). Our current work has evaluated an unstimulated animal and 2 additional monkeys, varying the proximodistal location of the stimulus. These animals were brush stroked either from the proximal interphalangeal joint to the tip of the left index finger or on the anterior midline of the left forearm (1.5x6cm). 2DG methodological details were after Sokoloff (J. Neurochem., 28; 1977). We found the size of modules and basic organizational pattern of metabolic activity to compare with that of our initial results in SI and SII. Contralateral modules in 3a and 3b averaged 360u, and in area 1, 320u. The modules for these two recently stimulated monkeys were: (a) confined to a more restricted cortical topological area (b) decreased in number and (c) primarily in areas 1 and 3b. This labeling corresponded to somatotopic representations in SI as previously described. In the control animal there was a paucity of modules in areas 3a, 3b, 1 and 2. In all four monkeys examined there was additional labeling in other cortical areas. These included areas 5 and 7, area 4 adjacent to 3a, the inferior bank of the lateral fissure, the insula, and both banks of the superior and inferior aspects of the superior temporal sulcus (STS). Activity in these areas was columnar in nature, but the modules were less regular in tangential extent and shape than those of SI. Also, many of these modules were present in the control animal. Thus, they may reflect cortical activation other than somatosensory. Some possibilities for the nature of this labeling include: (a) attempts by the paralyzed animal to tactually or visually manipulate its environment or (b) multiple somatosensory or polysensory areas (e.g. auditory input). The sizes of these modules were in the following ranges: areas 5 and 7 (360-600u), STS (600-890u); inferior bank, lateral fissure (540-720u); insula (600-720u); area 4 (540-660u).

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373 PREFRONTAL NEURON ACTIVITIES RELATED TO DIRECTION AND SIZE OF THE MOVEMENT DURING DELAYED-RESPONSE. K. Kubota and S. Funahashi*. Dept. Neurophysiol., Primate Res. Inst., Kyoto Univ., Inuyama, Aichi, 484, Japan.

Recent findings that the motor cortex PTN activity is modified by instructions given prior to movement onset prompted us to re-examine prefrontal (PF) neuron activities in a delayed-response in which motor responses are strictly controlled and PTN activities are well defined. It was aimed at extracting a specific PF neuron activity related to the choiced movement.

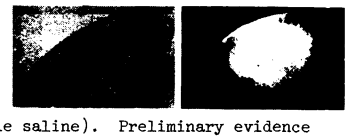
Monkeys performed wrist extension or flexion movement from a predetermined start zone to a predetermined target zone (displacement, 8°-40°), according to the visual cue presented prior to movement. The first task was a two-choiced visual tracking of single step. Rotating the handle to a central 8° zone, green lamp was on. Three sec later (preparatory period), its color was changed to red (GO signal). Simultaneously one of target lamps was on, indicating the direction of the movement (flexion or extension) of the same size. Then the monkey rotated the handle to an appropriate target zone (8°). The second task was a two-choiced delayed-response; one of target lamps (0.5 s) was on as soon as the green lamp was on, and succeeding 2.5 s was the delay period. In the third task only one of choiced movements was repeated, being the sequence the same as above tasks. In two macaque monkeys, 67 PTNs were rocorded from the hand motor area and 175 PF neurons were from the dorsolateral prefrontal cortex.

During a preparatory period of the first task both PF neurons and PTNs showed a steady activity and after GO signal they were activated in association with movement either unidirectionally or bidirectionally, though occurrence of unidirectional pattern was less frequent in PF neurons than in PTNs. With 0.3-0.7 s before GO signal of the third task there developed a preparatory activity (gradual increase on one direction of movement and decrease on the other). After GO signal these changes were further enhanced. During early phase of delay of the second task there appeared an activity, higher or lower than that in the first and third tasks. This activity superimposed upon preparatory or movement-coupled activities. Neuron activities were further tested in a task in which two different sizes of the movement of the same direction were presented. Size-dependent activity appeared in delay period in PF neurons but not in PTNs. Higher rates correlated to larger movement was observed in 26 and those to smaller one were in 8 PF neurons.

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

374 THE PREFRONTAL PROJECTION TO THE SUPERIOR COLLICULUS IN THE MONKEY. G.R. Leichnetz, R.F. Spencer and J. Astruc. Dept. of Anatomy, Medical College of Virginia, Richmond, Va. 23298.

A projection from the cortex within the concavity of the arcuate sulcus, the "frontal eye field," of the monkey, has been previously described by Kuypers and Lawrence (1967) and Astruc (1971) using silver methods, and more recently by Kunzle, Akert and Wurtz (1976) using the autoradiographic method. We have found that the use of our horseradish peroxidase gel (Griffin et al Br. Res. in press) in combination with the TMB procedure produces superb delineation of both anterogradely and retrogradely transported enzyme, such that one can virtually study the afferent and efferent projections of a given cortical area in the same experimental case. In this study, HRP gel placements were made within the arcuate cortex of both rhesus and cebus monkeys. The animals were allowed to survive for 24 hrs. and were processed according to the Mesulam TMB protocol (1978). The results illustrated below in both bright and dark field demonstrate the heavy projection of the frontal eye field to the stratum intermedium of the ipsilateral superior colliculus. While some anterogradely transported HRP was present in the stratum opticum above, and stratum profundum below, clearly the heaviest projection is to the intermediate stratum. The HRP technique did not appear to confirm the projection to the stratum zonale suggested in autoradiographic studies.



In an effort to determine the source of prefrontal projections to the tectum we injected HRP into the superior colliculus in a series of cebus monkeys (0.05 µl 25% HRP in sterile saline). Preliminary evidence confirms the projection from the frontal eye field to the colliculus. Large numbers of small pyramidal and stellate elements of Area 8 were labelled ipsilateral to the injection. Remarkably, the labelled cells were heavier in the inferior portion of Area 8, and large numbers of labelled small and medium-sized pyramids were present in layers III and V of the inferior bank of the principal sulcus and inferior convexity rostral to the arcuate cortex. While some labelled pyramids were often present in the dorsal convexity, it appears to us that the prefronto-tectal projection is primarily from inferior Area 8 and the prefrontal convexity inferior to the principal sulcus. The possible significance of these findings will be discussed. Supported by NSF Grant 78-22971.

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

The manner in which such stimulus dimensions as orientation, direction and wavelength affect the responses of the cells of striate cortex has been described in detail in recent years. Less is known about the manner in which stimulus intensity is encoded. We have found that different functional cell classes show different sensitivities as a function of luminance. In particular, we have found that orientation specific cells are differentially sensitive over a narrower range of luminances than non-orientation cells.

Single unit responses were recorded extracellularly in the awake rhesus monkey trained in a visual fixation task. Receptive field organization was determined using stimuli of high contrast which varied in size, shape, color, orientation and velocity.

Following classification, the responses of these cells to moving bars and large spots of light (exceeding receptive field boundaries) were tested. Responses to these stimuli were recorded at 9 different luminances which covered a range exceeding 3 log units. Background luminance was maintained at .15 ft lamberts and the maximum stimulus luminance was 10.8 ft lamberts. We analyzed both peak response frequency and total number of spikes.

Orientation specific non-color cells discriminate only a small range of stimulus intensities. When tested with an optimal stimulus they are sensitive only over a range from .5-1.5 log units, from threshold to saturation. In general these cells were unresponsive to or weakly inhibited by large spots.

The majority of cells lacking orientation specificity responded both to white moving bars and large white spots. Cells which were excited by large spots continued to discriminate luminance differences throughout the range tested. Some of these cells would likely discriminate even higher intensities. The range of luminances of a moving bar discriminated by non-oriented cells is somewhat narrower, but most cells show differential responses over a range in excess of 2 log units. These results suggest that orientation specific cells contribute no differential information about luminance at higher stimulus luminances. The wide sensitivity ranges of most non-oriented cells suggest that these cells may encode luminance information for both patterned and diffuse stimuli. Supported by NIH Grants 1 RO1 EY02230-02 and 5 T32 EY07019-04.

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

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

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

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

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

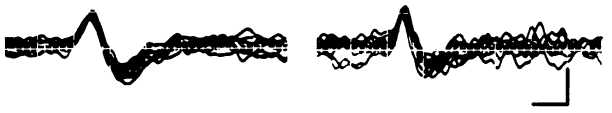


Fig. 1. Neuronal activity recorded from two of the electrodes 3 years and 49 days after implantation. Calibration .2 ms, 50μv.

377 VISUAL RECEPTIVE FIELD OF DORSOLATERAL PREFRONTAL NEURONS IN CHRONIC MONKEYS. A. Mikami*, S. Ito* and K. Kubota. Dept. of Neurophysiol., Primate Res. Inst., Kyoto Univ., Inuyama City, Aichi, 484, Japan.

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

Four rhesus monkeys were trained to perform a visual fixation task; fixating their eyes for 0.5-5 s on a small circular spot (0.3°, 1.0 cd/m^2) at the center of a tangent screen (1.5 X 1.2 m). The trial was started by depressing the lever. After a fixed waiting period, a spot appeared and then 0.5-3 s later, a slit stimulus (2.5 cd/m^2), unrelated to reinforcement, was presented for 0.5 s. Within 0.5-3 s after the slit, spot brightness was changed. Releasing his hand from the lever, he was rewarded.

A total of 197 neurons was sampled from the dorsolateral PF cortex while the monkey was performing a task in which moving slit stimulus of relatively large size (6°-40°/s, 3° X 30°) was routinely presented in the contralateral visual field. Seventy neurons showed clear changes of their discharge rates in response to the fixation spot with latency of 100-300 ms. Of these, 48 neurons responded also to extrafoveal slit stimuli with similar latency. Presence of neurons responding exclusively to the fixation spot suggested a mechanism related to the foveation. Further, 127 neurons responded only to the slit.

The RF was determined in 35 neurons, using 1° X 5° or 1° X 1° slit stimuli. Its size varied from about 10° X 10° to 60° X 60° and in 28 neurons it was larger than 30° X 30°. Often it was difficult to draw a boundary line between responsive and unresponsive areas because responses became gradually weaker when the slit was moved away from the RF center. Only 5 neurons showed a clear surround inhibition. In 16 neurons the RF was contralateral to the recorded side and in 2 it was ipsilateral. In 17 neurons it expanded from contralateral to ipsilateral side. As for topography of recorded neurons, rostrally located neurons tended to have larger RF. Responses to stationary and moving slits were compared in 26 neurons, and 7 showed stronger responses to the moving slit (6°-12°/s) than stationary one. Differential selectivities of rate changes depending on the direction of movement were found in 8 neurons.

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

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

Single neuron studies were made in the cortex of the inferior parietal lobule (area 7) in 8 hemispheres of macaque monkeys trained in tasks requiring maintained, directed visual attention. Area 7 contains a number of sets of cortical neurons, including those active during projection and manipulation with arm and hand; those active during visual fixation, tracking, or saccading; and those responsive to visual stimuli, per se; and those displaying various combinations of these properties. A large set of the visual neuron group is responsive to stationary and/or moving lights within response areas that commonly extend towards the contra lateral perimeter of the binocular visual field, and centrally towards but rarely to include the fixation point, where lights may evoke suppression. These response areas may be maximally sensitive close to the binocular perimeter, and may be bilateral. A smaller group is responsive to stimuli within the monocular portion of the field. Another is activated, not suppressed, by foveal and immediately peri-foveal stimuli, but not by small visual targets adequate for fixation and detection. Cells of all these sets show a remarkable directionality when tested with moving lights, and the response areas determined by moving and stationary lights are frequently not coincident. The responses of light sensitive cells frequently are changed in amplitude or even reversed in direction from excitation to suppression, or vice versa, by changes in background illumination. A small number of light sensitive cells appears to be related to response areas located in head-body, not retinal coordinates. No instance of orientation selectivity has been observed.

379 HUMAN LANGUAGE CORTEX: IDENTIFICATION OF COMMON SITES FOR SEQUENTIAL MOTOR MOVEMENTS AND PHONEMIC PERCEPTION, SEPARATE SITES FOR MEMORY, SYNTAX. George A. Ojemann, Catherine Mateer*. Dept. Neurological Surg. RI-20, UW, Seattle, WA, 98195.

Electrical stimulation mapping of human periSylvian cortex during standard tests of naming, reading, short-term verbal memory, single and sequential oral facial movements, and phonemic perception was undertaken during craniotomy under local anesthesia for resection of epileptic foci. The evoked changes in each of these tests were measured at 8-15 preselected sites in left brain of 5 patients and right brain of 1 patient, all of whom were shown to be left brain dominant for language by preoperative intracarotid amytal testing. The procedures used for obtaining informed consent for the research portions of this study in these patients had been reviewed in advance by the institutional biomedical sciences review committee in accordance with the applicable Public Health Service guidelines for human experimentation. Stimulation currents were at the highest level that did not alter discharges (mean 4.8mA).

The ability to mimic sequential oral facial movements and to perceive phonemes was altered by stimulation of the same left brain sites, at least 2 in each patient. This identifies a cortical sequential motor-phonemic perception language system that provides a link between speech production and understanding. This is an anatomic basis for the motor theory of speech perception (Liberman, et al., Psychological Reviews 47:431, 1967). These sites are in periSylvian cortex of frontal, temporal and parietal lobes, an area that would be included in almost every cortical lesion that gives rise to persisting language disorder. Phonemic perception was not disturbed at sites where motor movements were intact. Surrounding these cortical sites is a separate series of at least 2 left brain sites in each patient, where only short-term verbal memory was altered by stimulation, with stimulation during storage altering memory at parietal and temporal lobe sites and retrieval stimulation at frontal sites. Between the sequential motor-phonemic perception and memory sites are left brain sites in each patient where only naming or reading are altered by stimulation, including sites with changes only in the syntactic organization of language. In motor and immediately premotor cortex, are sites where all types of oral movements are altered and arrest of all aspects of language evoked, identifying a final motor pathway for speech. Stimulation mapping of the periSylvian cortex in nondominant right hemisphere showed no changes in any of these tests outside of motor cortex.

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380 CONNECTIONS OF THE FRONTAL EYE FIELDS IN THE CAT. G.J. Pascuzzo* and L.C. Skeen. Inst. Neuroscience and Behavior, Univ. Del., Newark, DE 19711.

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

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

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

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

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

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

381 A COMBINED GOLGI-EM STUDY OF THE SYNAPTIC RELATIONSHIP BETWEEN A SMOOTH STELLATE CELL AND A PYRAMIDAL NEURON IN RAT VISUAL CORTEX. Alan Peters and Charmian C. Proskauer*. Dept. Anat., Boston Univ. Sch. Med., Boston, MA 02118.

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

In addition we encountered a synapse between an axon terminal of the stellate cell and one of that cell's own dendrites. Although synapses of this kind have been supposed to exist in the cerebral cortex, so far as we are aware this is the first electron microscopic demonstration of one of them. Supported by NINCDS Research Grant NS 07016.

382 SUBCORTICAL PROJECTIONS OF THE PERICRUCIATE GYRUS IN CAT. E. Ramon-Moliner. Dept. Anat., School of Medicine, Univ. of Sherbrooke, Sherbrooke, Quebec, Canada, J1H 5N4.

Following injections of radioactive aminoacids in various points of the precentral, postcentral and coronal gyri, the brains were studied for the autoradiographic demonstration of efferent connections in sagittal and coronal sections. A massive efferent system terminates in the ipsilateral thalamus within which a somatotopic distribution can be envisaged. The reticular nucleus of the thalamus appears well outlined on the basis of what appears to be a specific plexus. There is a bilateral projection to the head of the caudate nucleus, the contralateral one being reached through fibers present in the rostral corpus callosum. Within the subthalamus one can see the origin of a sizeable vertical system of collateral fibers (the prerubral bundle of Cajal) in the form of ascending fibers which are labeled by the slow component of axonal transport. It could terminate in certain isolated islands within the pretectal tegmentum. It does not send fibers to the red nucleus. The latter center was devoid of evident afferent fibers, except in one case where the injection had been placed in the most lateral portion of the gyrus pericruciatus (coronalis). The contribution to the nucleus subthalamicus was uncertain but the region dorsal to this center (zona incerta) received a sparse by unquestionable projection. Within the lower brain stem the motor nuclei were devoid of afferents. Only the contralateral "lateral reticular nucleus" was labeled. The pyramidal tract was labeled by the slow axonal transport throughout its length. It could be followed until its decussation in the lower medulla oblongata. This indicates that a sizeable projection must reach the spinal cord. This structure, however, has not yet being processed for autoradiography and only further research will clarify the precise distribution of these cortico-spinal fibers. The contrast between the lack of projections to brain stem motor nuclei and this postulated projection to the spinal cord appears to indicate that if the cranial motor nuclei receive direct afferents from the cerebral cortex in cat, it must be from regions other than the pericruciate gyrus. However, the possibility cannot be excluded that they may receive indirect afferents via some relay station for the fibers of Cajal's prerubral bundle.

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

Injections of horseradish peroxidase (HRP, 20-30% Type VI, .1-1.6 µl) in the inferior olivary complex of 10 cats resulted in the retrograde labeling of cells in the motor cortex. The labeled cells were found in layer V ε were more numerous ipsilaterally. They were mainly in the presylvian gyrus and the banks of the cruciate sulcus and were sparsely distributed. A few were also present in the orbital, coronal and proreal gyri.

Injections of tritiated amino acids (^{3}H-ℓ-leucine [150-330 µCi/µl] or ^{3}H-ℓ-proline, leucine, lysine and ℓ-amino acid mixture [200 µCi/µl] NEN, vols. .2-2.0 µl) were made in the pericruciate region of 8 cats. Five injections involving the anterior sigmoid gyrus resulted in the labeling of the inferior olive. In the inferior olive, the caudal medial portions of the medial accessory olive (MAO) excluding subnucleus β were bilaterally labeled in two cases. The grain density was greater ipsilaterally than contralaterally. In three other cases, similar but very weak deposits were present. Previous studies had indicated terminations from the cortex in the rostral principal (PO) and dorsal accessory (DAO) olive (Sousa-Pinto and Brodal, Exp. Brain Res. 1969, 8: 364). Numerous fibers of passage were observed to pass through the rostral PO and DAO in our material and the presence of terminations was equivocal. Further experiments using shorter survival times are planned in order to resolve this question.

In the mesencephalon, deposits of silver grains over the nucleus of Darkschewitsch, the interstitial nucleus of Cajal and the rostro-medial pole of the red nucleus were present ipsilaterally in four cases. These cellular groups were previously identified as major sources of input to wide areas of the homolateral MAO and PO (Saint-Cyr and Courville, Neurosci. Abst. 1978, 4: 168). It is suggested that the motor cortex may influence widespread areas of the cerebellar cortex via the climbing fiber system principally through these mesencephalic relays.

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384 THE INTRAHEMISPHERIC CONNECTIVITY BETWEEN MOTOR CORTICES (MI AND MII) AND SOMATOSENSORY CORTICES (SI AND SII) IN THE RACCOON. Sharleen T. Sakai* and Paul Herron* (SPON: L. O'Kelly) Neuroscience Program and Depts. of Psychology and Biophysics, Michigan State University, East Lansing, MI 48824.

The purpose of this study was to investigate the interconnections of MI and MII with SI and SII in terms of the topography of body representations, specifically, with regard to the connections of the same body representation (homofunctional) or different body representation (heterofunctional). The cortico-cortical connections were investigated using the horseradish peroxidase (HRP) retrograde transport technique in the raccoon. Electrophoretic/pressure injections of 30-50% HRP were made into electrophysiologically defined regions in MI, MII, SI or SII in chloralose anesthetized raccoons. Following a survival period of 12-72 hours, the animals were intracardially perfused with a buffered aldehyde mixture. The brains were processed for HRP histochemistry using tetramethyl benzidine and dihydrochlorobenzidine as the chromogens on adjacent sections. Following an injection into MI forepaw/arm area, labelled cell bodies were observed in SI forepaw/arm area, MII and SII while following an injection into SI forepaw area, labelled cells were found in MI forepaw area. Injections into MI trunk area resulted in reactive neurons in a homofunctional area of SI and caudal SII. Following an injection into the SI hindlimb region, HRP positive cells were found in MI hindlimb area. After an injection in SII hindlimb region, labelled afferents were observed in a homofunctional area of MI. SII hindlimb area also received projections from an area caudolateral to MI forepaw and rostral to SI, a region which may correspond to area 3a. Following an injection into the distal limb area of MII labelled cells were observed in MI hindlimb area; HRP positive cell bodies were also found in the inferior and superior banks of the suprasylvian sulcus, a region which corresponds to the boundary region between SI and SII. The intrahemispheric cells of origin appear to be laminae specific. The SI and SII cells of origin primarily arise from layers III and V. The intrahemispheric projections of MII originate predominately from layers II and III, while the MI cells of origin largely arise from layer III. The cells of origin are distributed into clumps or clusters. Typically, a waxing and waning of labelled cells is observed interspersed by a rather continuous band of labelled layer III pyramidal cells. The results indicate that while there are reciprocal cortico-cortical connections between homofunctional areas of motor and somatosensory cortices, there are other interconnections as well. (Supported by Grant No. BNS 78-00879 from the National Science Foundation).

385 DIFFERENTIAL CONNECTIONS OF SOMATOSENSORY AND MOTOR CORTEX IN THE RAT. Jean Schoenen*, J. Neal Naranjo*, and Valerie B. Domesick. Department of Anatomy, Harvard Medical School, Boston, Massachusetts 02115 and Mailman Research Center, McLean Hospital, Belmont, Massachusetts 02178.

In a previous study, HRP injected into the spinal cord was found to label pyramidal cells in a cortical region much larger than previously described. This region includes at least three cytoarchitectonically distinct strip-like zones arranged sagittally alongside each other. According to neurophysiological studies, the medial of these strips corresponds to motor cortex (R.D. Hall and E.P. Lindholm, Brain Research 66 (1974) 23-38), while the more lateral correspond to the somatosensory cortex (C. Welker, Brain Research 26 (1971) 259-275). In order to define the thalamic dependencies of each of these strips, HRP was injected into each separately, whereas their efferent connections were demonstrated autoradiographically following injection of tritiated leucine-and-proline. Results indicate that the medial strip has reciprocal connections with the thalamic nuclei VL, VM, and PO, and the more lateral strips with VB and PO. Corticospinal fibers to the ventral horn originate only from the medial strip, whereas the spinal projection from the more lateral strips does not extend ventrally beyond the dorsal horn. The corticostriate projection from each of the three strips distributes bilaterally to a long sagittal zone of caudatoputamen; the three zones are somewhat different but overlap widely. Cortico-cortical fibers from the medial strip project to the two, more lateral strips, and in each are distributed in a discrete vertical column. In addition, the medial strip projects to a narrow perirhinal region of cortex which, as revealed by our previous HRP study, projects to the spinal cord. The two more lateral strips project to the medial strip in a more diffuse pattern. Contralateral (callosal) associations mirror the ipsilateral connections, indicating a greater heterotypy than previously suggested. The present findings tend to identify the medial cortical strip as motor and the two lateral strips as somatosensory cortex. Electrophysiological evidence of sensory-motor overlap may require a re-evaluation on the basis of overlap of projections from the medial and the two lateral strips in PO and striatum, as well as cortico-cortical interconnections. (Supported by USPHS grant 1-PO1-MH31154.)

386 EARLY EXPERIENCE, BRAIN ASYMMETRY, AND MURICIDE IN THE ALBINO RAT. Gordon F. Sherman*, James A. Garbanati*, Michael J. Hofmann*, Glenn D. Rosen*, David A. Yutzey*, and Victor H. Denenberg. Dept. Biobehav. Sci., Univ. Conn., Storrs, CT. 06268

Previous research (Denenberg et al. Science, 1978, 201, 1150-1152) has found that stimulation in infancy can produce an asymmetrical brain organization in the rat. This asymmetry was apparent in open-field activity and indicated the preferential involvement of the right hemisphere. The open field measures both exploratory behavior and emotional reactivity. Therefore, mouse-killing, a species-specific spontaneous behavior, was used in the present study as a more direct test of emotional reactivity.

Animals were handled or left undisturbed for the first 20 days of life. In adulthood 4 male littermates were given a right neocortical ablation, a left ablation, sham surgery, or no surgery. Approximately 8 months after this the rats were tested for mouse killing. The rats were individually housed and, 24 hours later, a mouse was put into each rat's cage. Latency to kill, in hours, was recorded. The means are given below (Exp. 1) with number of animals in parentheses. An asymmetrical effect was found in the Handling condition (t for Right vs Left lesion = 2.90, $p<.01$). However, the Handled sham group differed from the no-surgery group (t = 2.60, $p<.05$). There was no evidence of laterality in the Nonhandled groups.

We carried out a partial replication to further substantiate our findings and to explore the sham-control difference in our Handled groups. As shown in the table below (Exp. 2), the right-left difference is still present in the Handled animals (t = 2.69, $p<.02$). Therefore, we have replicated our finding that Handled rats with only an intact left hemisphere are significantly slower to kill than their littermates with an intact right brain. The sham difference found in Exp. 1 appears to be due to sampling error. Again no differences were found among the Nonhandled rats.

Handling induces brain laterality with respect to mouse killing by rats, with the right hemisphere preferentially involved. This is the same pattern as reported by Denenberg et al., and thus replicates and extends their prior findings.

Latency to Kill (in hours)

		R	L	Sham	Control
Exp. 1	H	66.74 (19)	31.87 (22)	54.80 (15)	21.74 (23)
	NH	61.47 (19)	63.11 (18)	29.65 (20)	26.40 (20)
Exp. 2	H	54.00 (8)	26.88 (8)	11.25 (4)	9.75 (4)
	NH	34.38 (8)	43.63 (8)	13.25 (4)	35.25 (4)

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

The connections between the neocortex (NC) and the superior colliculus (SC) have been studied in the gerbil by a combination of two methods: horseradish peroxidase injected into the SC and tritiated amino acids injected into a majority of the cytoarchitectonic fields of the NC.

The corticotectal projection arises widely from layer V of the occipital, parietal, temporal, and frontal fields. This extensive projection includes at least eight, and probably more, individual projections; by individual projections is meant a projection which arises in one, in some instances two or three adjacent complete cytoarchitectonic fields and has its terminals distributed in a single plane throughout the tangential dimensions of the SC. To some extent these planes are complementary, to some extent overlapping, in the radial dimension of the SC. Individual projections arising in occipital fields 17, 18a, and 19 project in overlapping succession to the deeper levels of the stratum griseum superficiale, stratum opticum and the superficial levels of the stratum griseum intermediale. Parietal fields 3 and 1, temporal fields 22, 36 and 41, medial cortical fields 8 and 24 and parietal fields 40 and 14 project in overlapping succession to the stratum griseum intermediale and stratum album intermediale. Finally, frontal fields 6 and 4 project to the stratum griseum profundum and stratum album profundum. Projections which arise in the occipital fields and those arising in the frontal fields terminate in a continuous plane in the superficial and deep strata of the SC, respectively. The remaining projections, by contrast, terminate in a "puffs and holes" pattern, i.e., a discontinuous pattern within the intermediate collicular strata.

Each projection appears to be topologically organized; the cytoarchitectonic boundaries of the fields of origin of each projection are directed to the margins of the SC. In some instances, adjacent points in the cortex on opposite sides of a common cytoarchitectonic border are in radial register with each other at the margins of the SC, e.g., at the 17/18a, 18a/19 borders. As a consequence of this principle of alignment, homologous points in the separate visual cortical representations are probably in register with each other within the SC. In other instances, adjacent points on opposite sides of a common border appear not to be in register with each other in the SC. This occurs where cytoarchitectonic borders probably separate projections affiliated with different functional systems, e.g., at the 22/40a and 8/6 borders. These borders probably lie between fields associated with the AI and SII and the Eye field and MI cortical representations, respectively.

388 A MODEL OF CORTICAL CIRCUITRY IN THE TURTLE BASED ON MORPHOMETRIC ANALYSIS OF THALAMIC FIBER INPUTS AND THEIR CORTICAL CELL TARGETS. Leslie M. Smith* and Ford F. Ebner, Neuroscience Sect., Div. Biol. Med. Sci., Brown University, Providence, RI 02912.

Evoked responses in turtle cortex to optic nerve or thalamic stimulation habituate rapidly when the stimuli are repeated more frequently than 0.5 Hz (Belekhova and Kosareva, '71). Our experiments provide a model that explains why habituation is the dominant cellular response in this neural system. The model requires knowing whether the visual pathways to cortex synapse on more than one cell type, and, if so, what the ratio of thalamic synapses are onto each cell type. We studied the ultrastructure and distribution of thalamic synapses, plus the density of thalamic terminals and cortical cells in equivalent volumes of cortex. Fourteen days after unilateral removal of the thalamus, we prepared the ipsilateral cortex for routine EM in 15 Pseudemys turtles. All thalamic fibers end within 100 um of the cortical surface, contain round agranular vesicles and form asymmetrical (type I) contacts. 86% of thalamic contacts are on dendritic spines and 14% are on dendritic shafts. Unequivocal evidence was found for thalamic contacts on the scattered cells located in the molecular layer. We calculate that there are 6.15 million thalamic synapses in a 1 mm cube of turtle cortex. Morphometric analysis of the cells in an equivalent volume of cortex leads to an estimate of 15,000 cells in the main cell lamina and 400 cells in the overlying molecular layer. Golgi preparations show that neurons in the main cell lamina have numerous spines on their apical dendrites. These dendrites ascend through the molecular layer into the thalamic input zone. In contrast, the vast majority of the cells in the molecular layer have smooth (aspinous) dendrites. For this calculation, we assume that the 86% of thalamic contacts on dendritic spines are onto main lamina cells (pyramidal cells) while those 14% onto dendritic shafts contact molecular layer cells (stellate cells). Given these assumptions, there are 5.29 million thalamic contacts onto the 15,000 pyramidal cells and 0.86 million thalamic contacts onto 400 stellate cells. The final step in this calculation produces a surprising and counterintuitive result, namely, that there are 6X as many thalamic synapses on each stellate cell as on each pyramidal cell (5.29 million ÷ 15,000 = 353 contacts/pyramid and 0.86 million ÷ 400 = 2150 contacts/stellate). We hypothesize that stellate cells in the molecular layer are inhibitory interneurons that receive 6X more excitatory thalamic input per cell than pyramidal cells. A single afferent volley activates both cell types, but the secondary effect of stellate cell activation is to leave the pyramidal cells hyperpolarized and refractory to further stimuli for periods in excess of a second. (Supported by NSF grant BNS 78-15933.)

389 PERFORMANCE OF NEMESTRINA MONKEYS WITH BILATERAL INFEROTEMPORAL CORTEX LESIONS IN A SIMULTANEOUS VISUAL PATTERN DISCRIMINATION TASK DEMANDING OF ATTENTION. Henry V. Soper, Susan Zweizig*, Thomas Gilman* and Donald B. Lindsley. Depts. of Psychol., Physiol. and Brain Res. Inst., UCLA, Los Angeles, CA. 90024.

Typically, monkeys with inferotemporal cortex lesions exhibit deficits in ability to learn visual pattern discriminations that are difficult. Recently, Gross (J.comp.physiol.Psychol., 1978, 92, 1095-1109) has reported that Rhesus monkeys with inferior temporal lesions are relatively unimpaired in learning difficult, rotated-pattern discriminations, involving pattern orientation differences rather than pattern differences.

Using 90° rotated patterns (N vs Z) presented tachistoscopically (10 ms) on two adjacent lucite panels by rear projection, we have studied the pre- and post-operative performances of three Nemestrina monkeys with emphasis upon retention, relearning and distractive stimulus additions (N and Z with annular surrounds). The task demands attention and fixation of the panel displays. The monkey with gaze directed to the panels, presses a lever to the right and after a 300 ms "alerting" delay receives the N and Z patterns. A press on the correct panel brings a banana pellet reward followed by a 15 sec inter-trial interval. Incorrect presses are penalized only by the inter-trial delay without reward. Each daily session consists of 20 practice trials followed by 100 training or test trials. Criterion performance is three successive days at 90% or better.

Monkeys A and B exhibited nearly identical pre-operative learning performances (28 and 25 sessions to criterion), typical of other monkeys we have trained on the same task. Both monkeys reacted similarly on pre-operative distraction and retentions tests and, following posterior inferotemporal lesions, showed a retention loss which only gradually recovered with retraining, requiring 80 to 100 daily sessions. Postoperatively, neither monkey could perform the task at criterion level with the distracting annular surrounds even after the prolonged retraining to criterion. Retention after a two-week interval, following criterion performance on retraining, was quickly re-attained in both. Monkey F was atypical on original learning pre-operatively, requiring twice as many sessions to reach criterion performance, but showed stabilized retention on pre- and post-operative tests.

In this attention-demanding task the postoperative performances of monkeys A and B indicate that posterior inferotemporal lesions cause partial loss of retention and require prolonged retraining to attain criterion. We believe this is not because of memory loss or pattern discrimination inability per se, but may rather be due to an attentional loss which penalizes those functions. Supported by USPHS grant MH-25938 and by grant from Grant Foundation. H.V.S. held NIMH fellowship 1-F32-MH05051.

390 EFFERENT ORGANIZATION OF VISUAL AREA I IN THE RABBIT. Harvey A. Swadlow* and Theodore G. Weyand* (SPON: A.L. Brickman). Dept. Psychol., Univ. Conn., Storrs, Conn. 06268.

Cortico-fugal and cortico-cortical efferent neurons of visual area I (V-I) in Dutch Belted rabbits were studied using anatomical (HRP) and physiological (antidromic) methods. Anatomical studies showed: (a) nearly all efferents to the lateral geniculate nucleus (LGN) are found in layer VI; (b) layer V is the origin of most neurons which project to the superior colliculus (S.C.) though significant numbers are found in layer IV; (c) layer II-III is the origin of most callosal efferent neurons, but some of these cells are also found in layers IV and V. Preliminary analysis of neurons projecting to visual area II (V-II) indicate that most of these cells are found in layer II-III.

In physiological studies efferent neurons were identified by antidromic activation following electrical stimulation via electrodes located within target structures of the efferent systems. The conduction velocities of most V-I→V-II and many V-I→LGN axons were very low (i.e., < 1.0 m/sec), suggesting that most axons of these systems were non-myelinated. In contrast, the conduction velocities of >90% of V-I→SC axons were >3.0 m/sec, suggesting that these axons were predominently myelinated. The number of cells studied (n) and the range (r) and median (M) conduction velocities of efferent axons were as follows: V-I→LGN (n=100, r=0.39-10.0 m/sec, M=1.2 m/sec; V-I→SC (n=121, r=1.1-23.6 m/sec, M=8.4 m/sec; V-I→callosum (n=69, r=0.55-8.0 m/sec, M=2.44 m/sec; V-I→V-II (n=69, r=0.23-5.7 m/sec, M=0.64 m/sec).

Antidromic tests for branching axons (utilizing collision techniques) provided no evidence for multiple destinations of the V-I→V-II, V-I→callosal or V-I→LGN efferent systems. Thus, although the laminar origins of the V-I→V-II and V-I→callosal systems overlap significantly, these systems are apparently independent. However, approximately 1/3 of V-I→SC cells were shown to project a collateral branch into the thalamus. The thalamic destination of these collaterals has not yet been determined.

391 PHYSIOLOGICAL PROPERTIES OF PROJECTION FROM AREA 3a TO 4γ OF THE CEREBRAL CORTEX IN THE CAT. R. Waters*, H. Yumiya*, K.D. Larsen and H. Asanuma. The Rockefeller University, New York, N.Y. 10021.

It has been shown, anatomically and physiologically, that neurons in the area 3a of the sensory cortex project topographically to area 4γ of the motor cortex. The present experiments were undertaken to further characterize the properties of this projection. Under Halothane anesthesia, the pericruciate and colonal sulci were exposed and a double-barreled closed chamber was installed over the sulci. All the wound tissue was infiltrated with a long lasting local anesthetic and experiments were carried out with a sedative dose of Nembutal (10mg/kg). Two independent manipulators were attached to the chamber and one electrode was inserted into area 3a and the other into area 4γ. Intracortical microstimulation (ICMS) was delivered to area 3a and the effects were examined by averaging the discharge of neurons in area 4γ. In one group of experiments, ICMS was delivered to a fixed site in area 3a. In these cases the facilitatory effects appeared in a small region of 4γ which extended along the direction of radial fibers constituting a columnar shape. The diameter of these columns ranged from 0.5 to 0.8 mm. In the other group of experiments the 4γ electrode was fixed to a position where the electrode picked up unitary activity of a given neuron or a given pair of neurons. ICMS was then systematically delivered to various sites in 3a. The sites which facilitated a target neuron were always restricted to a small region which extended along the direction of radial fibers with the diameter being less than 1.0 mm. In no one case did we find a 3a site which projected to more than one region in the motor cortex nor a 4γ neuron which was facilitated from more than one locus in 3a. Since both electrodes could be used for either stimulation or recording, receptive fields of neurons in both areas could also be examined. In the majority of cases, neurons in limited regions in 3a and 4γ which were connected received peripheral inputs from the same part of the body or adjacent areas. The results indicated that the projection from 3a to 4γ is highly localized, suggesting some particular built-in function for this system. However, the role played by 3a neurons in specifying the receptive fields of neurons in 4γ seems to be minor because the modalities of adequate stimuli were often different and ICMS did not elicit obligatory discharges in 4γ neurons but simply facilitated their activity. (Supported by the Grant NS 10705).

392 **SYNAPTIC PROFILES OF SPINY AND NON-SPINY STELLATE CELLS.**
Edward L. White and Michael P. Rock*. Dept. Anat., Boston Univ. Sch. Med., Boston, MA. 02118.

Thalamocortical (TC) afferents to the posteromedial barrel subfield (PMBSF) of mouse SmI cortex synapse with several different types of neurons (White, '79). The most frequent recipients of TC input are the stellate cells whose somata occur in layer IV and whose dendrites are mostly restricted to barrel hollows. The purpose of this study is to assess the locations of TC and all other synapses onto the cell bodies and dendrites of a spiny and a non-spiny stellate cell of mouse PMBSF cortex. The method used to identify TC axon terminals was lesion induced degeneration; a lesion was used to destroy the cortical projections from the nucleus ventralis posterior pars lateralis and the nucleus posterior thalami in a 2 month old CD/1 mouse. Lesion induced degeneration is generally considered unreliable for the identification of TC axon terminals because in most systems TC terminals degenerate over a varied time course such that some terminals have been phagocytosed before others have begun to degenerate. This is not true of terminals degeneration in mouse PMBSF cortex where all affected terminals - ~20% of the terminals in layer IV neuropil - degenerate simultaneously. A Golgi impregnated spiny stellate cell from layer IV of PMBSF cortex ipsilateral to the lesion was gold-toned and deimpregnated (Fairen et al., '77) so that its synaptic connections could be examined with the electron microscope. A series of thin sections containing the deimpregnated cell was cut and electron micrographs were taken of each part of the spiny stellate cell and of a neighboring non-spiny stellate cell which was not impregnated, but which could be identified on the basis of its cytology and synaptic connections. These cells were then reconstructed from wooden sheets of appropriate thickness at a final magnification of 20,000 X. TC and synapses of unidentified origin are depicted on the reconstructions. About 10% of the asymmetrical synapses onto the spiny stellate cell are from the thalamus; all of these synapses are with spines. By contrast, the non-spiny stellate cell receives a much larger share of its input from the thalamus and these synapses contact both its cell body and dendrites. An intriguing finding is that TC inputs are distributed in a non-random fashion over extensive regions of the spiny stellate cell. For example, the necks of 5 spines which receive TC input attach to one primary dendrite at intervals of 5± 0.5 μm. Our interpretation of this periodicity is that it reflects the spacing of TC axodendritic "junctions" prior to the time of spine formation.

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

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

Extracellular recordings were made from cortico-cortical and pyramidal tract neurons in cats lightly anesthetized with Nembutal. Stimulation of forelimb nerves was frequently followed by a change in the shape of the antidromic action potential, even in the case of neurons which did not fire in response to the peripheral stimulus. The change in the antidromic spike most often consisted of a clear decrease in the time from onset to peak. The decrease was especially evident for neurons whose antidromic spike was fractionated, in which cases the inflection of the rising phase was reduced or eliminated. This modification of the antidromic spike could be easily distinguished from those resulting from changes in presynaptic excitability (Dubner and Sessle, Exp. Neurol. 30: 223, 1971) or axonal excitability (Swadlow et al., Exp. Brain Res. 32: 439, 1978).

We conclude that the decrease in time from onset to peak is a result of an increase in the excitability of the soma. Therefore, monitoring changes in the time from onset to peak of extracellularly recorded antidromic spike potentials is a more sensitive method of detecting synaptic inputs than is collision-extinction testing. We have now used this method to investigate the patterns of sensory convergence upon identified populations of cortico-cortical and pyramidal tract neurons, as will be described in the following abstract.

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394 CONVERGENCE OF SOMATOSENSORY AND PROPRIOCEPTIVE INPUTS UPON CORTICO-CORTICAL AND PYRAMIDAL TRACT NEURONS. P. Zarzecki and D. Wiggin*. Department of Physiology, Queen's University, Kingston, Ontario, Canada K7L 3N6.

This study asked whether the same group of cortico-cortical neurons which relay group I muscle afferent input from area 3a to the motor cortex (Zarzecki et al., Exp. Brain Res. 33: 269, 1978) also receives input from cutaneous afferents. Excitation of pyramidal tract (PT) neurons of area 3a was also examined.

In cats under light Nembutal anesthesia, extracellular recordings were made in area 3a from neurons antidromically activated by microstimulation of the motor cortex (area 4γ) or from the medullary pyramid. PT neurons were tested for a projection to the spinal cord (C2-3). Identified cortico-cortical and PT neurons were tested for excitation from up to five contralateral forelimb nerves; deep radial (muscle), superficial radial (cutaneous), median (mixed) and ulnar, the latter divided into its dorsal cutaneous and palmar (muscle) branches. Volleys recorded from proximal nerve trunks were used to grade stimulus intensities (usually less than 2 x T) and to control for stimulus spread between nerves. Excitatory input to a cortico-cortical or PT neuron was concluded when nerve stimulation either elicited spikes which collided with the antidromic response or was followed by a decrease in the time from onset to peak of the antidromic spike (for justification see Wiggin and Zarzecki, this volume).

Among cortico-cortical neurons, 22 of 28 (79%) showed alterations of antidromic spike shape from each of two or more nerves. Sixteen (57%) showed this evidence of subliminal excitation from both muscle and cutaneous afferents. On the other hand, spike responses were evoked in only 11 of 59 cortico-cortical neurons (19%) by stimulation of two or more nerves and in only 4 (7%) from both muscle and cutaneous nerves.

Among PT neurons of area 3a, 11 of 18 projected to the spinal cord, 15 (83%) showed alterations of spike shape by two or more peripheral nerves, and 14 (78%) by both muscle and cutaneous afferents.

In conclusion, the majority of cortico-cortical and PT neurons of area 3a showed evidence of convergence from muscle and cutaneous forelimb afferents, although at the nerve stimulus intensities used these inputs were very often subthreshold. Neurons of these two types could be involved in cortical processing of information of several sensory modalities. This possible role might not be suspected from studies which require neurons to fire for the detection of an input.

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CHEMICAL SENSES: SMELL AND TASTE

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

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

396 **OROPHARYNGEAL AND HYPOTHALAMIC INPUT TO THE SAME NTS NEURONS.** **D.A. Bereiter, H.-R. Berthoud* and B. Jeanrenaud*. Lab. Res. Med., Univ. Geneva, SWITZERLAND.**

Oropharyngeal sensory input is capable of evoking an early, so-called cephalic phase secretion of immunoreactive insulin (IRI). Hypothalamic electrical stimulation is also reported to alter serum IRI levels. The neural mechanisms within the CNS underlying such modulation of IRI levels have not been fully investigated. We now report that these two seemingly different mechanisms may share a common neural substrate at the brainstem level. Single neurons located in the nucleus tractus solitarius (NTS) responsive to lingual nerve (L) or chorda tympani (CT) electrical stimulation (ES) were often responsive to lateral hypothalamic (LHA) ES. Caudal brainstem units were recorded using glass micropipettes filled with 2 M NaCl and Fast Green dye in male rats anesthetized with pentobarbital sodium. NTS cells responding to L- or CT-ES were subsequently tested for hypothalamic sensitivity. Among the diencephalic structures stimulated (50 - 350 μA, 0.2 msec, coaxial bipolar) the LHA was most effective in evoking short latency (< 15 msec) orthodromic unit activity in NTS cells responsive to L- or CT-ES. Diencephalic stimulation sites located in the dorsomedial hypothalamus, ventromedial hypothalamus, optic tract or medial amygdaloid nuclei generally had no influence on NTS cells responding to L- or CT-ES. Within the LHA, sites effective in evoking NTS unit activity extended anteriorly from the retrochiasmatic level and posteriorly to the ventral premammillary nucleus. These results demonstrate a significant degree of excitatory convergence between LHA and both trigeminal and CT afferents onto single NTS neurons. This convergence may provide a basis for a common neural substrate underlying LHA-induced and cephalic phase IRI secretion.

397 CHEMORESPONSIVE NEURONS OF THE GOAT GENICULATE GANGLION. James C. Boudreau, Joseph Oravec* and Nga Kieu Hoang*. Sensory Sciences Center, University of Texas at Houston, TX 77025.

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

398 CHANGES IN TASTE RESPONSES FROM SHEEP CHORDA TYMPANI NERVE DURING DEVELOPMENT. Robert M. Bradley and Charlotte M. Mistretta. Dept. Oral Biol., Sch. Dent., U. Mich., Ann Arbor, MI 48109.

We previously reported that response characteristics of taste neurons in the sheep solitary tract and nucleus change during fetal development (Science 202, 535-537, 1978). When the tongue was stimulated with 0.5 M NH_4Cl, KCl, NaCl and LiCl, solitary neurons in fetuses <114 days of age only responded to NH_4Cl and KCl, whereas neurons in older fetuses, lambs and adults responded to all four salts.

To determine if these results relate to peripheral or central taste system maturation, recordings were made from the chorda tympani nerve in 8 fetal sheep aged 108-110 days of gestation (term=147 days), and 4 lambs aged 44-50 days after birth. Both whole nerve and few or single fiber (n=13) responses were recorded while stimulating the tongue with 0.5 M NH_4Cl, KCl, NaCl and LiCl. The whole nerve responses were passed through an AC to DC converter, and the frequency of single and few fiber responses was counted using a rate meter. Effectiveness of chemical stimulation was then evaluated on the basis of height of the whole nerve response, or frequency of unit responses.

Analysis of both whole nerve and unit data demonstrated that in 108-110 day fetuses the most effective stimuli are NH_4Cl and KCl, in that order. Although responses were recorded to lingual stimulation with NaCl and LiCl, they were minimal. In the lamb, NH_4Cl remains the most effective stimulus; however, compared to the fetus, KCl becomes less effective and NaCl and LiCl become much more effective. Thus, the preliminary data indicate that the order of effectiveness of the four salts is:

Fetus: $NH_4 > K > Na = Li$.
Lamb: $NH_4 > K = Na = Li$.

The mean response frequency for all four chemicals increases with age. The average frequency for NH_4Cl in the fetus is 47 impulses/sec while the frequency in the lamb is 82 impulses/sec, an increase of 74%. The change in frequency for KCl is much less, 48%. However, the increases for NaCl and LiCl are very striking, at 248% and 284%, respectively.

These changes in peripheral taste nerve function during development confirm the reported central nervous system changes. At both levels, NaCl and LiCl become more effective stimuli as development progresses. The results support our hypothesis that the developmental change in salt taste responses is related to receptor maturation rather than maturation of taste synapses and fiber tracts. (Supported by NSF Grant BNS 77-09920 and National Institute of Dental Research, N.I.H., Research Career Dev. Award DE00066 to C.M.M.)

399 Depressed chorda tympani responsiveness in zinc deficient rats. Frank A. Catalanotto and Marion Frank, Dept. of Ped. Dent., Univ. Conn. Sch. Dent. Med., Farmington CT. 06032 and Rockefeller Univ. New York, NY

Previous behavioral studies in zinc deficient rats(Zn-) demonstrated abnormal preferences for sodium chloride (N), sucrose (S), quinine (Q), and hydrochloric acid (H) solutions when tested in a 2-bottle preference procedure(J.Nutr. 109:436-442, 1977).We hypothosized that the altered preferences were the result of a decrease in taste sensitivity and therefore measured the integrated whole nerve chorda tympani responsiveness to tastant solutions in control (Zn+) and Zn- rats. The experimental animals were male rats initially weighing 125-150 grams; they were fed either a Zn- diet (1.3 ppm Zn) ad libitum or pair fed a Zn+ diet (100 ppm Zn) for 4-6 weeks before being studied. This time period allowed for moderate sysmptoms to develop including anorexia, failure to grow, alopecia, and mild inflammation and scaling of the paws; control rats appeared normal.

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

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

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

400 SEXUAL AND HORMONAL INFLUENCES ON ODOR PREFERENCE MODIFIABILITY IN RATS AND HAMSTERS. Catherine Cornwell-Jones, Gina Dunston*, Cheryl Holder* and Kathryn Kovanic*. Dept. Psych., Princeton Univ., Princeton, NJ 08544.

Olfactory preference modifiability declines with age in male rats and hamsters (Cornwell-Jones, J. Comp. Physiol. Psych., 93, #4, 1979). The present experiments determined whether a similar decline in modifiability occurs in females of the two species, and if either neonatal or postpubertal testosterone manipulation influences the decline. In each of three experiments, odor preferences of rats and hamsters were examined in a two-choice situation immediately following three days of differential olfactory exposure. The testing apparatus allowed animals to smell but not touch or taste differently scented wood shavings placed in two compartments beneath a screen floor.

Animals reared in pine shavings generally avoided the odor of cedar shavings in favor of pine shaving odor, regardless of age at testing, sex, species, or hormone treatment. Housing normal animals in cedar shavings for three days before testing increased preference for cedar odor in male infants but not male juveniles of both species, and in females of both species at both developmental stages. The results indicate that olfactory preference modifiability declines less with age in females than males of both species.

Some olfactory-innervated brain regions are sexually dimorphic. The dimorphism depends on the presence or absence of neonatal androgens (Raisman & Field, Brain Res. 54: 1-29, 1973). In the second experiment, rat pups 2 days old and hamster pups 4 days old were injected with 300 µg testosterone propionate in .05 cc oil or with oil alone. Animals were differentially exposed and tested as juveniles. Neonatal treatment did not significantly influence exposure-induced differences in juvenile preferences for cedar vs. pine in females of either species. The results suggest that sexual differences in the anatomy of olfactory-innervated brain regions cannot, alone, account for sexual differences in juvenile odor preference modifiability.

In the third experiment, postpubertal male rats and hamsters were either castrated or sham-operated, allowed to recover from surgery in pine shavings in the isolated olfactory environment, and then were exposed differentially and tested. Housing in cedar shavings increased preference for cedar odor in rat castrates and sham operates, and in hamster castrates but not sham operates. The results suggest that in normal hamsters, age-dependent increases in endogenous testosterone levels may contribute to declining odor preference modifiability during development.

401 TEMPORAL CODING OF SENSORY QUALITY: EVIDENCE FROM SINGLE UNIT TASTE RESPONSES IN THE RAT NTS. Ellen Covey and R.P. Erickson. Dept. Psychology, Duke Univ. Durham, NC 27706.

The temporal charachteristics of responses by single neurons of the rat NTS to 50 widely different stimuli were compared, and their similarities computed by comparing the direction and amount of change in firing rate from one 0.1 sec interval to the next for 2 sec. The overall similarity value was the algebraic sum of the individual 0.1 sec comparisons.

Individual neurons did not respond with their own charachteristic temporal patterns; rather the temporal pattern seemed to be largely determined by the identity of the stimulus. Similarity values for repititions of the same stimulus across many different neurons were significantly higher than those for different stimuli, even within the same neuron.

To determine whether it is possible to identify a stimulus solely on the basis of its temporal response pattern, multiple responses to 4 different stimuli (NaCl, HCl, Quinine HCl, and sucrose) were averaged, giving "standard responses" for each stimulus. Individual "test responses" to the 4 stimuli were compared to the standards, and the standard giving the highest similarity value was taken to be the predicted stimulus identity for the test response. Identification was correct at least 70% of the time, and this was true over a fairly wide range of test response concentrations. When the number of stimuli was increased to 25, test stimuli were still most often identified with the correct standard, and errors in identification usually involved confusion with stimuli known to be similar in taste(e.g.NaCl might be identified first as another Na or Li salt, but showed very low similarity to HNO_3).

These results strongly suggest that temporal aspects of the neural response make an important contribution to the coding of stimulus quality in taste, and electrical stimulation studies to demonstrate whether these temporal patterns are actually used by the animal to discriminate stimulus quality are presently underway.

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402 TASTE AND TACTILE RECORDINGS FROM THE RAMUS RECURRENS IN THE CATFISH. Cynthia J. Davenport* and John Caprio. Dept. of Zoology and Physiology, Louisiana State University, Baton Rouge, LA 70803.

The ramus lateralis accessorius (ramus recurrens of VII) innervating the flank of the channel catfish, Ictalurus punctatus, responded to both amino acid and mechanical stimulation of the flank skin. Results were obtained employing conventional extracellular electrophysiological technique. Tactile stimuli consisted of fine streams of water directed to the flank by glass pipettes as well as slight mechanical deformation and stroking of the skin surface with glass probes. Most nerve twigs responded only to the mechanical stimulation, but taste recordings when obtained were correlated with the region of highest mechanical sensitivity. The time-averaged phasic gustatory responses increased exponentially with logarithmic increase in stimulus concentration. L-alanine and L-arginine were the most effective compounds tested, with thresholds estimated below 10^{-6}M. Individually identified taste units responed best to L-alanine or L-arginine. These data are similar to the results obtained from maxillary barbel taste recordings in the same species (Caprio, J. J. Comp. Physiol. 123:357, 1978), suggesting an analogous chemical response profile of facially innervated taste buds irrespective of their anatomical location in the catfish.

The ramus recurrens is believed to be the sole innervator of flank taste buds, with tactile stimulation being supplied by adjacent cutaneous free nerve endings, thought to originate in the dorsal root ganglion (Herrick, J. J. Comp. Neurol. 15:375. 1905). In the present experiments both intra- and extra-cranial neural recordings from the ramus recurrens indicated that mechanical responsiveness was not attributable to spinal innervation. It would thus appear that the ramus recurrens is a gustatory nerve with high mechanical responsiveness. Recent studies in other vertebrate species have also revealed mechanical sensitivity of taste units (Marui, T. Brain Res. 130:287, 1977; Yamane, S. Comp. Biochem. Physiol. 61A:451, 1978). These data raise the question whether taste cells share both mechano- and chemoreceptive properties, or whether taste cells are innervated by branches of the same sensory nerve fiber that forms mechanically sensitive free nerve endings in the surface epithelium.

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

A radioautographic investigation of the fiber projections of the olfactory bulb of the teleost Macropodus opercularis revealed that bulbar neurons enter the olfactory nerve and terminate selectively in the olfactory receptor epithelium. Four adult Macropodus were administered 0.2 to 0.5 μCi of L[^{3}H-2,3] proline on a dry 60 to 80 μm diameter bead of Dowex resin which was implanted unilaterally in the olfactory bulb (Davis, R.E. and Agranoff, B.W., 1977, Brain Res. 124:341-346. Following a 10 day survival period, the head was removed, fixed in formalin, decalcified and embedded in paraffin. Ten micron horizontal or transverse sections were dipped in Kodak NBT-2 emulsion and stored for 3 weeks. Following development of the emulsion the tissue was lightly stained with cresyl violet acetate. The implanted bulb was heavily labeled by grains of reduced silver. Labeled protein was distributed in the ipsilateral olfactory nerve and olfactory receptor epithelium in the nasal sac. The segments of indifferent epithelium were lightly labeled. The contralateral olfactory bulb and nerve were also only lightly labeled. The pattern of labeling in the nerve suggested that axons of efferent neurons intermingle with the afferent primary receptor neurons. Similar results have been obtained in the goldfish, Carassius auratus.

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

The olfactory bulb on the side which received the HRP and the contralateral bulb showed similar distributions of endogenous peroxidase in capillary walls and erythrocytes. Fibers in the superficial layer of the anterior zone of the ipsilateral bulb contained reaction product. The primary receptor neurons terminate in this layer. The ipsilateral bulb also contained many labeled cells embedded in the superficial layer and in the periglomerular region. The peroxidase-labeled cells in Carassius closely resemble the amphibian efferent neurons described by Rubaschkin (1903, Arch. mikr. Anat. 62, 207). The data indicate that the olfactory efferent neurons in fish terminate selectively in the receptor epithelium, which suggest a receptor modulation function. However, whether the efferents synapse with receptors or some other cells remains to be investigated.

404 DEVELOPMENTAL CHANGES IN NEURAL TASTE RESPONSES IN POSTNATAL RATS. Fay Ferrell*, Robert M. Bradley, Charlotte M. Mistretta and Kathy Miklossy*. Dept. Oral Biol., Sch. Dent., U. Mich., Ann Arbor, MI 48109.

Response characteristics of taste neurons in fetal sheep have been shown to change during development (Mistretta and Bradley, Science, 1978, 202, 535). Older fetuses respond to a greater number of salts and acids than do younger fetuses, and responses to specific salts and acids develop in a particular sequence. To learn whether functional changes also occur in a species in which the taste system develops postnatally, we examined taste responses in 11 preweaning (8-21 day), 20 weaning (22-60 day) and 10 adult (>60-day) rats.

Rats aged 8-30 days were anesthetized with Chloropent and older rats with Nembutal. Responses were recorded from the whole chorda tympani nerve while stimulating the tongue with 0.1M NaCl, LiCl, KCl and NH_4Cl, 0.01N HCl, 0.0025M citric acid, 0.5M sucrose, and 1.0M urea. Neural responses were passed through an AC to DC converter, and the height of the steady-state component of the response was measured. Ratios of the responses to each stimulus compared to the NaCl response were calculated.

As age increases, the order of effectiveness of the monochloride salts changes. Our preliminary data indicate that whereas the order in preweaning rats is NH_4 > Li > Na > K, in adults the order is Li > Na > NH_4 > K. Thus, NaCl and LiCl become more effective stimuli; NH_4Cl, less effective. HCl, citric acid and sucrose usually elicit larger responses than NaCl in preweaning rats, but equal to, or smaller responses in adults. Responses to LiCl and urea seem to remain stable with respect to NaCl.

The transition from preweaning to adult response characteristics seems to occur between about 20 and 30 days. By 40-50 days, responses relative to NaCl are essentially the same as for adults. These changes in taste function in rats after birth confirm the developmental changes observed in fetal sheep. Such changes in stimulus effectiveness may reflect maturation of taste receptor membranes. (Supported by NIH Postdoctoral Fellowship DE-05154 to F.F., and by NSF Grant BNS 77-09920 to R.M.B. and C.M.M.).

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

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

406 IONIC BASIS FOR REGULATION OF OLFACTORY RECEPTOR CILIARY MOVEMENT. R. C. Gesteland, D. L. Blank and K. M. Hanlon*. Northwestern Univ. Dept. of Biological Sciences, Evanston, IL 60201 and Naval Research Laboratory, Washington, DC 20375.

Movements of the cilia which grow out of the apical dendrites of olfactory receptor neurons in the frog are dependent upon odorous stimulation. Control of the motion appears to be by way of ciliary membrane conductance changes analagous to those which occur in some ciliated protozoans. Ciliary activity was observed at the edge of a fold of isolated epithelial tissue immersed in amphibian Ringer viewed with differential interference contrast optics. When the membrane was depolarized by reducing external (Cl^-) motion slowed and stopped. Changes in external (Na^{++}) had no effect. Reduction of external (Ca^{++}) increased the rate of ciliary motion. Addition of EDTA or EGTA in the absence of Ca^{++} blocked motion after a brief increase in activity. 0.01% Triton X (which probably reduces membrane ionic diffusion barriers) also first increased then blocked activity. Chelators plux Triton do the same only more rapidly. A wide variety of odorous substances presented at low concentrations modulates ciliary motion. These may increase activity, reduce activity or induce successive periods of increase and decrease. In the unstimulated state each cilium beat consists of a power stroke followed by a recovery stroke. There is no metachronal synchrony between neighboring cilia on the same cell or on adjacent cells and no resulting directed mucus transport. Stimulated increase in rate of activity does not affect either the stroke direction or asynchrony. Slowing of rate of movement is accompanied by metachronal synchrony and directed fluid transport. The direction of the power stroke also appears to change. These observations are consistent with a model of the ciliary membrane which is quite permeable to K^+ and/or Cl^- and slightly permeable to Ca^{++} in the unstimulated state. Factors which cause hyperpolarization are associated with increased beat frequency and asynchrony. Increase in membrane Ca^{++} conductance and depolarization result in slowing of rate and metachronal synchrony. All motion is blocked by removal of an essential ion (Mg^{++}?) from the cilium interior. The control processes postulated here for olfactory receptor cilia motion are the ones that have been shown to be responsible for motion reversal upon mechanical contact in *Paramecium caudatum*. The mechanical stimulus initiates a Ca^{++} action potential. We suggest that olfactory receptors are excited by odor-evoked increases in cilium membrane Ca^{++} conductance and inhibited by evoked increases in K^+ and/or Cl^- conductance and that a single receptor process both initiates a useful transport process and generates the neural signal. Supported in part by NIH Grant No. NS 14663 and NSF Grant No. BNS 78-17479.

407 UPTAKE PATTERNS OF 2-DEOXYGLUCOSE ASSOCIATED WITH ELECTRICAL STIMULATION OF THE OLFACTORY NERVE IN RAT AND IN VITRO TURTLE OLFACTORY BULB. C.A. Greer, W.B. Stewart, J.S. Kauer, K. Mori* and G.M. Shepherd. Sections of Neuroanatomy, Neurosurgery and Gross Anatomy, Yale Univ. Sch. Med., New Haven, CT, 06510.

Odor stimulation elicits distinct patterns of 2-deoxyglucose (2DG) uptake in the glomerular layer of the rat olfactory bulb, as detected by the Sokoloff autoradiographic method. The results suggest that increasing odor concentration is correlated with increased activity in the axons of specific groups of receptor cells and that the axons project to groups of glomeruli in the olfactory bulb (Stewart, Kauer and Shepherd, J. Comp. Neurol., In Press). We have sought further information about the contribution of receptor axons to the patterns by examining 2DG uptake associated with selective electrical stimulation of the olfactory nerve. The nerves were exposed in rats and stimulated through a bipolar electrode while recording evoked potentials in the bulb. Stimulation of the nerve bundles in the dorsal recess of the nasal cavity produced evoked potentials limited to the lateral anterior aspect of the ipsilateral bulb. Autoradiography revealed an intense focus of 2DG uptake in that region. Low frequencies (1/4 sec.) and low intensities of stimulation yielded uptake patterns centered in the glomerular layer. Increasing frequency (up to 10/sec.) and higher intensities resulted in progressive extension of the 2DG focus into neighboring laminae of the bulb. Stimulation of other olfactory nerve bundles, or antidromic stimulation of the lateral olfactory tract, produced readily distinguishable patterns. The general characteristics of 2DG uptake found here are similar to those observed following odor stimulation, which supports the interpretation that odor induced 2DG uptake is primarily due to increased activity in olfactory axons.

Similar experiments have been carried out in the isolated turtle olfactory bulb. The Sokoloff method was adapted to the in vitro preparation by continuously infusing the bath with ^{14}C-2DG (50μC/100ml) during the 45 min. period of stimulation. This was followed by a 15 min. washout with turtle Ringer prior to freezing and standard preparation for autoradiography. Stimuli were submaximal in intensity and delivered at a rate of 1/2-5 sec. while recording evoked responses in the bulb. Stimulation of an entire nerve bundle, to either the dorsal or ventral aspect of the bulb, produced extensive 2DG uptake in the corresponding part of the bulb. Following these relatively intense volleys, the induced 2DG uptake could be seen throughout most of the bulbar layers. The results demonstrate that the Sokoloff method can be applied successfully to in vitro preparations of the CNS, and moreover that the results are qualitatively similar to those observed in vivo.

408 NEURAL ONTOGENY OF CHORDA TYMPANI TASTE RESPONSES IN THE RAT. David L. Hill* and C. Robert Almli. Dept. Psychol., Ohio Univ., Athens, Ohio 45701.

We are in the process of examining the neural taste responses of the chorda tympani (CT) in the developing rat. In order to examine the developmental changes in the CT responses that may be associated with important developmental stages of consummatory behaviors, we have obtained summated multi-unit CT responses from male and female adult rats (90-120 days), postweaning-prepubertal rats (34-35 days), weanling rats (19-20 days), and preweaning rats (12-13 and 9-10 days). We have recorded responses from a few 6-day-old rats and are presently investigating the CT responses of rats at this age, as well as in younger rats.

To examine the developmental changes of CT responses to qualitatively different stimuli, 10 ml each of 0.5M NH_4Cl, 0.5M NaCl, 0.5M LiCl, and 0.1M citric acid were applied to the anterior two-thirds of the tongue. In addition, a concentration series of 0.1M NH_4Cl, 0.25M NH_4Cl, 0.5M NH_4Cl, and 1.0M NH_4Cl were applied similarly to assess the ontogeny of quantitative characteristics of the CT responses. Following each stimulus presentation, the tongue was washed with 20 ml of distilled water. A standard stimulus of 0.5M NH_4Cl was periodically applied to establish the stability of the preparation.

Preliminary results indicate that the CT is responsive to all of the above stimuli in rats as young as 6 days of age. We have found that as the rat progresses from the preweaning ages, through the weanling and postweaning-prepubertal ages, and finally to adulthood, the CT responses to 0.5M NaCl and 0.5M LiCl become progressively larger in magnitude relative to the CT responses to 0.5M NH_4Cl. In contrast to the equimolar salt response comparisons, the CT response to 0.1M citric acid appears to decrease in magnitude relative to that of 0.5M NH_4Cl as the rat matures, although the decrease in the response to citric acid does not seem to be associated with consummatory stages of development. In response to the concentration series of NH_4Cl, CT responses at all ages examined thus far increase in response magnitude as the concentration increases.

Developmental changes of CT responses to equimolar salts suggest that important changes are occurring in the development of taste buds. In addition, these data suggest that after the buds are mature, changes in their sensitivities to salts may occur concomitantly with important periods in development of consummatory behaviors. Supported by: OURC Grant No. 520.

409 AIR/OLFACTORY MUCOSA PARTITIONING OF ODORANTS. D. E. Hornung*, M. M. Mozell and J. A. Serio*. Dept. Physiology, Upstate Medical Center, Syracuse, NY 13210.

To better define the mucosal sorptive events, we have developed a technique, using tritium labeled odorants, to measure partition coefficients. Partition values for odorants can now be obtained, not only between air and water (often accepted as representative of the air/mucosa partition) but also between air and the mucosa itself. For various periods of time samples of frog mucosa or water were exposed in a closed environment to humidified air saturated with either tritiated butanol or tritiated octane. The radioactivity recovered from each of these samples, as determined by liquid scintillation counting, was used as a measure of odorant uptake. For butanol, the air/water partition coefficient was in good agreement with that reported by Amoore and Buttery (Chem. Sens. Flavor, 3:57, 1978) who used a gas flow technique. This agreement supports the validity of the present technique. We determined that, after equal exposure times, the water and mucosa samples sorbed equivalent amounts of butanol. This suggests that for butanol, uptake by olfactory mucosa is not significantly different from uptake by water. Therefore, the air/water partition coefficient can be taken to represent the air/mucosa partition coefficient. However, for tritiated octane, the mucosal uptake was about 1.5 times that of water, indicating that olfactory mucosa may have an increased ability over water itself to sorb octane molecules. Perhaps, then, for slightly water soluble odorants like octane the air/water partition coefficient may not be a very good indicator of mucosal uptake.

This technique allowed us to generate curves showing the uptake of odorant by the mucosa as a function of exposure time. Therefore, we were able, by extrapolation, to estimate the mucosal uptake during exposure times short enough to represent sniffs like those artificially produced in our earlier in vivo studies (Hornung and Mozell, J. Gen. Physiol. 69:343, 1977). This work suggests that the air/mucosal partition coefficient may be basic to the establishment of the different odorant distribution patterns earlier observed across the mucosa.

In experiments designed to study odorant desorption, mucosal or water samples were again exposed to a humid environment saturated with tritiated odorant. The samples were then quickly transferred to a humidified environment containing no odorant where desorption was allowed to occur for ten minutes. The remaining radioactivity in the samples was counted. For octane, the amount of desorption from the mucosa was significantly less than from water. This again suggests that the mucosa has an increased ability, over that of water itself, to sorb and retain certain types of odorant molecules. (NIH Grant NS 03904)

410 OLFACTORY FUNCTION IN BIRDS: NEUROPHYSIOLOGY AND BEHAVIOR Larry V. Hutchison and Bernice M. Wenzel. Dept. Physiol., Brain Res. Institute, Sch. Med., UCLA, Los Angeles, CA 90024.

Previous neurophysiological study has revealed major ipsilateral and contralateral forebrain centers which receive mono- and polysynaptic input from the olfactory bulb (OB) in the pigeon Extensive connections exist to sensory processing and relay areas and also to areas homologous with limbic structures and basal ganglia in mammalian forms. Characteristic evoked potentials and distinctive temporal patterns of unit responses to olfactory nerve (ON) stimulation are recorded in anatomically localized cell populations. In addition to topographical specificity, many possibilities exist for information coding in the olfactory pathway in terms of systematic changes in temporal parameters in unit activity. To examine some of these possibilities, detailed time series analyses are being conducted by computer on spontaneous activity and modifications of the firing rates of single cells. Recently, we extended anatomical and physiological study of the avian olfactory system to the Northern Fulmar (Fulmarus glacialis). This species, like other procellariiform birds, has relatively extensive olfactory conchae, mucosal area, and a larger olfactory bulb/cerebral hemisphere ratio than most other birds. Compared to the pigeon, the forebrain structures receiving fibers from the OB in the fulmar also appear proportionately more extensive. Further, the prepiriform cortex and olfactory tubercle are denser and more readily distinguished in Nissl stained sections. Bipolar stimulation of one bundle of ON twigs with 8-12 V pulses (single or train), 0.5-1.0 ms duration, at 0.1-1.0 Hz resulted in evoked responses in both OB's and in significant modifications of spontaneous unit activity (± 20% change in rate). Characteristic evoked potentials and modifications of unit activity were recorded from forebrain sites in areas comparable to those receiving first- and higher-order projections in the pigeon, viz., hyperstriatum, prepiriform cortex, parolfactory lobe, and the paleostriatal complex. Odor stimuli combined with ambient air blown directly into the nares produced significant changes from pre-odor baseline firing rates in several bulbar cells. The result was either enhancement or inhibition depending upon qualitative differences among stimuli. Unit activity was consistently inhibited by natural food odors (cod liver oil and krill homogenate) but not significantly modified by ambient air and control solvent. In our systematic program of experiments at sea we have found that procellariiforms including this species, detect and are attracted from downwind to sources of food-related odors with visual cues controlled. (Supported by USPHS grant NS 10353 to B.M. Wenzel and NINCDS Postdoctoral Fellowship NS 05896 to L.V. Hutchison).

411 PROCESSING OF CHEMOSENSORY AND MECHANOSENSORY INFORMATION BY APLYSIA NEURONS. Behrus Jahan-Parwar and Steven M. Fredman. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

Chemo- and mechanosensory input from the anterior tentacles of Aplysia project into the cerebral ganglion. In our previous work we have examined the responses of cerebral neurons to these modalities (1,2). We now present evidence that the identifiable cerebral B cluster neurons are second-order neurons in both pathways and that the tentacular mechano- and chemoreceptors are physiologically distinct. The responses of the B neurons to tactile and food chemosensory (seaweed extract, SWE) stimulation of the anterior tentacles, persisted after synaptic transmission in the tentacles had been blocked. Similarly, following blocking of polysynaptic pathways in the CNS, both tactile and food chemosensory stimulation still evoked EPSPs (many of which appeared to be unitary) in the B neurons. These data indicate that the tentacular mechano- and chemoreceptor cells synapse directly on the B neurons. B neuron tactile responses were phasic. Increased stimulus durations failed to produce proportionately longer responses. This appears to be due to decrement at the mechanoreceptor-B neuron synapses. B neuron responses to SWE were essentially tonic and lasted until the stimulus solution was washed out. The difference in the responses cannot be accounted for simply by the number of receptors activated. Seawater flowing over the tentacles which stimulated mechanoreceptors over the entire tentacle surface, still produced only phasic responses in the B neurons. This suggests that the mechano- and chemoreceptors are physiologically distinct and that the modalities are distinguished by the properties of the first-order synapses.

This work was supported by grants NS 12483, NS 14388 and BNS 77-24174 to B.J-P.

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412 STIMULATION AND INHIBITION OF TASTE RECEPTOR CELLS BY GYMNEMIC ACIDS AND ZIZIPHIN. Linda M. Kennedy and Bruce P. Halpern*. Depts. Psych., Harvard Univ. and Cornell Univ.; Dept. Psych. and Sect. Neurobiol. and Behav., Cornell Univ., Ithaca, NY 14853.

Gymnemic acids and ziziphin (from Gymnema sylvestre and Ziziphus jujuba) suppress fly behavioral and neural responses to sucrose in a manner similar to that in which they suppress sweetness perception in humans (Kennedy et al., 1975; Kennedy and Halpern, 1978, 1979). Aqueous solutions of purified components from G. sylvestre (KGE) (Bartoshuk et al., 1969) and Z. jujuba (ZjE-A)(Kennedy, 1977) stimulated two cells in single taste sensilla of the blowfly Phormia regina. Responses to KGE and ZjE-A presented either as single solute stimuli, in mixtures with sucrose, NaCl, or 50mM LiCl, or during mechanical stimulation, suggested that the two cells were not the "sugar," "salt," or mechanoreceptor cells. Responses to KGE, ZjE-A, 50mM LiCl, NaCl, or sugars after adaptation to sucrose, KF, or distilled water, confirmed that aqueous solutions of KGE and ZjE-A stimulate the "water" and "fifth" cells.

Responses of the "fifth" cell to 2 sec stimulations with KGE and ZjE-A were concentration dependent, but slow and not always phasic-tonic. Prolonged stimulation (≥ 2 min) often led to volleying and cessation of firing. A 3 min pretreatment with KGE or ZjE-A resulted in an initial depression, subsequent recovery, and eventual increase and volleying of action potential responses to sucrose.

Dose-response curves for stimulation of the "fifth" cell and inhibition of the response to sucrose were bell-shaped and suggested that mechanisms of stimulation and inhibition are related. Consequently, it is unlikely that KGE and ZjE-A suppress sucrose perception by "occupying," and thus "blocking," sweet receptor sites. The stimulation/inhibition effects suggest disruption of the plasma membrane. Similarities of stimulation/inhibition by the amphipathic molecules, KGE and ZjE-A, with stimulation/inhibition by amphipathic fatty acid salts (Dethier and Hanson, 1968) support the notion (Dateo and Long, 1973; Kennedy et al., 1975; Kennedy, 1977) of a role for surface active properties in the action of gymnemic acids and ziziphin.

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413 INVOLVEMENT OF GUSTATORY NEOCORTEX IN THE RAT'S NEOPHOBIC AND ASSOCIATIVE RESPONSES TO TASTE AND ODOR STIMULI. Stephen W. Kiefer and Kenneth W. Rusiniak*. Dept. Psych., and Ment. Retard. Res. Ctr., UCLA, Los Angeles, CA 90024

In normal rats, odor alone is a weak conditioned stimulus (CS) for LiCl-induced illness (US); taste alone is an effective CS. However, if odor and taste are presented as a compound CS and followed by lithium illness, both odor and taste become effective CSs when tested alone. Odor and taste in compound also evoke a strong neophobic response before conditioning. The gustatory neocortex (GNC) has been implicated in both neophobia and taste aversion conditioning. In this experiment we tested the role of the GNC in compound conditioning that involved taste and odor.

One month prior to behavioral training, rats were given either bilateral suction ablation of the GNC or sham surgery. For aversion training rats were first accustomed to a restricted drinking schedule. On the two conditioning trials, intubations of LiCl (.15M, 2% B.W.) immediately followed the drinking period; two groups of rats, (GNC, n=7; sham, n=5), drank distilled water in the presence of an almond odor, and the other two groups (GNC, n=8; sham, n=6), drank .1% saccharin in compound with the almond odor on the conditioning days. After conditioning, extinction tests were given to all rats with the almond odor and the saccharin taste presented separately on alternate days.

Results showed 1) that GNC rats did not show a strong neophobia to the odor/taste compound that was found in the sham controls; consumption of the compound odor-taste fluid by the GNC rats was the same as that of the GNC rats given odor alone, 2) GNC rats did acquire significant odor aversions, but not as efficiently as sham controls, 3) both GNC and intact groups trained with the odor alone tended to extinguish faster on the odor component than the groups trained on the odor/taste compound, 4) only the control group trained on the compound displayed significant aversions to the taste. GNC rats trained on the compound acquired virtually no aversion to the taste, consuming saccharin at control levels.

Thus rats lacking GNC did not exhibit normal neophobia to odor-taste stimuli. In addition, following compound conditioning, GNC rats acquired aversions for the odor but not to taste in contrast to the sham controls, which acquired aversions to both components. These results have implications for the neural basis of odor-taste interaction during illness aversion conditioning.

414 REGIONAL PATTERNING OF RESPONSE TO ODORS IN THE SALAMANDER OLFACTORY MUCOSA. John L. Kubie and David G. Moulton. Dept. of Physiol., Univ. of Penn. and V.A. Med. Ctr., Phila. PA 19104

Electro-olfactograms (EOGs) were recorded from various mucosal positions to determine whether different odorants elicit different spatial patterns of response. To eliminate "chromatographic" effects, (i.e., uneven odorant distribution imposed by sniffing) odorants were delivered through a modified "punctate" device that was positioned immediately above the electrode tip.

Experiment 1. Two stationary electrodes, one anterior and one posterior, were positioned on either the dorsal or the ventral epithelium. Each odorant was presented to each position in an ascending series of ½ log concentration steps. Eight ventral and 3 dorsal surfaces were tested with butanol, limonene and occasionally other odorants. For each mucosal surface and at all concentrations the anterior electrode position was more sensitive to butanol while the posterior position was more sensitive to limonene. The average composite difference in sensitivity exceeded one order of magnitude.

Experiment 2. We mapped EOG responses to odorants on 17 ventral and 6 dorsal surfaces. Eighteen to 25 positions were selected on each surface and a fixed stimulation sequence was constructed. Each odorant was presented at one concentration (usually one that produced 1.5 mv EOGs in previous experiments) sequentially to each point on the epithelium. Each receptor sheet was mapped for limonene, butanol and several other odorants. On all mucosal surfaces (both dorsal and ventral) we recorded strongest responses to butanol anteriorly and strongest responses to limonene posteriorly. Point-by-point correlations between butanol and limonene response maps were always negative. Fifteen odorants exhibited anterior patterns (trimethyl amine, cinnamic acid, cyclopentanone, acetone, isoeugenol, butanol, methyl butyrate, cantharidin, napthaline, methly sulfide, vanillin, B-ionone, acetophenone, valeric acid, and terpineol); 4 odorants exhibited posterior patterns (limonene, camphor, pinene, and hexachloroethane); and 3 appeared to stimulate the epithelium evenly (amyl acetate, decyl acetate and cyclohexanone).

This is the first demonstration made by direct recording from both dorsal and ventral surfaces of the olfactory mucosa that there is a clear dissociation of regional sensitivity to odorants. Since these results are totally independent of chromatographic effects, they may account for previous findings attributed to chromatographic effects or they may complement the normal sniff patterns of odorant distribution. There is also some indication that patterns other than those described here may exist for some odorants.

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415 STIMULUS ACCESS TO AND ACTIVATION OF THE GUINEA PIG VOMERONASAL SYSTEM. Judith W. Lerman*, Gary K. Beauchamp*, Charles J. Wysocki and John L. Kubie. (SPON: John H. Teeter). Monell Chemical Senses Center, 3500 Market St. and Dept. Physiol., Univ. Pennsylvania, Philadelphia, PA 19104.

When presented with conspecific female urine, male guinea pigs spend considerable time investigating, sniffing, licking and head bobbing to the stimulus. Females, when presented with conspecific urine, also investigate, sniff, and lick the urine, but not with the intensity exhibited by males. Our hypothesis, generated from these observations, was that urine per se may be transported to the vomeronasal epithelium where subsequent sensory processing is initiated. If correct, then urine or some constituents thereof, should be localized in the vomeronasal organ after exposure of a guinea pig to urine. Additionally, if the vomeronasal system is utilized in the detection of urinary constituents then activation of the vomeronasal epithelium should be reflected as an increase in activity of central nervous system structures associated with the vomeronasal system, e.g., the accessory olfactory bulb.

The hypothesis that low volatile compounds can enter the vomeronasal organ during urine investigation was pursued through the use of fluorescent dye-adulterated urines. Guinea pigs were offered urine with or without a fluorescent dye, rhodamine (B or 6G) hydrochloride. After a short exposure to the urine (less than 10 min), the guinea pig was sacrificed and the vomeronasal organ was removed, frozen, sectioned, and examined with a fluorescence microscope. In animals exposed to adulterated urine, dye fluorescence was observed in the vomeronasal organ but not on the olfactory epithelium. In addition, fluorescence was consistently observed in the mouth and nasopalatine ducts. Dye fluorescence was not observed in tissues from animals exposed to unadulterated urine.

The hypothesis that urine investigation activated central structures in the olfactory and accessory olfactory pathways was investigated by using the [14C] 2-deoxy-D-glucose (2DG) technique. Male guinea pigs were injected with 2DG and exposed to a series of female urine samples during the 2DG incorporation period. Autoradiographs revealed activation of the vomeronasal organ and the accessory olfactory bulb after exposures to female urine. To our knowledge, this is the first demonstration of activation of the vomeronasal system through the use of radiolabeled 2DG.

These results suggest activation of the vomeronasal system by liquid borne, possibly high molecular weight, compounds.

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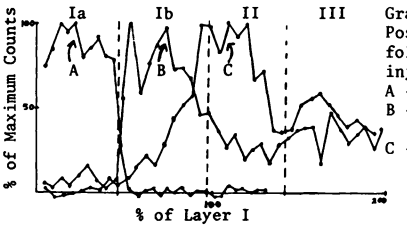
416 LAMINAR DISTRIBUTION OF ASSOCIATION FIBER SYSTEMS IN THE OLFACTORY CORTEX IN THE RAT. M.B. Luskin* and J.L. Price. Dept. Anat. & Neurobiol., Wash. Univ. Sch. Med., St. Louis, MO 63110.

Recent studies using the autoradiographic method have elucidated the laminar and topographic organization of the association fiber projections from subdivisions of the olfactory cortex. Two major laminar patterns of termination have been found. Previous studies have demonstrated that projections arising in the anterior olfactory nucleus (AON), piriform cortex (PC) and lateral entorhinal area (LEA) terminate in layers Ib and III (Price, '73) (curve B below); the projection to layer Ib is complementary to the projection to layer Ia from the olfactory bulb (curve A). These association projections are organized such that projections arising in more anterior structures project principally to areas deep to or near the lateral olfactory tract (LOT), while projections arising in more posterior structures terminate primarily in areas medial, lateral and caudal to the LOT (Haberly and Price, '78).

Another set of intracortical fibers has been found which terminates in layer II, the deeper portions of layer Ib, and in layer III (curve C). These projections arise from the ventral tenia tecta (VTT), dorsal peduncular cortex (DPC) and periamygdaloid cortex (PAC). Although the projection from the individual subdivisions terminate in different areas, collectively they innervate most of the olfactory cortex. The VTT projects principally to the AON; the DPC projects principally to the PC; and the PAC projects principally to the olfactory tubercle (OT), PC, anterior cortical amygdaloid nucleus (Co_a) and LEA.

Two further association projections have also been found: a projection from the Co_a to the PC, medial part of the AON, VTT and PAC which terminates throughout layer I, including both layers Ia and Ib, and a bilateral projection from the nucleus of the lateral olfactory tract to the medial PC and lateral OT, which is heavily concentrated over and around the cells of the edge of the PC and the superficial islands of Calleja.



Grain counts in the Posterior Piriform Cortex following ^{3}H-Leucine injections into:
A - Olfactory Bulb
B - Anterior Piriform Cortex
C - Dorsal Peduncular Cortex

417 RESPONSE PROPERTIES OF RAT OLFACTORY BULB NEURONS. Robert G. Mair* (SPON: W.J. McEntee). Psychology Dept., Brown University, Providence, R.I. 02912.

Neural activity was recorded from 65 units located near the mitral cell body layer in the rat olfactory bulb. These neurons were stimulated with 2 second odorant pulses delivered by an air dilution olfactometer and their responses were quantified as averaged peristimulus time histograms (PSTH's). Analysis of the PSTH data obtained from units driven by varying concentrations of single odorants suggests the existence of three discrete populations of neurons. Each of these are distinguished by characteristic temporal patterns of activity, evoked during the stimulus event, which change in a consistent manner as a function of stimulus concentration. Neurons driven by more than one of the test odorants exhibited similar patterns of activity in response to all effective stimuli. Type I responses are marked by an increase in activity within one second of stimulus onset. At low stimulus concentrations type I responses are characterized by a single burst of action potentials. At higher concentrations they consist of two shorter bursts of action potentials separated by a relatively quiet period. The activity of type II neurons is suppressed during the stimulus event when these units are driven by weak stimuli. Type II neurons driven by stronger stimuli exhibit a post-inhibitory burst of action potentials the latency of which decreases as a monotonic function of increasing concentration. The activity of type III neurons is decreased by all concentrations of effective stimuli. The extent and duration of this inhibition increases with stimulus concentration. Intensity related changes in type I and II responses were quantified in terms of three measures: number of action potentials fired during the stimulus event, latency of action potential bursts, and peak rate of activity during action potential bursts. The amount of evoked activity was not as consistently correlated with stimulus concentration as was the pattern of evoked activity. Increases in activity following stimulus offset (off responses) were apparent for some units and appeared to be of two types: phasic and tonic. Off responses occurred in some cells that did not respond during the stimulus event and in some of the cells exhibiting type I, II, and III responses during the stimulus event. It thus appears that the occurrence of off responses is not dependent on a unit's response during the stimulus event.

418 DEVELOPMENT OF TONGUE EPITHELIUM GRAFTS IN FETAL SHEEP. Charlotte M. Mistretta, Robert M. Bradley, and Hazel M. Stedman.* Dept. Oral Biol., Sch. Dent., U. Mich., Ann Arbor, MI 48109.

To study nerve-tissue interactions during development of lingual taste buds and papillae, grafts of tongue epithelium were made during sterile surgery in fetal sheep. In 16 fetuses, aged 52-107 days of gestation, anterior tongue epithelium containing fungiform papillae was transplanted to an external cheek site. In 4 fetuses, aged 62-73 days of gestation, posterior tongue epithelium containing circumvallate papillae was transplanted to the external cheek. Fetuses with grafts were replaced in utero to remain for the rest of gestation (term= 147 days). Near term or after birth, fetuses were perfused and graft tissues were prepared for light microscopic examination.

In anterior tongue grafts, filiform papillae acquired the structural characteristics of normal tongue. However, neither taste buds nor typical fungiform papillae developed. A few structures that resembled fungiform papillae were found in about one-half of the grafts; these structures were of smaller diameter than control papillae and usually had more keratin on the dorsum. Posterior tongue grafts contained the glandular structures found in control tongue; but only one or two papillae resembling circumvallates were found in each graft, and no taste buds were present. The papillae that resembled circumvallates were atypically small and were usually keratinized.

In the 4 fetuses with posterior tongue grafted to external cheek, the posterior site from which circumvallate papillae had been removed was examined. No circumvallate papillae developed in the smooth epithelium that covered areas from which lingual tissue had been dissected. However, several taste buds not associated with gustatory papillae were found in this epithelium.

We conclude that: 1. Gustatory papillae and taste buds will not develop under the influence of the trigeminal innervation to the external cheek. However, with trigeminal innervation, anterior and posterior tongue do retain some characteristics of the respective lingual tissue (for example, filiform papillae and glands). 2. Gustatory papillae will not regenerate if removed during development. 3. Taste buds will develop in tongue epithelium that does not contain gustatory papillae.

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419 NEURONAL RESPONSES TO l-CARVONE IN THE RAT PIRIFORM CORTEX: AN EXTRACELLULAR AND INTRACELLULAR STUDY. James W. Nemitz* and Stephen J. Goldberg. Dept. of Anat., Med. Col. of Va., VCU, Richmond, VA. 23298.

Bipolar electrical stimulation of either the lateral olfactory tract (LOT) or olfactory bulb (OB) in urethane anesthetized (1.5g/kg) male rats (≃300 grams) elicited characteristic field potential and single unit responses in the ipsilateral piriform cortex. An air-dilution olfactometer was used to deliver ≃250 msec. pulses of l-carvone to cells identified with electrical stimulation. A tracheal tube and a tube ascending to the choana were inserted. Controlled suction (≃8ml/sec.) applied to the ascending tube produced an artificial sniff during odor presentations. Recordings were made with beveled, potassium citrate (1.6M) filled micropipettes (20 MΩ). Electrodes were inserted from dorsal to ventral through the rats' cortices. The neuronal recordings were made in both anterior (3-4 mm anterior bregma) and posterior (at bregma) piriform cortex.

A total of 38 cells were studied in response to electrical stimulation. LOT stimulation was used to elicit responses in 9 cells which showed latencies to spike initiation ranging from 3 to 16 msec. with an average of 9.1 msec. OB stimulation was used to elicit responses in 29 cells with latencies to spike initiation ranging from 4 to 28 msec. with an average of 13.3 msec. 10 of these 38 cells were studied intracellularly and exhibited latencies of 1-6 msec. to initiation of the EPSP. 28 of the total 38 cells were subjected to odor stimulation with l-carvone (1 part/100). In 22 extracellularly recorded cells, 11 were excited and 11 showed no response to odor stimulation. In 6 intracellularly recorded cells, all showed excitatory responses although 2 neurons did not produce action potentials in response to odor stimulation, but EPSPs were present. Latencies to spike initiation ranged from 1.1 to 5.0 sec. in response to odor stimulation.

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

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420 TRANSECTION-INDUCED DECLINE IN IXth NERVE TASTE RESPONSES DEPENDS UPON SEASON BUT NOT AGE. Bruce Oakley and Lee B. Jones*. Div. Biol. Sci., Neuroscience Lab. Bldg., Univ. of Mich., Ann Arbor, MI 48109.

Following transection of the gerbil's (Meriones unguiculatus) IXth nerve, summated impulse discharges to taste solutions declined by 50% in 119 ± 44 min ($\bar{x}$ ± S.D., N = 15). Compound action potentials were still normal, indicating that the nerve trunk remained viable. We noted, however, that the taste responses of a number of the animals remained stable after nerve transection. We defined a stable response as one which was maintained longer than 3.5 hours (the mean decline time plus two S.D.); in fact, most stable responses showed no sign of declining in a 5 hour recording session. Over a four year period we have analyzed the influence of season of the year, age and sex on the likelihood that transection would produce a rapid decline in the nerve's taste response. 119 IXth nerves were transected in the months September through April. Taste responses declined in 98% (39 of 40) of the cut nerves recorded from September through December, yet declined in only 73% (58 of 79) of cut nerves recorded from January through April ($p < .01$, chi square). Gerbils were caged with littermates of the same sex and maintained on a 12/12 light/dark cycle. The occurrence of a significant population of nerves which failed to decline in January through April seems not to be related to the age or sex of the animal. Gerbils with stable and declining taste responses had identical age ranges (12 to 36 weeks) and no significant difference in mean age ($p > .15$, chi square). 57% of those nerves giving stable responses were from male gerbils while only 40% of the nerves with declining responses were from males. This difference is not significant ($p > .2$, chi square). We conclude that the physiological taste responses of the gerbil display seasonal variation which may be related to neurotrophic maintenance in the gustatory system.

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421 ACROSS FIBER SPECTRUM FOR SUGAR IN TASTE. Elizabeth Omand and Jacob Zabara, Temple University Health Sciences Center, Depts. of Physiology & Biophysics, Philadelphia, PA 19140.

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

Using standard tip recording through third molar LiCl six sugars were tested over a wide range of concentrations on "largest" labellar hairs (#1-12) of intact male Phormia regina, Meigen, 1-3 days old, unfed and reared in a 12:12 light cycle at 24°C, and 60% R.H. Sucrose and Maltose evoked receptor responses characteristic of a single unit. Monosaccharides at high concentrations commonly yielded irregular interspike intervals denoting two active units with similar impulse amplitudes. Spike "pairs" and single impulses alternated, the latter with amplitudes denoting degrees of coincidence. The pattern was consistent with the presence of two independent impulse trains. That appearing at lower concentrations had good correspondence with (tarsal) behavioral thresholds, and is assumed to be from the sugar receptor. Slope consistency with chemical class supported this interpretation. The train appearing at the higher concentration generated a response curve with a slope much greater than that by the other train to any sugar. Barring a facilitative electrolyte effect, "fifth" cell response to monosaccharides is indicated, with this order of effectiveness: Sorbose>D-arabinose>Glucose>Fructose. Apparently some carbohydrates can stimulate two receptor types, which therefore, exhibit spectral overlap. The overlap enhances the possibilities for across fiber patterning. Common action between carbohydrates and amino acids also occurs in mammals, and maybe be reflected in receptor responses which display mutuality for these chemical classes in the blowfly. The results are consistent with the concept of a continuum among taste stimuli. Discrimination among sugars appears possible in the blowfly based on across fiber patterns of discharge which differ between disaccharides and monosaccharides, especially those which are uncommon in nature.

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422 PONTINE UNIT RESPONSES TO TASTE MIXTURES IN THE HAMSTER. Susan E. Plock* and David V. Smith. Dept. Psychol., Univ. Wyoming, Laramie, WY 82071.

Taste-responsive neurons in the hamster brainstem have been shown to be more broadly responsive to the four classical taste stimuli (sucrose, NaCl, HCl, and quinine) than peripheral taste fibers (Smith, Travers & Van Buskirk, 1979; Travers & Smith, 1978). This increase in breadth of tuning is accompanied by greater response complexity (e.g., inhibition) at more rostral levels. The responses of pontine neurons to mixtures of the four basic stimuli may provide an indication of the way in which these neurons process information about more complex stimuli. Responses of neurons in the parabrachial region of the pons were recorded extracellularly from urethane-anesthetized hamsters. Stimuli were 0.1 M sucrose, 0.03 M NaCl, 0.003 M HCl, 0.001 M quinine hydrochloride (QHCl) and the six undiluted two-component mixtures of these compounds, delivered to the anterior portion of the tongue.

Predicted responses to the six mixtures were calculated for each neuron from the algebraic sum of the responses to each individual component. Approximately half of the responses to these mixtures were a linear summation of the responses to the two components, whether or not the components produced excitation or inhibition. Nonlinear interactions were complex. For example, in several cells, when sucrose elicited an excitatory response and QHCl suppressed the ongoing spontaneous rate, the response to the mixture was considerably less than the algebraic sum of the component responses, i.e., there was enhancement of the inhibitory effect. Thus, inhibition appears to be a more powerful phenomenon in these taste mixtures than is evident from the inhibition of the spontaneous discharge. When the responses to both stimuli were excitatory, the response to the mixture could be either suppressed or enhanced. For example, a mixture of sucrose and NaCl often produced no greater response than that elicited by the more effective component. This complete suppression occurred more frequently than partial suppression, in which the response to the mixture was less than the sum of the responses to the individual stimuli but greater than that produced by the more effective component. Less frequently, the response to a mixture was synergistic, being greater than the sum of the responses to the individual stimuli. Cases of nonlinear summation occurred with all possible mixtures of the four basic stimuli and sometimes varied for different mixtures within an individual neuron. Thus, the response to complex taste stimuli cannot be predicted readily from the responses to these four compounds.

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423 TASTE RESPONSES TO L-AMINO ACIDS IN RAT: A SINGLE NEURON ANALYSIS. Thomas C. Pritchard* and Thomas R. Scott (SPON: Jeffrey B. Malick) Dep't. Psychol. and Inst. for Neurosci. and Behav., U. Delaware, Newark, DE. 19711.

The physiological importance of l-amino acids (aa's) to animals implies that the gustatory system should be sensitive to their presence in the environment. Studies of bacterial chemotaxis and teleost taste responses confirm that chemical sensitivity to aa's in these species is indeed great. Humans report a wide range of taste experience from aa's, with a flat-bitter component predominating for many. The amplitude of whole-nerve chorda tympani responses from rats correlates well with human magnitude estimates of aa concentration, suggesting that human and rat sensitivities to these stimuli are similar. We sought to further analyze the rat's neural responses by recording the activity of single axons from the chorda tympani. Stimuli were 12 l-amino acids which were washed over the anterior half of the tongue at concentrations which evoked one-half the maximum whole nerve response for that chemical. The mean activity of 40 axons indicated that stimuli had the following order of effectiveness: .006 M CYS > .3 M ARG > .03 M LYS > 1.0 M GLY > 1.15 M PRO > .15 M ALA > .001 M HIS > .01 M LEU > .1 M THR > .003 M TRY > .03 M ISO > .06 M MET. This ranking correlates +0.78 with that established by whole nerve recordings. Most axons appeared to respond to this range of stimuli with a similar profile, with the overall responsiveness of the cell being the critical variable in determining breadth of sensitivity. For example, if a neuron's response to a standard solution (.1 M NaCl) was robust (> 20 spikes/sec), that cell was likely to respond to some degree to all 12 stimuli. Moderate responses (10 - 20 spikes/sec) to the standard indicated sensitivity restricted to the more effective aa's, and weak salt responses accurately predicted activity only to CYS, ARG and LYS. Thus for these stimuli there was basically one neuron "type" with varying degrees of sensitivity to stimuli in general. The relative similarity of any two aa's, as indicated by correlations among across-fiber patterns, was in general accord with that seen in human and rat psychophysical studies. (Supported by NIH grant NS 10405)

424 MORPHINE INHIBITION OF PARABRACHIAL TASTE UNITS REVERSED BY NALOXONE. G.H.Rogers, R.C.Rogers and D.Novin
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Immunohistochemical studies have localized endogenous opioid peptide activity within central nuclei (eg. nucleus tractus solitarius, parabrachial nucleus, ventromedial hypothalamus, amygdaloid complex) implicated in modulation of consummatory behavior as well as peripheral sites (eg. myenteric plexus and sympathetic ganglia). Behaviorally, systemic administration of morphine or its antagonist, naloxone, markedly reduced water intake in water-deprived animals (Rogers, et al 1978, W.Pharmac.Soc.).

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

Rats were anesthetized with urethane (1.5gm/kg) and provided with vena cava cannulae. The animals were then mounted into the stereotaxic frame; the skull trephenated and glass microelectrodes (filled with Pontamine dye) lowered into the PBN. Single unit neural responses were monitored with an oscilloscope coupled to an audio-amplifier and a pulse integrator-polygraphic chart recorder. Gustatory units were identified by increased activity in response to washing the tongue with 1.8%NaCl solution followed by a return to baseline activity in response to washing the tongue with water. Upon localization of such a taste activated unit, morphine was infused via the vena cava. Within 15sec, both baseline activity and responses to stimulation were greatly diminished. Although no further decline in baseline activity was seen, all responses to stimulation were lost 20min post-infusion. At this time, naloxone was infused via the vena cava and both baseline activity and responses to stimulation were readily returned to pre-morphine levels. The location of such identified cells was then marked by iontophoretic application of Pontamine dye. The responsive cells were thus identified following preparation for standard Nissl histology.

Perhaps the suppressant effects on feeding or drinking that are observed with systemic administration of morphine or naloxone are attributable to the altered activity of these gustatory units ie."blunting" of taste reception, which has previously been demonstrated to modulate consummatory behavior (Ernits & Corbit, 1973, JCPP).

425 EFFECT OF LESIONS ON AMINO ACID DISTRIBUTION IN RAT OLFACTORY BULB. C.D. Ross, D.A. Godfrey and J.A. Carter. Dept. Anat. and Neurobiol. and Dept. Pharmacol., Washington Univ. Sch. Med., St. Louis, MO. 63110.

Distributions of GABA, glutamate and aspartate were determined in the olfactory bulb of control and lesioned rats using quantitative histochemical mapping procedures. Data from these same rats concerning the cholinergic system in the bulb and amino acids in the piriform cortex have been reported previously (NS Abst. 4:86,91, 1978). In rats A and B, knife cuts were placed caudal to the anterior olfactory nucleus through the LOT, extending more deeply in B than A. In rats C and D, lesions were placed immediately caudal to the bulb in an attempt to separate it from the rest of the brain. Survival time was 1 week. Amino acid levels are given in mmoles/kg dry wt. in a control rat and in rats A-D (control, lesion side). (EPL, IPL: ext., int. plexiform layer; decreases significant: + at p= .05, * at p= .006).

LAYER	Control	Rat A	Rat B	Rat C	Rat D
GABA					
fiber	9			5, 8	
glomerular	24			23,27	
EPL, superficial	39			43,35	
EPL, deep	47	47,45	45,36+	51,43+	44,31*
mitral	48	51,50	50,42	55,36+	44,31*
IPL	54	46,49	45,38	54,35+	41,23+
granular	48	37,37	35,27	44,35	31,17*
periventricular	26	26,25	26,17	28,14	24,10
GLUTAMATE					
fiber	35			37,34	
glomerular	33			37,32	
EPL, superficial	42			44,36*	
EPL, deep	42	47,36	38,31	45,33*	37,22*
mitral	46	46,37	35,34	45,28*	35,22*
IPL	40	35,31	30,30	37,22*	36,20*
granular	39	36,39	31,30	39,26*	36,18*
periventricular	46	42,44	38,35	49,18	37,14
ASPARTATE					
fiber	7			8, 9	
glomerular	9			10,13	
EPL, superficial	14			18,18	
EPL, deep	18	19,20	16,17	20,18+	16,14
mitral	18	17,15	16,14	19,17	14,11
IPL	15	10,10	10,12	14,14	15, 8
granular	10	8, 9	8, 8	11,16	11, 8+
periventricular	7	6, 7	7,11	8, 8	8, 5

The significant decrease in glutamate in all layers deep to the glomerular layer in rats C and D is consistent with a centrifugal glutamatergic pathway entering the bulb in its deepest layers. (Amer. Cancer Soc. BS4S; USPHS NS-08862 and NS-08000).

426 THALAMO-CORTICAL MECHANISMS IN ODOR GUIDED BEHAVIOR: II. EFFECTS OF LESIONS OF THE MEDIODORSAL NUCLEUS AND FRONTAL CORTEX ON ODOR PREFERENCES AND SEXUAL BEHAVIOR IN HAMSTERS. R. M. Sapolsky* and H. B. Eichenbaum. Dept. Anth., Harvard Univ., Cambridge, MA. 02138, Dept. Bio. Sci. Wellesley Coll., Wellesley, MA. 02181, and Dept. Psych., MIT, Cambridge, MA. 02139.

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

Preoperatively, male hamsters demonstrated normal preferences to odors produced in the home cage and normal sexual performance In two separate experiments, subjects received either sham surgery or lesions limited to MD or to the frontal neocortex either of the medial wall (MW) or of the dorsal bank of the rhinal sulcus (RS). Post operative odor thresholds of all animals were normal. However, both attraction to pure odors and odors of mal and female conspecifics and discrimination among these odors was significantly reduced in hamsters who had received damage to MD and RS but not MW or controls.

In post-operative tests of sexual competence all subjects continued to mate successfully. However, male hamsters who receive lesions of MD or RS (relative to those who received lesions of MW or control surgery) spent more time sniffing the female's body rather than its genitals and often mounted an inappropriate body position of the female.

Only the medial subdivision of MD receives direct olfactory input and only this subdivision projects primarily to RS. It appears that disruption of this thalamocortical olfactory pathway disrupts discriminitive aspects of odor preference and sexual performance, but does not eliminate detection of odors or their potential for "priming" sexual behavior.

427 WAR GASES AS OLFACTORY PROBES. R. Schafer, D. W. Criswell*, and F. L. McClure*. Dept. Biol., N. Texas State University, Denton, TX 76203.

In most theories of olfaction, it is assumed that receptor sites exist on the excitable portions of olfactory receptor membranes where odorous molecules bind momentarily to initiate olfactory transduction. However, little is known of this chemical interaction and the nature of the odorant-binding receptor sites.

Any biochemical or physiological investigation is greatly enhanced when specific and irreversible inhibitors are available to use as chemical probes (e.g., alpha-bungarotoxin in studies of acetylcholine receptors). Although sulfhydryl reagents (in combination with protection techniques) have been used as olfactory inhibitors, there has been no report of marker agents or inhibitors which preferentially bind to specific classes of olfactory receptor sites. We are attempting to develop such agents from a novel source.

Chemical agents once used as war gases (mainly of pre-World War II vintage) are being tested as possible probes using the frog nose as a model system. For example, ethyl bromoacetate (a "fruity-smelling" tear gas) blocks electroolfactogram (EOG) responses to amyl acetate and other "fruity-smelling" esters, but does not block responses to isoamyl amine and other "fishy-smelling" amines. The blockage is irreversible inhibition, not simply sensory adaptation. The presumption is that ethyl bromoacetate preferentially deactivates ester-binding sites in the olfactory mucosa, but leaves amine-binding sites intact. Ethyl bromoacetate's effect is not totally specific, however, because it inactivates responses to sulfides such as isoamyl sulfide as well as responses to amines. Many other war gases are available, including mustard gas (sulfur-containing Bis(2-chloroethyl) sulfide, with a garlic-like sulfide odor) and nitrogen mustard (the amine mechloroethamine, with a "fishy" odor). These alkylating agents also inactivate olfactory responses in the frog nose with varying degrees of specificity. The advantages of these agents are (1) some specificity which can be experimentally augmented using protection techniques, (2) irreversible action over a period of at least several hours, and (3) application in the vapor phase. Since the agent is applied as a vapor, the experimenter can monitor the progressive effect of inhibitor application, unlike the application of inhibitors in liquid solutions which block testing.

428 COLLATERAL BRANCHING OF OLFACTORY TRACT AXONS. John W. Scott. Dept. of Anat., Sch. Med., Emory Univ., Atlanta, GA 30322.

Single neurons of the mitral cell and external plexiform layers of the olfactory bulb of the rat were activated antidromically by stimulation of the lateral olfactory tract, the olfactory tubercle and several points on the olfactory cortex. Antidromic activation was confirmed by collision tests. To date, 76 cells have been observed which responded antidromically to stimulation of one or more of the projection areas. Fifty-two (68%) cells were activated antidromically from both the olfactory tubercle and the olfactory cortex. Seventeen of these cells were shown by collateral collision tests to have separate collateral branches to the olfactory tubercle and to the cortex. These tests involved showing that stimulation at one point blocked antidromic responses to stimulation at the second point for a time period which could only be accounted for by invasion of a collateral by the action potential. Miscalculation of collision times due to spike suppression by reciprocal synapse inhibition was ruled out in each of these cases. Sixteen cells (21% of the total sample) could be antidromically activated only from the olfactory tubercle. These spikes were recorded almost exclusively from the ventral, posterior portion of the olfactory bulb (both mitral cell and external plexiform layers). These results indicate that many of the projection cells of the olfactory bulb have axon collaterals to two or more widely placed terminal areas. The results also indicate differential origin of axons projecting exclusively to the olfactory tubercle.

429 TASTE RESPONSES TO L-AMINO ACIDS IN RAT: A BEHAVIORAL ANALYSIS. Thomas R. Scott, Thomas C. Pritchard*, Kathleen Keene* and Margaret F. Parsons*. Dep't. Psychol. and Inst. for Neurosci. and Behav., U. Delaware, Newark, DE. 19711.

The relative similarities of 12 l-amino acids (aa's) to one another has been determined from single neuron data from the rat chorda tympani nerve. In this study we took a behavioral measure of stimulus similarity based upon generalization gradients around the conditioned stimulus (CS) in a conditioned taste aversion (CTA) paradigm. Stimuli were 12 aa's all at concentrations which evoked one-half the maximum whole chorda tympani nerve response for that chemical (see Pritchard and Scott, this volume). In addition, stimuli representing the four basic tastes (.1 M NaCl, 1.0 M sucrose, .03 M HCl, .001 M QHCl) were included to determine whether the tastes of the aa's could be encompassed within the dimensions of salt, sweet, sour and bitter. One hundred forty-four male rats under 18-hour water deprivation were trained to lick rapidly for water presented during a 15-minute period each day. When this was accomplished, each was offered a CS followed by an intraperitoneal injection of LiCl (426 mg/kg). Suppression ratios were calculated from the volume consumed by experimental rats divided by the volume consumed by matched controls (distilled water CS). The similarity between two stimuli was determined from the mutual suppression which each caused in the other. These behavioral similarity measures were in general agreement with similarity judgments based on across-fiber patterns from chorda tympani axons and with those from human psychophysical experiments. For example, ALA, GLY, PRO and sucrose are all reported to be predominantly sweet by humans, show highly correlated patterns of activity in the rat CT, and generalize well to one another in the CTA paradigm. Some aa's generalized strongly to a single basic taste stimulus. Others related less strongly to two or three basic stimuli, indicating a more complex taste, but one still manageable within the concept of four primaries. However four aa's (ISO, LEU, MET, TRY) showed no generalization to any of the four basic stimuli, suggesting that in the label-free language of licking, salt, sweet, sour and bitter are insufficient dimensions to describe all tastes. (Supported by NIH grant NS 10405)

430 THALAMO-CORTICAL MECHANISMS IN ODOR GUIDED BEHAVIOR: I. EFFECTS OF LESIONS OF THE MEDIODORSAL NUCLEUS AND FRONTAL CORTEX ON ODOR DISCRIMINATION IN RATS. K. J. Shedlack*, H. B. Eichenbaum and K. W. Eckmann*. Dept. Biol. Sci., Wellesley Coll., Wellesley, MA. 02181 and Dept. Psych., MIT, Cambridge, MA. 02139.

Olfactory information travels directly from primary olfactory cortex to the mediodorsal nucleus of the thalamus (MD) and then to prefrontal cortex. These, in turn, are intimately connected with limbic structures crucial to cognitive and affective behavior. Thus MD and prefrontal cortex appear to form an interface between sensory and limbic systems. As such, these structures may be involved in both sensory and cognitive functions.

In a number of experiments, rats were tested for their abilities of olfactory detection and discrimination. Individual experiments involved lesions either of MD or of its two cortical projection targets. Animals with lesions in MD were significantly impaired relative to sham operated controls in post-operative discrimination of three different odor pairs. Only the lateral part of frontal cortex, the frontal pole and dorsal bank of the rhinal sulcus, receives input from the part of MD which is the target of olfactory cortex efferents. Lesions of the frontal pole and rhinal sulcus (RS) produced a similar significant deficit in olfactory discrimination of these three odor pairs. No deficits were associated with lesions of the frontal cortex target at the medial wall (MW).

Detection and threshold tests revealed that the discrimination deficit observed with lesions in MD and RS is not secondary to a general anosmia or an elevated sensory threshold. Futhermore, the degree of discrimination deficit observed in MD-lesioned animals is sensitive to factors of problem novelty and difficulty. The impairment is diminished by pre-operative odor pair training and is enhanced in discriminations involving highly similar odors. Thus, the thalamocortical olfactory pathway is not crucial to odor detection but seems to play a role in higher order cognitive functions such as stimulus assimilation or value assignment.

431 NEUROGENESIS IN THE OLFACTORY EPITHELIUM. Peter A. Simmons and Thomas V. Getchell. Dept. Physiol., Yale Univ., Sch. Med., 333 Cedar St., New Haven, CT 06510 and Dept. Anatomy, Wayne State Univ., Sch. Med., Morin Memorial Lab., 550 E. Canfield, Detroit, MI 48201.

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

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432 EFFECTS OF INTRANASAL $ZnSO_4$ IRRIGATION ON OLFACTORY BULB MORPHOLOGY AND BEHAVIOR IN THE RAT PUP. Pauline Singh, Pat Model, George Pappas, and A. Marie Tucker. Albert Einstein Col. of Med., Bronx, NY and Queens Col., Flushing, NY

Morphological changes in the glomerular layer of the main olfactory bulb and latency to suckling were investigated after chemical lesion of the olfactory neurons. Wistar rat pups from 7 litters were subjected to intranasal irrigation with 5% $ZnSO_4$ solution at 7 or 8 days of age. Within each litter 4 pups were $ZnSO_4$-treated (Zn) and 3 were normal or saline controls. Body weights were taken daily, and latencies to suckling on the anesthetized mother were observed. Pups were killed at 1, 4, or 7 days post treatment. The olfactory bulbs of both control and experimental animals were then prepared for light and electron microscopic study.

Light microscopy indicated the following changes in various days post treatment: 1 day, discrete fibers were visible in the medial glomeruli, but most of the peripheral glomeruli appeared disorganized; 4 days, no distinct glomeruli were visible indicating that degeneration had occurred; 7 days, medial glomeruli were again present, although no discrete fibers were visible. This may indicate early stages of regeneration. Electron microscopy showed degenerating terminals with distorted synaptic vescicles, dense cytoplasm, and swollen mitochondria at 1 day post treatment. Some glial-engulfed material and membranous whorls were also present. At 4 days post treatment, few or no degenerating terminals could be distinguished. Instead, glial-engulfed material was present suggesting that the degenerating terminals had been phagocytosed.

The weights of Zn pups were significantly lower than those of control littermates on all days following treatment ($p < .05$). The Zn pups killed at 1 and 4 days post treatment exhibited a deficiency in nipple orientation and attachment. Their median latencies to suckling were 180 sec. (maximum duration of observation), whereas for control littermates they were 45 sec. or less ($p < .05$). At seven days post treatment, there was no significant difference. The behavioral effects of peripheral olfactory deafferentation appear to be correlated with the morphological changes observed at the light and electron microscopic levels.

433 DEVELOPMENTAL STUDIES OF THE OLFACTORY BULB: 2-DEOXYGLUCOSE UPTAKE PATTERNS IN SUCKLING RAT PUPS. Martin H. Teicher*, William B. Stewart, John S. Kauer, and Gordon M. Shepherd. Sections of Neurosurgery, Neuroanatomy, and Gross Anatomy, Yale University Sch. of Med., New Haven, Ct. 06510.

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

Rat pups 5-15 days of age have been used. Following intracardiac injection of ^{14}C-2DG (20 μC/100 g) the pups were placed on their anesthetized mother, in direct contact with a nipple. Attachment occurred within a few minutes, and suckling continued for 45 minutes. Preparation of the olfactory bulbs for autoradiography was by standard methods.

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

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

Numerous myelinated neurons and dendritic segments were found in the olfactory bulbs (OB) of 5 normal squirrel monkey (Saimiri) brains prepared for electron microscopy by routine procedures. Most profiles occurred in the periglomerular region (PGR), but a considerable number were also present in the external plexiform layer (EPL), especially in its uppermost region. The granule cell layer (GCL) contained the least number of myelinated processes. The more than 100 myelinated neurons studied exhibited different cytological characteristics. Since no ultrastructural study of neuronal types in primates exists, criteria established by Pinching and Powell (J. Cell Sci. 9, '71) in rat OB for the classification of different cell types were employed. Although not every cell could be unequivocally categorized, it was possible to discriminate between 4 different types of myelinated neurons. In the PGR and the EPL, myelinated somata resembled external granule cells, and this cell type was the most numerous of all myelinated cells. Neurons with the characteristic appearance of short axon and medium to small tufted cells were also found. These cell types occurred in about equal numbers. The few myelinated perikarya in the GCL were morphologically indistinguishable from adjacent granule cells except for the myelin sheath. Profiles of myelinated dendritic segments were sectioned in both the transverse and longitudinal planes, indicating that they were not running in a preferred direction. Some myelinated dendritic segments were in continuity with somata that resembled tufted or external granule cells. The myelin sheath either completely enveloped a perikaryon or was interrupted by myelin-free gaps in the plane of section. The myelin also varied in thickness, the thin sheaths exhibiting about 8-12 dense lines. Dendrites also displayed gaps in myelination. Frequently, at these gaps, the dendritic segment was engaged in a synaptic relationship and was either in a pre- or postsynaptic position. Occasionally, the synapse was reciprocal. The OB of a 44-year-old chimpanzee also contained myelinated somata and dendritic segments of similar morphology and distribution as the squirrel monkey OB. In the ape, however, myelinated processes occurred less frequently. The functional significance of these myelinated profiles in the primate OB is obscure. Supported by NIH Grant RR-00165 to the Yerkes Regional Primate Research Center and NIH Grant EY-00638.

435 EVIDENCE FOR SUBCORTICAL ADEQUACY IN THE ACQUISITION AND RETENTION OF A SODIUM TASTE-PLACE ASSOCIATION. Celeste R. Wirsig* and Harvey J. Grill (SPON: R. M. Beckstead). Dept. of Psychology, University of Pennsylvania, Phila. Pa. 19104.

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

Male Sprague-Dawley rats (n=9) were decorticated by aspiration in 1 or 2 stages and recovered 2-3 weeks before training. Each water deprived rat was trained to bar press for water (VI60) during the first phase of the experiment and then for .15 M NaCl. Immediately after the last training session of each phase rats were injected with sodium depleting drugs (DOCA and furosemide) and given salt-free food and water; 24 hrs. later bar presses were counted during a 1 hr. extinction session (Krieckhaus & Wolf, 1969). The results indicate that the presence of the neocortex is not necessary for the salt-trained rat to demonstrate need relevant resistance to extinction. Salt-trained rats pressed the bar at a high rate during extinction while water-trained rats did not at all. In addition, a very brief exposure to saline (1 min. during the last two days of water training) causes resistance to extinction (n=2).

In another experiment the same paradigm was used except that rats (n=3) were trained before a one-stage decortication. After 2½ weeks of recovery rats were injected with sodium depleting drugs and tested 24 hrs. later. Pilot tests had shown that decorticates recover bar pressing by this time and that controls would retain the taste-place association for at least 26 days. Results show that removal of the neocortex does not interfere with the rat's capacity to associate the presurgically tasted sodium with a need condition that was never concomitant with the taste experience. In summary, it was demonstrated that subcortical structures are adequate in the acquisition and retention of a sodium taste-place association. (Supported by NIH AM 21397).

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

Although the endogenous (autorhythmic) nature of the circadian is generally accepted, little is known concerning its possible basis in neuronal circadian oscillators. Data previously reported from our laboratory is analyzed to present the chemoreceptor neuron as an experimental model of the circadian system of the fly. The observations relating light and feeding interactions on chemoreceptor discharge is summarized graphically and compared to behavioral observations. The circadian clock is described as an endogenous rhythm, possibly genetically determined, composed of an ordered sequence of autorhythmic units summating to a circadian time period. The summation is accomplished by integrating mechanisms which are expressed as endocrine, or central excitatory states. The independent neural units ($A = A_1,\ldots,A_n$) are represented as derivative functions:

$$A_1 = f_1 \frac{de_1}{dz_1}$$

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

$$f \frac{de}{dz} = K_1 \frac{d(I)}{dt} + K_2 (I)$$

where I = interspike interval or spike frequency
and K = proportionality constant
For simplicity we have restricted this formulation to a first order derivative, although higher orders are involved. It is possible to consider the neuro-membrane as simulated by a simple physical model of a variable capacitor (dielectric)-resistance network. Discharge recordings from the chemoreceptor in light and feeding conditions will be presented to illustrate this aspect of the model. The polarized state of the membrane and the condition of its dielectric as well as the channels for sodium and potassium represent critical elements of the circadian system. The average value (S_i) of a time varying excitability factor (E) giving rise to a circadian rhythm over a time interval (0,T) is represented by the following:

$$S_i = \frac{1}{T} \int_0^T E_o(t) \sum_{i=0}^{n} f_i(t)\ (Z_i)dt$$

This formulation, for instance, represents enhanced excitation in a fasting fly where the locomotor activity increases to "override" the ordinary circadian rhythm. (Supported by NIH NS14209).

436 EVIDENCE FOR A CHOLINERGIC PROJECTION TO THE OLFACTORY BULB FROM THE MAGNOCELLULAR PREOPTIC AREA. William M. Youngs, N. Suzan Nadi*, Barry J. Davis, Frank L. Margolis and Foteos Macrides. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545 and Department of Physiological Chemistry and Pharmacology, Roche Institute of Molecular Biology, Nutley, NJ 07110.

Our previous HRP studies have shown that the magnocellular preoptic area (POM; "nucleus of the horizontal limb of the diagonal band") is a major source of centrifugal afferents to the olfactory bulb in the hamster. In the present study we characterized this projection with the autoradiographic tracing technique and examined the possibility that cholinergic afferents to the olfactory bulb originate in the POM. After injections of tritiated amino acids into the POM, labeled axons could be observed to travel rostrally in the medial forebrain bundle, course diffusely through the olfactory peduncle and enter directly into the main olfactory bulb (MOB). A small component of the projection was observed in the medial aspect of the lateral olfactory tract. The projection appeared to terminate in all layers of the MOB, including the glomerular layer. To assess whether the projection contains cholinergic fibers, unilateral electrolytic lesions were made in the POM of fourteen hamsters. Seven additional hamsters were sham-operated. Nineteen to twenty-eight days following surgery, the animals were sacrificed by decapitation and choline acetyltransferase (CAT) activity in the olfactory bulbs was measured by radiometric assay using carbon-labeled acetyl CoA. The CAT activity levels in the bulbs contralateral to the lesions did not differ from levels in the bulbs of sham-operated animals. Activity levels in the bulbs ipsilateral to the lesions were significantly reduced, and for individual animals ranged from 22% to 65% of the levels in the contralateral bulbs. Histological examinations of the extents of the POM lesions indicated that these reductions could not be attributed to an involvement of the vertical limb of the diagonal band. In another set of hamsters, HRP was injected into the ipsilateral bulbs following typical POM lesions in order to evaluate whether damage to fibers of passage might account for the reductions. The nucleus of the lateral olfactory tract and the piriform cortex contained heavily labeled neurons comparable in numbers to those labeled after HRP injections in intact animals. The labeling in the raphe nuclei and locus coeruleus was reduced substantially, but these projections to the MOB are thought to be serotonergic and noradrenergic, respectively. These various results support the hypothesis that the POM is a major source of cholinergic afferents to the olfactory bulb.

(Supported in part by NSF grant BNS78-06248 and NINCDS grant NS 12344.)

COMPARATIVE NEUROBIOLOGY

438 THE ROLE OF PROTOCEREBRUM IN THE MODULATION OF CIRCADIAN RHYTHMICITY IN THE CRAYFISH VISUAL SYSTEM. B. Barrera-Mera, J. Cibrian Tovar*and D.E. García-Díaz*. Depto. de Fisiología, Fac. Med. U.N.A.M. y Colegio de Postgraduados, Chapingo, México.

We have previously demonstrated that bilateral neural connections of crayfish visual system at protocerebrum level have an important role in the central modulatory process of electroretinographic (ERG) circadian oscillations (Brain Res. Bull. 3: 101, 1978; Comp. Biochem. Physiol. 61A: 427, 1978). In order to explore the role of that area of the cerebral ganglion (cg) as the probable site of synthesis and or regulatory releasing of neurosecretions involved in the control of such retinal changes, animals with protocerebrum only, were submitted to continuous recordings of ERG and eye glow area throughout the day. Fig. 1 and figure 2 illustrate typical cases in which we can see the circadian changes of EGA size as well as ERG voltage in animals with protocerebrum only. Notice the clear changes of both EGA size and bilateral ERG rhythm in these conditions in which only 30per cent of cg tissue remains neurally attached to both eyestalks. Since in the animals without cg the rest phase of both ERG and EGA size rhythm was strongly diminished (Barrera-Mera, Physiol. Behav. 17, 59, 1976) one possibility is that protocerebrum is the site of synthesis of hormonal neurosecretions directly involved in the ERG and EGA diminution observed during the rest phase of both rhythms. On the other hand, since the injection of sinus gland extracts obtained from brainless animals induced light adaptation, we believe that protocerebral area of cg controls the releasing of neurosecretion from the sinus gland.

Fig. 1

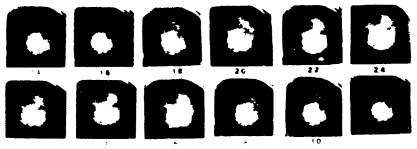
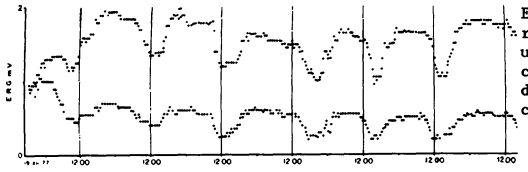


Fig. 2



EGA and ERG were recorded under continuous darkness conditions

439 DESTINATIONS OF AFFERENTS AND SOURCES OF EFFERENTS IN VIIITH NERVE BRANCHES OF MORMYRID FISH. Curtis Bell, Neur. Sci. Inst., Good Samaritan Hosp. & Med. Cent., Portland, OR, 97209.

Eighth nerve branches supplying the three canals, sacculus, and lagena were labeled with HRP. The utricular branch was labeled in association with the anterior canal nerve. For comparison, anterior and posterior lateral line nerves were also labeled.

Central structures where afferents ended included; n. descending, n. octavius, n. tangentalis, n. magnocellularis, eminentia granularis, n. anterior, and the cerebellar crest. The last two structures may be considered as a unit. Descending, tangentalis and magnocellularis received afferents almost exclusively from canals and utriculus. Eminentia granularis received input from lateral line nerves and all N. VIII end organs except the sacculus. Octavius received afferents from lateral line nerves and all branches of N.VIII. Saccular afferents went both ipsilaterally and contralaterally to a separate group of cells within this nucleus. Projections to cerebellar crest – n. anterior were noteworthy in that utriculus, sacculus, lagena, anterior lateral line nerve and posterior lateral line nerve went to different regions of the horizontal sheet defined by these cell groups, with afferents from canals overlapping those from utriculus.

The nearly indistinguishable projections of the utriculus and canals are consistent with their common role in equilibrium. The distinct saccular projection with its strong contralateral component and lack of endings in eminentia granularis, a probable cerebellar structure, is consistent with an auditory role. The rather intermediate pattern of lagenar afferents is at least consistent with present uncertainty as to whether this otolith has an equilibrium or auditory role. Our results also indicate that in the mormyrid N. VIII and lateral line nerves project to the same central structures.

Retrograde labeling showed that efferents in the lateral line nerves and all N. VIII branches arise from the same nucleus, a group of large cells above the medial lemniscus. The central course of the axons of these cells parallels that of facial (N. VII) motoneurons and the octavolateral efferent axons exit with the motor root of N. VII. Single efferent axons must branch to supply different end organs since more than half of the ipsilateral neurons in the nucleus were often filled with HRP after labeling single branches of N. VIII or one lateral line nerve.

440 SOME CONNECTIONS OF THE LATERAL OLFACTORY AREA OF THE HORN SHARK. David Bodznick and R. Glenn Northcutt. Dept. Neurosci's., UCSD, La Jolla, CA 92093 and Div. Biol. Sci's., UM, Ann Arbor, MI 48109

The lateral olfactory area (LOA) in the telencephalon of the horn shark (Heterodontus francisci) was reexamined using experimental anatomical and physiological techniques. A relatively large amplitude, short latency (40 msec) positive evoked potential following electrical stimulation of the olfactory tract was recorded from the lateral 1/3 of the ipsilateral telencephalic hemisphere corresponding to the extent of LOA. Fink-Heimer procedures revealed degenerating olfactory tract fibers and terminals throughout the rostral-caudal extent of LOA in animals with unilateral olfactory peduncle transection after 14-21 days at 14° C. Comparable projections were observed with autoradiography following intrabulbar injections of tritiated proline (60 μCi at 20 μCi/μl) and 7 days survival at 14°C. The LOA of Ebbesson and Heimer (Brain Res., 1970) is not a uniform cytological field but can be divided into a dorsal pallial field (lateral pallium) and a more ventral nuclear group (Field A). The lateral pallium is characterized by 3 laminae: a pronounced periventricular lamina of small densely-packed cells, a middle lamina of larger bipolar and polygonal neurons, and an outer lamina of smaller neurons and olfactory fibers. Field A does not possess a pronounced periventricular cellular lamina; it is characterized by an outer fiber layer and an inner zone of scattered cells which ventrally becomes more compact and grades into the cell plate of area superficialis basalis. Injections of HRP (0.1-0.3 μl of 20% Sigma VI) into the lateral pallium revealed that primary olfactory projections arise from 2 distinct populations of olfactory bulb neurons: mitral cells and smaller neurons located within the internal granule cell layer. The injections also revealed additional afferents to the lateral pallium from the ipsi- and contralateral dorsal and medial pallia and from the contralateral lateral pallium. These results suggest that olfactory information reaches the lateral pallium by a direct ipsilateral olfactory projection and an indirect contralateral projection via the contralateral lateral pallium. The latter is also evidenced by a relatively long latency (120 msec) negative evoked potential recorded in the lateral pallium after electrical stimulation of the contralateral olfactory tract. Unilateral injections of HRP (0.5-1.0 μl) into the olfactory bulb revealed projections from ipsi- and contralateral lateral and dorsal pallia but not from Field A. (Work supported in part by USPHS Postdoctoral Fellowship to DB and Rackham Faculty Research Grant (UM) and NIH NS11006 to RGN. Research was performed in accordance with NIH Guidelines, Vol. 7, #17, Nov., 1978.)

441 SOME CONNECTIONS OF THE TORUS SEMICIRCULARIS IN THE BOWFIN, AMIA CALVA: A HORSERADISH PEROXIDASE STUDY. Mark R. Braford, Jr. and Catherine A. McCormick. Department of Anatomy, Georgetown University Schools of Medicine and Dentistry, Washington, D.C. 20007

Following unilateral injections of horseradish peroxidase (HRP) into the torus semicircularis, several populations of neurons were retrogradely labelled. At the level of the obex labelled cells were present in the medial part of the dorsal horn of the medulla. Nearly all of these cells were contralateral to the injected torus. Nucleus medialis of the octavolateralis area receives primary input from the anterior and posterior lateral line nerves. A large number of neurons in this nucleus were labelled following toral HRP injection. Most of the cells labelled in nucleus medialis were the so-called Purkinje-like cells which lie immediately deep to the cerebellar crest into which some of their HRP-filled dendrites ramify. About 90% of these labelled cells were found contralateral to the injection. A few cells more ventrally located in the nucleus medialis were also labelled, again mostly contralaterally. At and rostral to the level of entrance of the eighth nerve, a population of cells was labelled in the central portion of the basal plate. These cells, which may represent the superior olivary nucleus, were labelled predominantly ipsilateral to the injection. In the isthmal tegmentum a group of neurons, termed here the isthmal reticular group, were labelled almost exclusively contralateral to the injection.

Anterograde labelling of fibers and terminals was also present showing both descending and ascending toral efferents. The descending pathway was ipsilateral and formed a compact bundle on the ventral surface of the medulla. Its most prominent terminations appear to be in the superior olivary nucleus. An ascending pathway courses rostrally through the lateral tegmentum and terminates bilaterally in the caudal diencephalon, the contralateral fibers crossing in the supraoptic decussation. The terminal field lies laterally adjacent to the central posterior nucleus of the dorsal thalamus dorsally and over a population of large cells in the glomerulosus complex ventrally. (Supported by NIH Grant 2 R01 NS11006 To R.G. Northcutt and NSF Grant BNS 78-22411.)

442 AFFERENT AND EFFERENT FIBERS OF THE VESTIBULOCOCHLEARIS NERVE OF THE LARVAL ANURAN. D.L. Brown, J.T. Hackett, and S.L. Cochran. Dept. of Physiology, University of Virginia School of Medicine, Charlottesville, VA 22908

Neuroanatomical and electrophysiological techniques were used to study fibers of the vestibulocochlearis (VIIIth) nerve of premetamorphic *Rana catesbeiana* tadpoles. Lesions of selected branches of the anterior and posterior rami were made after opening the otic capsule; horseradish peroxidase (HRP) was applied to the cut nerve. In some experiments HRP was pressure injected into the nerve in place of lesion techniques. The capsules were then sealed. After 1-4 days survival, the brains were processed for the HRP reaction product. Stained primary afferent fibers could be traced from the site of the HRP application through the ganglia of the VIIIth nerve to extensive projections in the rhombencephalon--from the granule cell layer of the cerebellum to the level of the XIIth nerve. Fibers as large as 10 µm could be seen entering the brainstem and turning to form the ascending or descending tracts of the VIIIth nerve, or bifurcating to form both. Collaterals from these tracts could be seen branching perpendicularly into the medial zone of the alar plate. The largest fibers tended to course more medially. More of these fibers were observed from the posterior than the anterior ramus. Neither decussation of fibers to contralateral regions nor differential specificity of projections from various branches was observed. Efferent fibers and somata were labeled in these experiments also. Application of HRP to the anterior ramus revealed a nucleus with more than 20 labeled cells ventral to the sulcus limitans, in the region of the motor nucleus of the VIIth nerve. This efferent nucleus extends from the level of the rostral edge of the VIIIth nerve caudally to the lateral line nerve and is composed of small and medium size cells (8-20 µm). Fine processes from these cells cross the midline into the central grey, as well as ramifying ipsilaterally. Labeling of the posterior ramus showed a different localization of efferent somata. At the level of the VIIIth nerve the cells were found in a more dorsolateral position relative to the anterior ramus efferent nucleus described. Other cells were found in the lateral reticular zone as far caudally as the Xth nerve. Additional evidence for the anterior ramus efferent nucleus was obtained by injecting HRP into cells identified by antidromic stimulation. Location of these cells was accomplished by first identifying the maximum extracellular field potential evoked by VIIIth nerve stimulation. Then a more medial recording site was found where efferent cells could be penetrated. Recovered cells were found in the efferent nucleus, with presumed axons projecting towards the VIIIth nerve.

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443 AFFERENT PROJECTIONS TO THE ANTERIOR DORSAL VENTRICULAR RIDGE IN THE LIZARD IGUANA iguana. Laura L. Bruce* and Ann B. Butler. Dept. Anat., Georgetown Univ., Washington, D.C. 20007.

The reptilian anterior dorsal ventricular ridge (aDVR) was traditionally regarded as a homologue of the mammalian basal ganglia. However, recent connectional, histological, and developmental studies indicate that instead it may be a homologue of portions of the mammalian neocortex which receive ascending thalamic sensory projections. To study the projections of the aDVR further, injections of horseradish peroxidase (HRP) into the aDVR of Iguana iguana, processed after deOlmos and Heimer (Neurosci. Let., '77), were used to identify retrogradely labeled cells.

After large injections of HRP into the aDVR, the distribution of labeled cells corresponded to previous descriptions of projections to the aDVR in other reptiles. Thalamic nuclei with labeled cells included nucleus rotundus, nucleus medialis, and several nuclei surrounding nucleus rotundus, such as the nuclei dorsomedialis anterior and dorsolateralis anterior. In addition labeled cells were found in several regions within the lateral mesencephalic tegmentum.

Very small, restricted injections of HRP were also made in various regions of the aDVR. After a small injection limited to the middle third of the rostral aDVR, labeled cells were found in the aDVR ventral to the injection site. Several large cells lying close to and within the lateral forebrain bundle were also labeled. A few scattered cells were labeled in the lateral hypothalamus and in the lateral mesencephalic tegmentum. The labeled fibers course predominantly in the lateral forebrain bundle and several labeled fibers were also in the lateral part of the medial forebrain bundle. When a small injection was limited to the middle third of the caudal aDVR, labeled cells were found in the posterior DVR and in the rostral parts of the aDVR, particularly in the superficial areas. A few labeled cells were scattered in the lateral hypothalamus and in the lateral mesencephalic tegmentum. Labeled fibers coursed along the lateral aspect of the medial forebrain bundle.

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

444 THE RETINA OF THE WEST INDIAN MANATEE (SEACOW). Joel L. Cohen, Gail S. Tucker* and Daniel K. Odell*, University of Miami, Rosenstiel School of Marine and Atmospheric Science, and the School of Medicine, William L. McKnight Vision Research Center, Miami, Florida 33149.

The retina of the West Indian manatee (Trichechus manatus), an endangered marine mammal, has never been accurately described. In order to determine the cellular makeup of this retina with special reference to photoreceptor cells, eyes were fixed in phosphate-buffered gluteraldehyde (pH 7.3), postfixed in 2% OsO_4, and embedded in Epon 812. Sections were stained with toluidine blue or para-phenylenediamine for light microscopy, and with lead citrate and uranyl acetate for electron microscopy.

Pigment granules were present in the pigment epithelium layer. Two types of photoreceptors were seen. One cell type is typically rod-like with a long cylindrical outer segment and an inner segment of the same diameter as the outer segment. A second cell type has a conically tapering outer segment. Using light microscopy, the nuclei of the two photoreceptor types stain differently with toluidine blue. With the electron microscope, two types of synapses were observed. One type, termed a spherule, is associated with rod-like cells, while the other, a pedicle, is associated with cone-like cells. Typical ribbon and conventional synapses were seen in the inner plexiform layer. The ganglion cell layer is remarkable due to the presence of cells with large perikaryon ($\sim$ 50 µm). This retina can be considered to be anatomically duplex.

445 ORGANIZATION OF SOMATOSENSORY INPUT TO THE MIDBRAIN OF THE FROG. Christopher Comer and Paul Grobstein. Dept. Pharmacolog. Physiol. Sci., Univ. Chicago, Chicago, IL 60637.

The existence of an orderly representation of visual information in the midbrain tectum of the frog has long been known. More recently an orderly representation of auditory inputs in the underlying torus semicircularis has been described (Pettigrew et al., Nature 272:138, 1978). We were interested in whether an orderly representation of somatosensory information is also present in the frog's midbrain. We here report electrophysiological studies on the organization of cutaneous inputs to the midbrain of Rana pipiens.

Multiunit responses to visual, acoustic, and tactile stimuli were recorded with tungsten microelectrodes in adult frogs anesthetized with tricaine methanesulfonate. In some animals the electrode was lowered through the tectum and tectal ventricle, and then into the underlying torus seimcircularis. In others, the tectum was first aspirated permitting direct access to the torus. Significant recording locations were marked with electrolytic lesions and subsequently located in histological sections.

All of the animals recorded from showed similar patterns of sensory responsiveness in the midbrain. Only visual activity was reliably recorded from the optic tectum. In the torus, weak visual activity was occasionally encountered; auditory activity (responses to clicks and snaps) and tactile activity (responses to gentle stroking of the skin) were prominent and reliable.

Penetrations near the midline in the torus yielded purely auditory responses; lateral penetrations, in the region of the magnocellular nucleus of the torus, yielded purely tactile responses. Penetrations between these extremes yielded responses to both modalities. Multiunit tactile receptive fields were large and located primarily on the contralateral body surface. More rostrally in torus, receptive fields were located on the snout and forelimbs; more caudally, receptive fields were located on the hindlimb and flank. There is thus at least a crude topographic map of the body surface in this region.

We conclude that there is a somatotopic representation in the lateral torus semicircularis and hence that not only the visual and auditory but also the somatosensory modality has an orderly representation in the midbrain of the frog. Auditory and somatosensory modalities seem to be principally represented in the torus semicircularis. Our observations suggest an exclusively auditory projection to the medial torus and an exclusively tactile projection to the lateral torus with an overlapping intermediate polymodal region.

Supported by PHS Grant EY-01658 and an Alfred P. Sloan Research Fellowship to PG.

446 PREFRONTAL "CORTEX" IN THE PIGEON BRAIN. Ivan Divac. Institute of Neurophysiology, University of Copenhagen, Denmark.

Certain regions of the bird "striatum" are now considered to be comparable to the sensory and motor cortical areas of mammals. It is not known whether the bird brain has a region comparable to the mammalian prefrontal cortex (PFC). In mammals, PFC is defined as the target of the mediodorsal thalamic nucleus which in turn is topographically identified. The bird thalamus, however, has no lamina medullaris interna and thus no topographically definable mediodorsal thalamic nucleus. It was recently discovered that PFC in mammals of three different orders receives uniquely rich dopaminergic innervation. §) Dopaminergic innervation may thus serve as a guide in search for "PF" tissue in the bird telencephalon.

Examination of pigeon brains prepared for catecholamine fluorescence revealed in the posterodorsolateral "neostriatum", just under the ventricle, a somewhat poorly delimited region with rich fluorescent innervation forming perineural nests. This region was clearly distinguishable from the paleostriatum augmentatum, accumbens, and septum. Additional experiments indicated that this "neostriatal" innervation is dopaminergic rather than noradrenergic. Thus, the posterodorsolateral "neostriatum" in the pigeon brain may correspond to PF in mammals.

§) Divac, I., Björklund, A., Lindvall, O. and Passingham, R.E. Converging projections from the mediodorsal thalamic nucleus and mesencephalic dopaminergic neurons to the neocortex in three species. J.Comp.Neurol. 1978, 180, 59-72.

447 HISTOCHEMICAL STUDY OF THE MONOAMINE CELL GROUPS OF THE AVIAN BRAIN STEM. Lorraine Dubé* and André Parent (SPON: R. Boucher). Lab. Neurobiol., Fac. Med., Univ. Laval, Québec, Canada.

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

In the mesencephalon of the chick, four CA cell groups are found at the level of the III nerve. First, a few large-sized neurons occur in the periaqueductal gray. These neurons do not display any significant AChE activity. Numerous multipolar CA cell bodies are also present in the tegmental pedunculo-pontine nucleus (TPP). These neurons stain moderately for AChE. Numerous other CA neurons (without AChE activity) are scattered dorsolaterally to the TPP group. Finally, a dense population of large, multipolar, CA cell bodies occurs along the lateral border of the III nerve root fibers. They display a very high AChE activity. More caudally, a CA and a 5-HT cell group are intermingled together within the decussation of the cerebellar peduncles. The CA cells are small, round or oval and display a very high AChE activity. The 5-HT neurons are larger, multipolar and also strongly stained for AChE. At the level of the nucleus of the IV nerve, numerous 5-HT cells occur along the midline and around the MLF. This group is composed of medium-sized and fusiform neurons that stain moderately for AChE. It extends itself laterally and intermingles with the CA cells of the locus coeruleus (LC). The LC is composed mostly of closely-packed and strongly fluorescent cells which also stain intensely for AChE. At metencephalic level, two CA cell groups occur one above the other in the lateral portion of the brain stem. The ventrally located neurons are small and lie in the area of the nucleus of the VII nerve, whereas the more dorsally located neurons are larger in size and are scattered beneath the nucleus of the Vth nerve. Some 5-HT cells are also scattered among the root fibers of the VI nerve, ventrally. At the same level, numerous 5-HT cells are present in the raphe region. These neurons display a moderate AChE activity. More caudally, another group of 5-HT cells is found within the raphe region at the level of the inferior olivary nucleus. Finally, two groups of CA cells can be visualized in the medulla oblongata. First, a few closely-packed CA neurons occur dorsolaterally to the motor nucleus of the X nerve, in an area containing numerous highly reactive AChE neurons. Second, multipolar CA cells are scattered within the central portion of the medulla.

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

448 A THALAMIC RELAY NUCLEUS FOR THE LATERAL LINE SYSTEM IN TELEOST FISH. Thomas E. Finger. Dept. Anat., U. Colo. Med. Ctr., Denver, CO, 80262.

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

Anterograde transport methods (HRP and tritiated amino acids) were used to determine the projections of the torus semicircularis (mesencephalic target for the lateral line sense) in two species of teleost fish (Carassius auratus and Ictalurus nebulosus). In both species, the torus semicircularis projects to a nucleus in the ventral thalamus possibly equivalent to a portion of the nucleus anterior tuberis of Sheldon (J. Comp. Neur., 1912). A small portion of the toral efferents bypass the ipsilateral thalamic nucleus and cross the midline in a supraoptic (ansulate) commissure to end in the contralateral nucleus anterior tuberis and torus semicircularis. This pattern of connection is similar to that seen in mormyrid fish (Finger, Bell & Russell, in preparation) except mormyrids possess two or three thalamic lateral line nuclei instead of the one seen in goldfish.

Large HRP injections into the lateral half of the telencephalon in catfish (Ictalurus) retrogradely label neurons of the thalamic lateral line nucleus. This thalamic area does not exhibit retrograde label following injections confined to the medial third of the telencephalon and median forebrain bundle. The exact telencephalic target area for the thalamic lateral line efferents remains to be determined. Some of the thalamic neurons are retrogradely labeled following HRP injections confined to the ipsilateral torus. Whether these same neurons also project to the telencephalon could not be determined.

In summary, the central connections of the lateral line system in teleosts appears to be organized similarly to the auditory lateral lemniscus in amniotes. A discrete lemniscal pathway for the lateral line sense can be identified from the lateral line lobes via torus semicircularis and ventral thalamus to finally reach the telencephalon.

This work supported by BRSG RR-05357 awarded by the Biomedical Research Grant Program, NIH.

449 DIFFERENTIAL PROJECTIONS OF THE AMPHIBIAN AND BASILAR PAPILLAE IN THE LEOPARD FROG (RANA PIPIENS): AN ANATOMICAL BASIS FOR TONOTOPIC ORGANIZATION. Zoltan M. Fuzessery* and Albert S. Feng. Dept. of Physiology and Biophysics, Univ. of Illinois, Urbana, IL 61801

The central projections of the two anuran auditory organs, the basilar and amphibian papillae, were examined using horseradish peroxidase (HRP) tracing technique. The HRP (Sigma Type II) was pressure injected into the roots of individual organs. We modified Mesulam's HRP protocol by directly transferring frozen sections from microtome knife to glass slides, and developing the reaction product on slides. This modification facilitates tissue processing without affecting the sensitivity or resolution of stain development. The majority of fibers from both papillae terminated on the dorsal nucleus; a small portion descended to the solitary nucleus at the XIIth nerve level. Termination sites of the two papillae differed within the dorsal nucleus. Terminals of basilar papilla fibers were restricted to the extreme dorsomedial area. The amphibian papilla termination site was more extensive, occupying discrete dorsal and ventral regions in the lateral two thirds of the nucleus. Terminstion sites of posterior vertical canal fibers were examined as a methodological control. They were limited to the ventral nucleus and lateral reticular zone. In addition, fibers from this organ ascended to the nucleus cerebelli and cerebellar granular layer and descended into dorsal gray at the XIIth nerve level. Since the basilar and amphibian papillae are sensitive to different frequency bands, their separate termination sites in the dorsal nucleus provides an anatomical basis for a rudimentary tonotopic organization. (Supported by a Biomedical Sciences Support Grant RR-07030 to the University of Illinois and a RIAS Study Grant from NSF.)

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

Still photography, time lapse motion pictures, and behavioral observations of a species of communal spiders, Mallos gregalis (M. g.) Simon, were used in examining spatial distribution, activity level and interactions under varied laboratory conditions. M. g. reside together, communally catching prey and feeding, but exhibiting no intraspecific aggression, in a web which may, in natural settings, house several thousand animals. Though other animals (e.g. bees, ants) also live in large groups, M. g. (apparently morphologically isomorphous) do so without obvious behaviorally based castes, division of labor, or other systematic organization. Moreover, the relative lack of interdependence of individual animals has been indicated by naturalistic observations of M. g. in a large terrarium in which separate colonies ranging in population from 2 to 70 have successfully coexisted for months. Measures of animal distribution (inter-animal distances) were used as an index of social organization in artificially created M. g. colonies varying in group size and density. These measures showed variability in distribution approaching randomness among colonies with differing numbers and density, within groups of similar colonies, and within individual colonies. The effects of external factors on group behavior were examined by analyzing films of colonies with and without water present under three conditions: heavy fed, light fed, or dextroamphetamine treated. The presence of water generally increased activity and the number of interactions across conditions. Amphetamine treated groups exhibited relatively more activity and interactions than did heavy fed groups, with light fed colonies being intermediate. These differences, especially the amphetamine related behavioral changes, suggest further experimentation with other drugs in this social context as an extension of studies of psychotropic drug effects in solitary spiders. (This research was supported by National Science Foundation grant BNS 74 09915 to Peter N. Witt).

451 SPINAL AFFERENTS TO THE DORSAL COLUMN NUCLEI IN A GARTER SNAKE. Virgil L. Jacobs and Raymond F. Sis*. Dept. Vet. Anat., Coll. Vet. Med., Texas A&M Univ., Coll. Sta., TX 77843.

An investigation was made of the ascending spinal degeneration to the dorsal column nuclei (DCN) of a non-limbed reptile. Garter snakes (Thamnophis siritalis) received spinal cord hemisections at levels ranging from segment 2 to 31 and were maintained postoperatively for 11 to 28 days. Following perfusion and fixation the brains and spinal cords were processed for degenerated axons according to the Nauta silver methods.

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

The dorsal column nuclei (DCN) consist of a thin lamina of cells medially contiguous with a relatively large population of cells laterally. The ventrolateral nuclear portion is wedge shaped and bordered laterally by tractus and nucleus descendens trigemini. The medial boundary at caudal levels is formed by nucleus and tractus solitarius and more rostrally by nucleus descendens vestibuli. A lesion at segment 2 shows dense degeneration throughout the DCN. An overall reduced preterminal degeneration in the DCN follows a segment 13 lesion that is related mainly to the caudal and medial regions of the DCN. Only a couple of preterminals are present in the medialmost cells after a lesion at segment 31. Based on the somatotopic projection of DC fibers to the DCN it appears that these nuclei have homologous features to nucleus gracilis and cuneatus of mammals. Other preterminal degeneration from the DC passes to nucleus descendens vestibuli, nucleus commissura infima and area postrema.

452 THE COURSE OF THE DORSAL LATERAL OLFACTORY TRACT AS AN INDICATOR OF DICHOTOMY IN THE PHYLOGENY OF PLACENTAL MAMMALS. John Irwin Johnson, Robert C. Switzer III, and John A. W. Kirsch*. Neuroscience Program and Zoology, Biophysics and Psychology Depts., Michigan State Univ., E. Lansing MI 48824; Lab. of Brain, Evolution and Behavior, NIMH, Bethesda MD 20014; and Dept. of Biology and Peabody Museum of Natural History, Yale Univ., New Haven CT 06520.

A neuroanatomical feature provides evidence which divides the placental orders of mammals into two major groups, thereby implying common ancestry to each set, and providing a basis for supraordinal grouping which has heretofore been difficult and controversial due to discontinuities in the paleontological record. This dichotomy is concluded from the alternate trajectories taken by the dorsal component of the lateral olfactory tract (LOT) at the level of the accessory olfactory formation (AOF). Our examination of 171 specimens representing 121 species of 15 orders shows that the dorsal LOT passes THROUGH the AOF (separating the large perikarya from the internal granule cells) in rodents, bat (Desmodus), insectivores, colugo, primates, tree shrew and elephant shrew. These orders are in the supraordinal cohorts Unguiculata and Glires in Simpson's 1945 classification, and would be derived from Leptictid-like insectivores in McKenna's 1969 scheme. In the other orders of placentals, Simpson's cohort Ferungulata and derived from Paleoryctid-like insectivores in McKenna's scheme, the carnivores, perissodactyls, hyrax and most artiodactyls, the dorsal LOT passes UNDER the AOF. Marsupials and monotremes exhibit the UNDER condition which leads us to conclude that this is the primitive or plesiomorphic trait and the THROUGH state is the derived or apomorphic trait. These observations indicate the role that developmental heterochrony can play in the evolutionary process. In the primitive state the LOT axons pass UNDER the AOF after the granule cells have migrated into place. In the "new" derived state, the LOT axons pass THROUGH the AOF before the granule cells have completed migration. Both routes are found in edentates and lagomorphs (e.g. of the anteaters Tamandua shows THROUGH and Myrmecophaga shows UNDER), suggesting that these groups may represent intermediate stages wherein the arrival of LOT axons and granule cells arrive nearly simultaneously and minute heterochronic variations switch the adult condition to one or the other trajectories. (Specimens were examined in the collections of the Univ. of Wisconsin Dept. of Neurophysiol. courtesy of W. I. Welker and in that of Georgetown Univ. courtesy of A. Butler and O. Solnitzky, in addition to our own collections.)

453 THE CORTICAL FIELD OF ORIGIN OF THE ANTERIOR COMMISSURE OF THE RHESUS MONKEY. Marc L. Jouandet and Michael S. Gazzaniga. Div. of Cognitive Neuroscience, Dept. of Neurology, Cornell Univ. Med. Col., NY, NY 10021.

The full extent of the cortical field of origin (FO) of the anterior commissure (AC) of the rhesus monkey was mapped by horse radish peroxidase (HRP) histochemistry. Following complete callosal commissurotomies and an eight month survival, two monkeys underwent a second operation involving the massive unilateral injection of HRP into the entire left temporal lobe. Since the AC was the only neocortical fiber system ramifying to the contralateral hemisphere following callosotomy, only the cells giving rise to the AC were labeled in the uninjected hemisphere. Cytoarchetecturally striking pyramidal cells were found whose proximal axons, apical, and basilar dendrites were clearly apparent. The FO extended from the temporal pole to the occipito-temporal sulcus and from the inferior depths of the insula to the parahippocampal gyrus. The FO was not entirely continuous for cells sometimes appeared in clumps separated by quiet zones; more often, however, entire layers were active within certain gyri. The density of pyramidal cells was invariably greatest in the lateral portions of the superior and inferotemporal gyri. In the superior temporal gyrus, pyramidal cells were widely scattered within layer III; in the inferotemporal gyrus, they were restricted to a tight laminar organization, 4 to 6 cells deep, within layer III. The presence of these cells in the insula, the depths of the superior temporal sulcus, and the parahippocampal gyrus appeared to vary with rostro-caudal location. Proceeding caudally, the FO moves progressively out of the inferior half of the insular cortex and down the lateral surface of the superior temporal gyrus, and moves inferiorly and medially into the inferotemporal and parahippocampal gyri. The FO appears to be much more extensive than the terminal projection field of the AC as delineated by previous investigators in rhesus (Zeki, Journal of Comparative Neurology, 148, 1973) and squirrel (Pandya et al., Brain Research, 15, 1973) monkeys. These investigators found the terminal projection field limited to the anterior temporal cortex. If the AC, in actuality, projects in a truly commissural homotopic fashion, then the previous delineations are clear underestimations of the terminal field's true extent. It may be, however, that the extensive FO may project through the AC to a highly compact terminal zone in the contralateral hemisphere. This heterotopic organization would be consistent with Van Alphen's (Acta Anat (Basel), 57, 1969) work on the AC of the rabbit. Aided by USPHS Grant No. NS-15053 and the McKnight Foundation.

454 TRACING THE COMPONENTS OF THE LINGUAL BRANCH OF THE GLOSSOPHARYNGEAL NERVE IN THE FROG BY ANTEROGRADE AND RETROGRADE TRANSPORT OF HORSERADISH PEROXIDASE. F.A. Kutyna and P. Conte*. Dept. of Physiology; Dept. of Anatomy, Uniformed Services University, School of Medicine, Bethesda, MD 20014.

The lingual branch of the IX cranial nerve (glossopharyngeal) of the frog carries in addition to afferent sensory fibers an efferent sympathetic (Chernetski, J. Neurophysiol. 27:493, 1964) as well as an efferent cholinergic (Morimoto and Sato, Proc. IUPS, XIII: Abs. 1563, 1977) component. Electrical stimulation of the first sympathetic ganglion results in enhanced sensitivity of gustatory afferents to chemical stimulation while electrical activation of the cholinergic component results in a depression of IX nerve chemosensory fiber discharge.

Horseradish peroxidase (HRP) was either injected into the crushed IX nerve or applied as a dry powder to both peripheral and central cut ends of the nerve. HRP was transported out to the tongue as well as toward the central nervous system.

The majority of cell bodies accumulating HRP were found in the glossopharyngeal component of the pneumogastric ganglion (g. jugulare) and constitute the sensory component of the IX nerve. A smaller cell type containing HRP was found adjacent to the glossopharyngeal component at the entrance to the ganglion of the sympathetic connective from the first sympathetic ganglion. Very rarely was a cell containing HRP found in the first sympathetic ganglion itself. Electrical stimulation of the IX nerve could evoke ganglionic potentials in the pneumogastric but never in the 1st sympathetic ganglion. This suggests that the IX nerve sympathetic postganglionic fibers originate for the most part outside the 1st sympathetic ganglion and near or adjacent to the cell bodies of the sensory fibers in the pneumogastric g. HRP was never found in cell bodies of the vagal (X) component of the pneumogastric g.

Rarely a motor cell body containing HRP was found in the nucleus of IX. These cells were smaller than their neighbors and of teardrop shape. The axonal processes were seen to travel in the direction of the sensory root of IX suggesting that these may be preganglionic visceral efferents and probably cholinergic. These anatomical findings describe in the frog (Rana pipiens pipiens) the IX nerve with sympathetic, cholinergic and sensory components in agreement with one interpretation of current neurophysiological data.

455 LIGHT ADAPTATION IN THE CRAYFISH EYE INDUCED BY SUSTAINED PHOTIC STIMULATION OF THE HETEROLATERAL EYESTALK WITHOUT RETINAL PHOTORECEPTORS. J. Larriva-Sahd*, J. Cibrian-Tovar*, D.E. García Díaz* & B. Barrera-Mera. (SPON: G. Massieu).
Div. Invest., Deptos. de Histología y Fisiología, Fac. Med. U.N.A.M. y Colegio de Posgraduados, Chapingo, México.

Characterization of extraretinal photosensitivity at several levels of crayfish nervous system (NS) has been advanced by means of electrophysiological (Prosser, 1934; Kennedy, 1958) and behavioral methods (Welsh, 1934; Harris & Stark, 1972). Bilateral influences of extraretinal nature involved in such photosensitivity were studied in these animals in which light adaptation of the retinal shielding pigments, was recorded as a reduction of the eye glow area (EGA). Adult crayfishes without retinal photoreceptors of either eye and with protocerebrum only in which caudal influences of the NS upon the visual system are suppressed were used.

Fig. 1

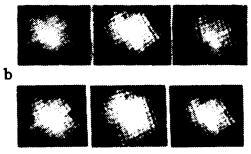
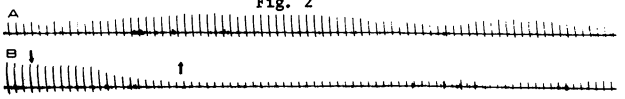


Figure 1 illustrates this changes few minutes after sustained photic stimulation (SuPS) of the heterolateral eyestalk, before (1a) and after (1b) the complete removal of its retina and lamina ganglionaris. Spontaneous variations of the electroretinogram (ERG) amplitude (Fig. 2A), as well as a ERG diminution (15-25 min) after a 60 min, SuPS (↓↑ 2B) were recorded in these conditions. We believe that the first result is probably due to the close relationship of the neuroendocrine system of both eyestalks involved in the control of release of hormon (es) which modulate the retinal sensitivity. On the other hand ERG diminution after SuPS seems to correspond to light adaptation and is probably mediated by extraretinal photosensitivity at the eyestalk level. The histological examination of the eyestalk without retina showed neurosecretory, pigmentary, and osmiophilic cells. The latter with ovoid granules (~ 1.5 µm).

Fig. 2



We postulate that the cells with osmiophilic ovoid granules which probably correspond to lipochondria (Krauhs et al. Biochem. Biophys. Acta, 471, 25, 1977), are involved in the extraretinal bilateral light adaptation of the visual system of these animals.

456 ELECTRON MICROSCOPIC DEMONSTRATION OF CAUDAL AFFERENTS TO THE RAT INTERPEDUNCULAR NUCLEUS. Nicholas J. Lenn and Viviana Wong*. Dept. Neurol. and Ped., Carnegie Labs of Embryol., U. California, Davis CA 95616.

Analysis of the synaptology of the rat interpeduncular nucleus is of interest, amongst other reasons, because of the morphological and biochemical evidence for sprouting of afferents produced by habenular nucleus lesions. Only the synapses arising from the habenulointerpeduncular afferent axons have been identified thus far.

The afferents from the dorsal and ventral tegmental nuclei, which form a tegmentopeduncular tract, have been studied by the electron microscopic degeneration method in adult rats. Electrolytic lesions were placed stereotaxically so as to destroy the tegmentopeduncular tract, as well as various portions of the dorsal or ventral tegmental nuclei. The degeneration of endings in the interpeduncular nucleus was the same in each case. This consisted of: 1.) occasional neurofilamentous type degeneration of axons forming axodendritic synapses at three days' survival; 2.) dense type degeneration of axons contacting either small (0.4-0.8 micron) or large (1.4-2.0 micron) dendrites with synaptic contacts which were symmetrical to moderatedly asymmetrical; 3.) occasional axosomatic synapses also showing dense type degeneration. The dense type degeneration, which was similar at 3 and 4 days' survival, was apparently greatest in the caudal portion of the interpeduncular nucleus.

Equally important were observations of structures which were unaffected by the lesions. These included the S and crest synapses. Some somatic synapses, and apparently normal numbers of flattened vesicle synapses were also seen to be normal.

The present observations clarify at the fine structural level the afferents from the tegmentum caudal to the interpeduncular nucleus. A part of those synapses previously classified as S or somatic synapses, and possibly as F synapses are of this origin. It can be hypothesized that these afferents are amongst those which undergo sprouting caused by habenular lesions. This may involve the formation of heterologous crest synapses, the increase in number of somatic synapses, or both. This hypothesis is amenable to testing in double and triple lesion experiments.

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457 IDENTIFICATION OF CATECHOLAMINERGIC PROJECTIONS TO AREA X IN THE ZEBRA FINCH (POEPHILA GUTTATA). James W. Lewis, Susan M. Ryan, Larry L. Butcher, Arthur P. Arnold. Dept. Psych. and Brain Res. Inst., UCLA, Los Angeles, CA 90024.

In some passerine birds, song is a behavior which is typical of males but not females, and is controlled by a group of discrete sexually dimorphic brain regions. Area X of the lobus parolfactorius (LPO) is a region which is sexually dimorphic and receives afferents from the caudal nucleus of the hyperstriatum ventrale (HVc), an area known to be involved in the control of song (Nottebohm et al, 1976; Nottebohm and Arnold, 1976). The possible role of Area X in the control of song remains to be determined.

Our injections of horseradish peroxidase (HRP) into Area X confirm the projection from HVc to Area X. Additionally, HRP injections centered in Area X indicate possible afferent projections from midbrain nuclei, either nucleus tegmenti pedunculopontinus (TPc), the area ventralis of Tsai (AVT), or both.

Using the specific glyoxylic acid histofluorescence technique of de la Torre and Sturgeon (1976) we have demonstrated dense fluorescence, due to catecholamine containing nerve terminals and axons, in Area X, LPO, and paleostriatum augmentatum (PA). Catecholamine containing neuronal cell bodies are found in TPC and AVT. Electrolytic lesions of TPc eliminate catecholamine fluorescence in PA, but not in Area X or LPO, when examined 7 days later. A lesion of AVT eliminates fluorescence in Area X and LPO but not in PA. Lesions placed immediately anterior to AVT eliminate fluorescence in LPO, Area X, and PA, presumably by interrupting the fibers to these regions from both AVT and TPc.

The HRP and histofluorescence results, when taken together, suggest catecholaminergic projections from AVT to Area X and LPO, and from TPc to PA. The projection to Area X is sexually dimorphic. In the male, terminal fluorescence in Area X is clearly more intense than that in the surrounding LPO; in the female this distinction is not apparent.

The avian paleostriatal complex, composed of LPO, PA, and paleostriatum primitivum, has previously been suggested to be homologous to the mammalian basal ganglia (Karten and Dubbeldam, 1973). Our results support and extend this homology. Anatomical location and the catecholaminergic projection from AVT suggest Area X - LPO to be the avian homolog of the mammalian nucleus accumbens and/or olfactory tubercle. Identification of sources of afferents to Area X and the neurotransmitters involved will allow further physiological interventions and pharmacological manipulations to assess the functional role of Area X within the paleostriatal complex and its possible significance in the control of song or related behaviors.

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458 NEAR-FIELD VISUAL ACUITY IN PIGEONS FOLLOWING LESIONS OF THALAMIC VISUAL NUCLEI. Kathleen A. Macko* and William Hodos (SPON: R. H. Wurtz). University of Maryland, College Park, MD 20742.

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

The present study was designed to determine whether this distinction in functional organization can be applied to the avian visual system. Visual acuity determinations were made for 10 pigeons trained to discriminate high contrast square-wave gratings of successively higher spatial frequencies from a blank stimulus of equal average luminance. The gratings and blanks were presented according to the method of constant stimuli. Video-taped motion pictures of the key-pecking response provided a measure of the eye to stimulus distance. The preoperative results indicated that the mean near-field visual acuity of the 10 pigeons was 2.3' (range 1.6 - 3.6') under conditions of photopic adaptation with the stimulus luminance at 70 cd/m^2. When performance was stable, bilateral electrolytic lesions were placed in 1) the OPT complex (thalamic component of the thalamofugal pathway), 2) nucleus rotundus (thalamic component of the tectofugal pathway) and 3) the OPT complex plus nucleus rotundus. The postoperative data indicated that lesions confined to the OPT complex did not impair resolution threshold. Lesions confined to nucleus rotundus produced permanent threshold elevations. When destruction of OPT was combined with nucleus rotundus the deficit was more severe. The results indicate that in contrast to the mammalian visual system, the integrity of the tectofugal pathway of the pigeon is required for normal performance of spatial discrimination near acuity threshold and demonstrate that wide receptive field neurons are capable of processing detailed spatial information.

459 HISTOCHEMICAL AND ULTRASTRUCTURAL STUDY OF THE PRIMATE CLITORIS. JoAnn McConnell. Dept. Neurobiol. and Anat., U.T. Med. Sch., Houston, TX, 77025.

As with many other genitourinary organs, the structure of the clitoris has not been studied in any detail. The clitoris is formed by two corpora cavernosa which arise from two crura lying on the ischiopubic ramus. Although there is no corpus spongiosum the clitoris is considered to be the "homologue" of the penis in the male. The extent of this similarity, however, is difficult to ascertain since histological data is not available in the current literature.

Tissue for this study was obtained from female cynomolgus monkeys and from patients undergoing a partial clitorodectomy for hormone-related hypertrophy of the clitoris. Each sample was divided into two parts, the first being frozen immediately for use in various light microscopic analyses, and the other being fixed immediately by immersion in glutaraldehyde for EM analysis. Light microscopic techniques utilized included hematoxylin and eosin, phosphotungstic acid hematoxylin, acetylcholinesterase (AChE) and glyoxylic acid (GA) histofluorescence. In addition to routine EM preparations, Wood's (1963) glutaraldehyde-dichromate method was employed for specific localization of adrenergic vesicles.

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

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

The above data suggest that the morphology of the clitoris is significantly different from that of the penis and thus the two are not truly homologous in the adult primate.

Partially supported by USPHS Grant 1 F32 NS 06179-01.

460 IDENTIFICATION OF EIGHTH NERVE EFFERENT CELLS IN THE BOWFIN, AMIA CALVA. Catherine A. McCormick and Mark R. Braford, Jr. Department of Anatomy, Georgetown University, Schools of Medicine and Dentistry, Washington, D.C. 20007

Efferent cells of the anterior and posterior rami of the eighth (octavius) nerve were visualized by means of retrograde labelling. In a given animal, one of the two rami was injected with HRP at the level of its ganglion. Labelled cells were seen ipsilaterally after a survival time of four days at 27 C. These efferent cells are found near the branchial motor cell column, with the majority of cells in a periventricular position at or just medial to the sulcus limitans. These cells form a diffuse population, extending from a level just caudal to the entrance of the posterior octavius ramus to a region between the entrance of the anterior vagal rootlets and the glossopharyngeal nerve. The efferent cells are fusiform or multipolar in morphology, with diameters of approximately 50-80 μ and large dendritic extents. The axons of some of these cells emerge from the lateral aspect of the cell and course rostrally toward the entering octavius rami in the descending octavius tract. It also appears that some of the axons course toward the ventral border of the medulla before turning laterally to enter this tract.

Preliminary results on the location of the efferent cells of the lateral line system suggest that these cells occupy a region similar to that of the octavius efferent cells. Supported by NSF Grant BNS 78-22411

461 ANTERIOR AND POSTERIOR THALAMIC AFFERENTS IN THE BULLFROG, Rana catesbeiana. Timothy J. Neary and Walter Wilczynski. Anat. Dept., Creighton Univ., Omaha, NE 68178 and Sect. Neurobiol. Behav., Cornell Univ., Ithaca, NY 14853.

50-150 nl of HRP was injected into either the anterior or posterior thalamus of 14 bullfrogs. Because of diffusion from the injection sites, all injection zones encompassed several cytoarchitectonic fields. Anterior thalamic injections were centered in the anterior nucleus of the dorsal thalamus but spread into the adjacent habenular complex was present in every case. In most of these cases there was further spread into the ventromedial nucleus and into the ventrolateral complex (nucleus of Bellonci, dorsal and ventral ventrolateral nuclei, and superficial ventral nucleus). Structures consistently labelled after anterior thalamic injections include the pre- and supraoptic areas, posterior entopeduncular nucleus, ventral hypothalamus, posterior thalamic nucleus, pretectal grey, and posteroventral tegmental nucleus. In cases where larger amounts of HRP were injected, occasional labelled cells were seen in the medial amygdala, posterior tuberculum, dorsal hypothalamus, anteroventral tegmental nucleus, and a small group of reticular formation neurons medially adjacent to the motor nucleus of V. Numerous cells were also seen in the perisolitary band (Neary and Wilczynski (1977) Brain Res. 138:529). Nearly all labelled cells were ipsilateral to the injection sites. However, most of the labelled cells in the perisolitary band and many of the labelled cells in the pretectal grey were contralateral to the injection sites. Posterior thalamic injections were centered in the posterior thalamic nucleus, with spread into the pretectal grey, posterodorsal nucleus and posterodorsal division of the lateral nucleus. Following these injections labelled cells were seen in the anterior entopeduncular nucleus, pre- and supraoptic areas, ventral hypothalamus, nucleus of Bellonci, dorsal ventrolateral nucleus, optic tectum, and antero- and posteroventral tegmental nuclei. Nearly all labelled cells after posterior thalamic injections were ipsilateral to the injection sites. However, many labelled cells were seen in the contralateral posterior thalamic nucleus and pretectal grey. Anterogradely-filled fibers could be followed from the posterior thalamic injection sites into the dorsal ventrolateral and anterior nuclei.

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462 INTERVAL CODING OF TEMPERATURE BY PREOPTIC NEURONS IN SUNFISH. D.O. Nelson* and C. Ladd Prosser. Dept. of Physiology and Biophysics, Univ. of Illinois, IL 61801.

The frequency of discharge of neurons in the preoptic region of many lower vertebrates and mammals is highly sensitive to changes in peripheral and central temperature. These neurons are implicated in thermoregulation. Microstructural neural patterning may be involved in temperature sensing and/or integration necessary for temperature regulation in mammals. Extracellular recordings were made from single cells in medial and lateral preoptic regions of green sunfish, (Lepomis cyanellus), while altering forebrain and skin temperatures. Warm sensitive (22%) and cold sensitive (6%) cells were identified; some of these responded to forebrain temperature changes, some to skin temperature changes and others to both forebrain and skin temperature alteration. In addition, some temperature-insensitive (72%) cells were found. However, examinations of interspike interval patterns of these grossly insensitive cells showed complex alterations from unimodal firing patterns to bimodal patterns. These transitions, which consisted of changes from a continuous random firing pattern to a bursting pattern, occurred at different specific temperatures for different units; in one 25°C acclimated fish, neurons were found which showed transitions at 17.5, 23.0, 25.2 and 28.7°C. The temperatures of transition of different cells appear to comprise a continuum in all fish examined. Such a system of neurons may provide a coded temperature scale which may be used in behavioral thermoregulation. It is suggested that microstructural coding of temperature may be a property found in lower vertebrates as well as in mammals. Investigation of peripheral temperature alterations on firing patterns of preoptic neurons is in progress.

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463 COMPARATIVE ORGANIZATION OF REPTILIAN RETICULAR FORMATION. Donald B. Newman* and Antonia Gebert* (SPON: M. B. Carpenter). Dept. of Anatomy, USUHS, Bethesda, MD. 20014.

The brains of snakes of the genera Crotalus, Agkistrodon, and Natrix, lizards of the genera Tupinambis, Basiliscus, Dipsosaurus, and Gecko, turtles of the genera Pseudemys, Chrysemys, and Terrapene, and the crocodilian Caiman were processed using Golgi and Nissl techniqes.

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

In lizards, snakes and crocodilians, RI is subdivided into dorsal vs. ventral portions (RID vs. RIV). Turtles possess only the RID field. In all the reptiles studied, RM, RS, the MDL-MVL complex can be identified in the metencephalon.

The raphe nuclei varied considerably. In snakes, only medium-sized neurons are present in RaI, whereas RaI in lizards and turtles contains large cells, as does RaM in turtles. RaI in crocodilians contains giant cells.

Small reticular neurons (<30μ) have fusiform or triangular somas and bear two to three sparsely-branching dendrites which average 183μ in length. These dendrites exhibit no preferred orientation.

Large reticular neurons (>30μ) have fusiform or polygonal somas which bear three to six sparsely-branching dendrites which average 234μ in length. Dendrites of large neurons in RID show no particular orientation. Dendrites of large neurons in RIV course horizontally. Most dendrites of large neurons in RM course ventrally. RM neurons bear dendritic aborizations which span the dorsoventral width of the brain stem, intersecting the medial longitudinal fasciculus dorsally and touching the pial surface ventrally. Some large RM neurons bear dendrites which cross the midline and enter the contralateral RM field.

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

The reptilian reticular formation resembles that of mammals in that it consists of distinct sub-nuclei or fields which contain sparsely-branching, "isodendritic" neurons. An unexpected finding was the wide variation observed in the organization of the raphe complex in the various reptilian groups.

464 SOME CONNECTIONS OF THE SKATE DORSAL AND MEDIAL PALLIA. R. G. Northcutt and J. C. Wathey. Div. Biol. Sci's., UM, Ann Arbor, MI 48109 and Dept. Neurosci's., UCSD, La Jolla, CA 92093

Some pallial connections in adult thornback skates (Platyrhinoides triseriata) were revealed with HRP histochemistry (DIA or TMB substrates). Animals survived 8-14 days at 16°C after unilateral pallial injections of 200-400 nl of Sigma VI HRP (20-40%). Retrograde and anterograde transport were apparent. HRP-positive neurons were present in both an ipsilateral and contralateral rostral thalamic nucleus following pallial injections confined to the telencephalic central nucleus and dorsal pallium lying immediately dorsal to the central nucleus. Anterograde fibers could be followed over the caudal pole of the hemisphere where they turned caudally to terminate in the ipsilateral rostral thalamic nucleus that projects to the caudal telencephalic roof. Fibers were also followed from the roof ventrally and caudally where they decussate dorsal to the optic chiasm and terminate in the contralateral rostral thalamic nucleus. The rostral thalamic nucleus projecting to the caudal telencephalic roof has been identified as the lateral geniculate in sharks such as Ginglymostoma (Schroeder & Ebbesson, BBE; 1974). In sharks the LGN projection appears totally crossed, as are the primary optic projections. Skates, however, possess ipsilateral as well as contralateral retinothalamic projections (Northcutt & Boord, unpublished observations) and this difference may account for the bilateral projection of the rostral retinorecipient thalamic nucleus in skates. Pallial injections with greater rostro-caudal extent reveal HRP-positive neurons in an ipsilateral cell group immediately dorsal to area superficialis basalis, and in the septal nuclei and cells of the inferior lobe. Larger injections also revealed two additional cell groups more caudal in the thalamus. The more dorsal group probably corresponds to the central thalamic nucleus of Ebbesson; the second nucleus is more ventromedial and has not been previously named. These larger pallial injections appear to include parts of the medial pallium and reveal pallial efferents comparable to those reported by Ebbesson in sharks (CBP; 1972). Telencephalic efferents terminate bilaterally on the septal nuclei as well as exit the caudal hemisphere where part of the descending fibers decussate in the habenular commissure and supraoptic decussation. Fibers appear to terminate bilaterally in the habenular nuclei, dorsal thalamus, optic tecta, and tegmentum. Descending fibers were also traced to the contralateral inferior lobe where extensive terminal arborizations occur.

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465 IDENTIFICATION OF HOMOLOGOUS MUSCLES AND MOTONEURONS IN 2 SPECIES OF SANDCRAB BELONGING TO DIFFERENT TAXONOMIC FAMILIES SUGGESTS ANCESTRY OF SWIMMING BY UROPOD BEATING. Dorothy H. Paul. Hopkins Marine Station, Pacific Grove, CA 93950.

Uropod beating during swimming in Emerita analoga (Hippidae) is attributed to a central neural rhythm generator since the isolated abdominal nerve cord generates power-stroke (PS) motoneuron bursts which are appropriately phased within return-stroke (RS) periods (Paul, 1979, J.Neurobiol.10). But the most prominent feature of the motor program is an intricate pattern of rhythmic activity in motoneurons which innervate the ventromedial muscle (VM) and not bursting of PS and RS motoneurons. VM is a small muscle, a PS synergist and pronator of the uropod. An explanation of the unexpected prominance of VM neurons in the central motor program has emerged from examination of the neuromuscular organization subserving the tailfan of another sandcrab, Blepharipoda occidentalis which belongs to a different family (Albuneidae) and does not swim. Although their uropods have the characteristic sandcrab orientation, the arrangement of muscles in the telson of B.occidentalis resembles that found in decapods with conventional tailfans (e.g., crayfish) as closely as it does the highly specialized anatomy of E.analoga. The probable homologues of all 6 telson muscles of E.analoga have been identified in B.occidentalis by relative position, innervation and, in some cases, soma position and central morphology of motoneurons. Three pairs of homologous muscles in particular reveal striking morphological and function-differences (Table).

muscle*	% of all telson muscles (wet wt.) E.analoga	B.occidentalis	action on uropod E.analoga	B.occidentalis
RS	36	6	retract	elevate
PS	50	24	protract	depress
VM	4	28	protract & promote	none

*terminology as for Emerita

The larger mass of RS and PS muscles in E.analoga attests to the larger angular excursions of the uropods in this species. But the most interesting discovery is that the VM homologue in B. occidentalis is not a muscle of the appendage at all. It is a telson flexor and, therefore, functionally a part of the abdominal flexor system. Thus VM in E.analoga appears to be, historically, an axial muscle. The implication is that the intricate pattern of rhythmic bursting of VM motoneurons in E.analoga, the most prominent part of the central motor program, reflects activity of a neuronal mechanism which may have subserved rhythmic abdominal movements in an ancestral decapod.

466 THE PALEOSTRIATAL COMPLEX IN TURTLES. Anton Reiner. Dept Psychiatry and Behavioral Sciences, Health Sciences Center, SUNY, Stony Brook, N.Y. 11794.

The present study examined the histochemistry and efferent projections of the ventral portion of the reptilian lateral telencephalic wall (paleostriatal complex, PC), the presumed equivalent of the mammalian basal ganglia. Using catecholamine (CA) histoflourescence, acetylcholinesterase (AChE) histochemistry substance P immunocytochemistry, three subdivisions of the PC were recognized in the turtle (Chrysemys scripta). 1) A small-celled zone (paleostriatum augmentatum, PA) occupies most of the basolateral telencephalic wall, extending from the olfactory tubercle to the amygdaloid region. The neuropil of PA is rich in AChE, weakly positive for substance P and contains a fine network of CA terminals similar to that observed in the caudoputamen of mammals. 2) A medial small-celled zone (area d, Riss, Halpern and Scalia, BB and E, 1969), coextensive with PA along the rostrocaudal axis, is interposed between PA and the lateral ventricle. The neuropil of area d is also rich in AChE, but differs from that of PA in that it is more intensely positive for substance P and contains a network of thick CA fibers and terminals. 3) A group of large AChE-rich neurons (globus pallidus, GP) is interspersed with the fibers of the lateral forebrain bundle through intermediate PC levels. The neuropil of GP is rich in substance P, but nearly devoid of CA and AChE.

The efferent projections of the PC were examined using autoradiographic tracing techniques. The most prominent projection of the PC was to a tegmental cell field containing numerous CA neurons. Other targets of the PC included several subthalamic nuclei, the dorsal nucleus of the posterior commissure and parabrachial isthmic regions. Injections of horseradish peroxidase into the tegmental CA cell field indicated that the projection to this region from PC arises from GP and caudal area d. Only a few PA neurons were labeled by such injections. Other research (Parent, Br.Res., 1976) has shown that PA receives an extensive input from the tegmental CA cell field.

The projection of the PC upon the tegmentum is reminiscent of the projections of globus pallidus and nucleus accumbens upon the tegmentum in mammals. The present connectionistic and histochemical data are consistent with the hypothesis PA, GP and area d in turtles are similar to caudoputamen, globus pallidus and nucleus accumbens, respectively, in mammals. I thank Harvey Karten for his support during this research. Supported by USPHS 1 F NS 05682001.

467 AFFERENT AND EFFERENT CONNECTIONS OF THE BULLFROG MEDIAL PALLIUM. Mark C. Ronan* and R. Glenn Northcutt (SPON: M. B. Pritz). Neurosciences Program and Division of Biological Sciences, University of Michigan, Ann Arbor, MI 48109.

Medial pallial connections in adult bullfrogs (Rana catesbeiana) were investigated with HRP histochemistry (DIA or TMB substrates), autoradiography, and Fink-Heimer procedures. Animals were allowed to survive 3-6 days at 23°C after surgery or injection of the tracer. Injections of 100-150 nl of 20-40% HRP (Sigma VI) into the medial pallium revealed both retrograde and anterograde transport. Checks on the medial pallial efferents revealed by HRP were provided by autoradiographic analysis after ^{3}H-proline injections or lesions of dorsal, lateral, and medial pallia processed by Fink-Heimer techniques. The medial pallium receives ipsilateral projections from the dorsal and lateral pallia, lateral and medial septal nuclei, olfactory tubercle, pars medialis of the amygdala, bed nucleus of the pallial commissure, anterior thalamic nucleus, and the midbrain raphe nucleus. The medial pallium also receives contralateral projections from the medial pallium, medial septal nucleus, pars medialis of the amygdala, bed nucleus of the pallial commissure, and anterior thalamic nucleus. The efferent projections of the medial pallium consist of commissural connections with the contralateral medial pallium, and a projection to the contralateral bed nucleus of the pallial commissure, both via the pallial commissure. A precommissural fornix system passess to the ipsilateral lateral and medial septal nuclei, the olfactory tubercle, medial postolfactory eminence, internal granule layer of the olfactory bulb, pars lateralis and medialis of the amygdala, lateral pallium, and preoptic nucleus. Contralateral projections to the medial septal nucleus, olfactory tubercle, pars lateralis and medialis of the amygdala, and preoptic area decussate in the anterior commissure and travel in the contralateral medial forebrain bundle. A postcommissural fornix system consists of medial pallial efferents to the anterior thalamic nucleus and caudal hypothalamus. These postcommissural projections are bilateral and also appear to decussate in the anterior commissure. All telencephalic populations except the striatum, pars lateralis of the amygdala, olfactory bulb, and preoptic nucleus, converge on the medial pallium; the medial pallium forms reciprocal connections with most of these populations and constitutes one of the major efferent pathways of the telencephalon.

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468 EVIDENCE FOR CHOLINERGIC MECHANISMS IN BRAIN REGIONS RELATED TO BIRD SONG. Susan M. Ryan and Arthur P. Arnold. Dept. Psychol. and Brain Res. Inst., UCLA, Los Angeles, CA 90024.

Brain regions thought to be involved in the control of song in the zebra finch, Poephila guttata, were examined histochemically using the Karnovsky and Roots direct-coloring method for the detection of acetylcholinesterase (AChE). In order to control for the possible presence of nonspecific cholinesterase, two substrates, acetylthiocholine and butyrylthiocholine, and several enzyme inhibitors, tetraisopropyl pyrophosphoramide (iso-OMPA), BW284C51, and eserine sulfate, were reacted with the tissue. Manipulations with these compounds demonstrated that most of the reaction product with the substrate acetylthiocholine was AChE. In addition, some of the subjects were pretreated with diisopropylphosphofluoridate (DFP). DFP irreversibly inactivates AChE, which allowed observation of newly synthesized AChE in cell bodies only.

Several brain areas thought to be involved in vocal control in adult males and females contain AChE. These nuclei include Area X (or dorsolateral LPO), magnocellular nucleus of the anterior neostriatum (MAN), nucleus interface (NIF), caudal nucleus of the ventral hyperstriatum (HVc), intercollicular nucleus (ICo), robust nucleus of the archistriatum (RA), and tracheosyringeal portion of the hypoglossal nerve nucleus (nXIIts). In addition, several auditory nuclei, nucleus ovoidalis, dorsal portion of the lateral mesencephalic nucleus (MLd), and Field L also contain AChE. Of the vocal control nuclei, Area X, HVc, MAN, NIF, ICo, and nXIIts contain a denser reaction product than the surrounding tissue. All of the vocal control nuclei, except Area X, contain mostly AChE-synthesizing neuronal cell bodies; however, in Area X an extremely dense reaction product is present in the neuropil, suggesting possible cholinergic input.

Autoradiographic procedures were used to localize binding of tritiated quinuclidinyl benzilate (QNB), a specific muscarinic cholinergic antagonist, in the brain. In both males and females, QNB was localized in high concentrations in the neuropil of Area X (or dorsolateral LPO). This binding was displaced by preinjection with two specific muscarinic antagonists, atropine sulfate and scopolamine hydrobromide, but not by saline, suggesting that the binding is of limited capacity and specific to muscarinic cholinergic receptors. Taken together, the high concentrations of AChE and high level of specific binding of QNB in Area X strongly implicate cholinergic mechanisms in the function of Area X.

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469 STRUCTURAL CHARACTERIZATION OF THE ANTERIOR AND POSTERIOR PITUITARY OF THE WEST INDIAN MANATEE. Alan Schneyer, Robert Hinkley*, and Daniel Odell*. Department of Anatomy and The Rosenstiel School of Marine and Atmospheric Science, The University of Miami, Miami, Florida 33101.

The pituitary gland of the West Indian manatee, Trichechus manatus, a marine mammal, was examined by light and electron microscopy to determine the cell types present and organization at the ultrastructural level. For light microscopy, paraffin sections were stained by the acid fuchsin-analine blue method to distinguish acidophils, basophils, and chromophobes. The permanganate oxidation-aldehyde fuchsin method was used to demonstrate neurosecretory material in the neurohypophysis. For electron microscopy, tissue was fixed in glutaraldehyde in cacodylate buffer, post-fixed in osmium tetroxide, dehydrated, and embedded in Epon 812. Acidophils, basophils, and chromophobes occurred at relative frequencies of 34%, 22%, and 44%, respectively. Colloid-filled follicles surrounded by a layer of pale-staining cells were present in most sections. Ultrastructurally, at least six cell types, and possibly seven, could be distinguished on the basis of granule morphology. In descending granule size, cell type I contained granules averaging 630 nm in diameter, type II, 515 nm; type III, 410 nm; type IV, 320 nm; type V, 275 nm; type VI, 240 nm; and type VII, 180 nm.

Thin sections of neurohypophysis contain numerous profiles of neurosecretory cell processes and pituicytes. The pituicytes average 7-10 u in diameter, possess lengthy cell processes, and are characterized by one or more well-developed Golgi complexes, vesiculated endoplasmic reticulum, lysosomes, and few mitochondria. Bundles of filaments averaging 9-11 nm in diameter are present in pituicyte cell bodies and processes. The axoplasm of fibers comprising the hypothalamo-hypophysial tract contain numerous filaments, few microtubules, clear vesicles measuring 35-50 nm in diameter, and numerous electron-opaque secretion granules averaging 210 nm in diameter. No Herring bodies were seen in paraffin or Epon sections. Many neurosecretory processes terminate near non-fenestrated capillaries which provide the neurohypophysis of the manatee with a rich vascular supply.

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470 THE EFFECTS OF UNILATERAL LESIONS AND IPSILATERAL OR CONTRALATERAL EYE CLOSURE ON THE SOCIAL BEHAVIOR AND ACTIVITY LEVELS OF THE WESTERN FENCE LIZARD. Robert S. Tarr. Physiol. Dept., Chicago College of Osteopathic Medicine, 1122 E. 53rd Street, Chicago, Illinois 60615.

Territorial defense behavior was studied in Sceloporus occidentalis before and after unilateral telencephalic lesions noting the effects after closing either the ipslilateral or contralateral eye. An adult male animal was placed in a large (100 sq. ft.) enclosed seminatural environment. The animal occupied the territory alone for 48 hours, then another adult male was introduced. Activity levels (number of times changing position and number of times changing location) and agonistic behavior (number of assertion displays and challenge displays) were recorded. The animals were removed and the resident was either sham lesioned, lesioned in the anterior DVR, lesioned in the paleostriatum (and nucleus accumbens) or lesioned in the amygdala. All lesions were unilateral. The resident's ipsilateral (to the lesion) or contralateral eye was sutured shut, he was reintroduced into the cage, and 48 hours later the observations were repeated. The animals were again removed and the eye was opened and the other eye sutured shut. Again, 48 hours later the observations were repeated. Animals showing any change from normal were observed for at least one additional observation period. The intruding lizard was the same animal for all the residents. Light, temperature and diurnal activity cycles were all held constant.

Sham lesioning and anterior DVR lesions did not result in a change in activity level or agonistic behavior through either eye. Certain small lesions in the paleostriatum and/or amygdala, resulted in a drop in activity and display behavior when the contralateral eye was open: challenge displays were entirely absent, assertion displays nearly absent, activity was reduced 50% or more and dominance in the territory was lost. These animals could still see and move however, as noted by their normal visual predatory behavior. When the ipsilateral eye was being used social behavior and activity was either normal or reduced but since challenge displays were always present the animal never lost dominance with this eye.

These observations confirm the role of the reptile telencephalon in arousal, attention and agonistic behavior and the functional effect of nearly complete crossing of optic fibers. They further suggest that in these animals the mechanism of arousal is heavily dependent on visually processed information related to social behavior.

471. DISCRIMINATION OF MIRROR-IMAGE SYMMETRICAL STIMULI AFTER LESIONS OF THE VISUAL SYSTEM IN PIGEONS. Susan R. B. Weiss* and William Hodos. Dept. Psych., Univ. of Maryland, College Park, MD 20742

Mammals have been reported to have greater difficulty discriminating between stimuli that are mirror-image (MI) symmetrical than stimuli without such symmetry. Pigeons were trained to perform a simultaneous discrimination task with three pairs of stimuli: laterally MI-symmetrical,  vs.  ; vertically MI-symmetrical,  vs  ; and unsymmetrical, non-MI,  vs  . In contrast to mammals, pigeons had no greater difficulty discriminating MI-symmetrical stimuli than non-MI stimuli.

Following training, bilateral stereotaxic lesions were made either in visual wulst, which is a telencephalic component of the thalamofugal visual pathway or ectostriatum, which is a telecephalic component of the tectofugal visual pathway. After surgery, both groups were retrained to the preoperative criterion. Visual wulst lesions resulted in minor, transient deficits on all three stimulu pairs. MI-symmetrical stimuli showed no greater impairment than non-MI stimuli. Lesions of ectostriatum resulted in moderate to severe impairment in the discrimination of all three stimulus pairs. All subjects with ectostriatum lesions exhibited a greater impairment in the discrimination of lateral MI-symmetrical stimuli than the vertical MI-symmetrical or unsymmetrical stimuli.

Intact mammals require more training to discriminate pairs of MI stimuli than any other type of stimulus pair differing only in spatial orientation. In contrast, intact pigeons learn to discriminate MI and non-MI stimuli at the same rate. Interruption of the tectofugal visual pathway in pigeons results in a differentially greater impairment in the discrimination of lateral MI stimuli than non-MI stimuli. The differences between birds and mammals in their ability to discriminate lateral MI stimuli may be related to differences between them in the organization of their hemispheric interconnections.

472 STRIATAL EFFERENTS IN THE BULLFROG, RANA CATESBEIANA. Walter Wilczynski and R. Glenn Northcutt. Sec. Neurobiol. and Behav., Cornell University, Ithaca, N.Y., 14853, and Div. Biol. Sci., University of Michigan, Ann Arbor, Michigan 48109.

In bullfrogs (Rana catesbeiana) efferents from the thalamic nuclei receiving tectal and toral projections terminate heavily in the striatum (Wilczynski and Northcutt, Anat. Rec., 193: 721, 1979). In order to determine routes by which the striatum could in turn influence midbrain sensory structures, we investigated striatal efferents in adult bullfrogs using autoradiographic and degeneration techniques, and the anterograde transport of horseradish peroxidase (HRP). Results from each technique were essentially identical. Except for the small ventral eminence of the lateral pallium, no pallial area receives a striatal input. The striatal efferents descend in the lateral forebrain bundle (LFB) through the anterior entopeduncular nucleus (AE) where a large fascicle leaves the LFB to cross in the anterior commissure and terminate in the contralateral AE and caudal half of the ventral striatum. A much smaller projection leaves at this level to enter the ipsilateral lateral amygdala. The remaining efferents continue caudal in the ipsilateral LFB, passing through the posterior entopeduncular nucleus. In the diencephalon, small projections to the ventrolateral (pars lateralis), ventromedial, and posterior tuberal nuclei were apparent. A much larger projection leaves the LFB in the pretectum to run dorsally through the lateral nucleus and lateral edge of the posterior nucleus. Striatal efferents continue caudally through the superficial tegmental nuclei (nucleus profundus mesencephali and superficial isthmal reticular nucleus) and into the ventral anterodorsal, lateral anteroventral, and rostral pole of the posterodorsal tegmental fields. A small superficial projection continues caudally to isthmal levels, but not beyond. Tegmental HRP injections reveal retrogradely filled cells in the dorsal and ventral striatum, nucleus accumbens, and AE, and pretectal HRP injections reveal filled cells in the caudal ventral striatum and AE, thus confirming striatal projections to these areas. Striatal HRP injections have failed to demonstrate the source of the commissural striatal connections.

Striatal influence reaches the midbrain roof through several disynaptic pathways: via AE, the pretectum, and the tegmentum, all of which project to the tectum and torus (Wilczynski, Neurosci. Abst., 4: 301, 1978; Wilczynski and Northcutt, J. Comp. Neur., 173: 219-230, 1977). Additional trisynaptic routes are present via AE and its pretectal and tegmental connections, which parallel the striatal pathways.

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473 WHAT IS A HOMOLOGY IN THE CENTRAL NERVOUS SYSTEM? GOLGI STUDY OF THE MEDIAL GENICULATE BODY OF THE OPOSSUM AND THE CAT. Jeffery A. Winer and D. Kent Morest. Department of Anatomy, University of Connecticut Health Center, Farmington, Connecticut 06032.

Concepts of homology in the central nervous system of different species are central to comparative neurology. All students of neuroscience rely on such concepts in relating the significance of their findings. Yet there are few critical evaluations of its definition and practical application. The present study explores the premise that the shapes of neurons and axons can be used to demonstrate homologous cell groups in the medial geniculate body of the opossum and cat. Morest (Anat.Rec., 1965, 151, 390) compared the structure and connections of these neurons with the Golgi and Nauta methods to derive homologous nuclei. We have confirmed and extended this work. In the ventral division, the thalamo-cortical neuron in both species has tufted dendrites which are arranged in parallel laminae. A smaller Golgi type II cell with a locally arborizing axon is also present. In the dorsal division, the opossum has two types of principal neurons, as does the cat, and there is close morphological correspondence between the species; in the cat, there are also two kinds of Golgi type II cells, while the opossum probably has only one type. In the medial division, both species have numerous (as many as five) cell types, including Golgi type II cells, and a corresponding richness of afferent inputs. A major difference between the species is the relative abundance of Golgi type II cells in the cat, and their paucity in the opossum in each of the thalamic subdivisions. In addition, certain subdivisions are proportionately expanded or elaborated in the cat (e.g., pars lateralis of the ventral division), while others are relatively more prominent in the opossum (e.g., pars ovoidea of the ventral division.) We conclude that patterns of connectivity, the topographical position of nuclei, their developmental history or functional organization are insufficient to confirm homology in the nervous system; while they can be useful adjuncts in the determination of homology, we propose that a more reliable criterion is neuronal structure.

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DEVELOPMENT

474 CONSTANCY OF SPATIAL PREFERENCES OF GRASSHOPPER MOVEMENT DETECTOR NEURONS DURING POST-EMBRYONIC EYE DEVELOPMENT. Tom Abrams* (SPON: J.S. Lockard). Dept. of Zoology, Univ. Washington, Seattle, WA 98195.

The eyes of grasshoppers grow during each instar by adding new ommatidia at their anterior margins. The number of ommatidia increases approximately from 2500 in the 1st instar to 9300 in the adult in Schistocerca americana. Because the field of view of each eye is relatively constant throughout development, the number of ommatidia viewing a given angle in space must increase with age. This was verified by measuring the angle between the optical axes of adjacent ommatidia (interommatidial angle) in particular regions of the retina at different developmental times. The interommatidial angle in the horizontal plane for ommatidia present at hatching was reduced from 5.0° in the 3rd instar to 3.4° in the adult. Any one size stimulus is viewed by 2.2 X more facets in the adult than in the 3rd instar.

The essential piece of information necessary for size discrimination by a visual system is the amount of retina stimulated by an object. If the processing in optic lobe is unchanged during development, then one consequence of the reduction in the area viewed by a given number of facets must be a shift in the size preferences of higher order visual neurons towards smaller stimuli than were optimal for the same cells in younger instars.

I have tested this prediction by determining the size preferences of the Movement Detector (MD) neurons in grasshoppers at various stages of development. The MD cells receive input in the 3rd optic ganglion from across the whole visual field. These neurons are optimally excited by small moving targets, and they selectively fail to respond to stimulation of large areas of the retina. Size preference data were obtained for identified regions of the eye in 2nd and 3rd instar and adult animals. The stimulus used was the motion of a horizontally oriented rectangular grating (11° period; 60°/sec motion). The grating was viewed by the insects through a square window of varying width.

In juveniles, response was maximal with a 5° wide window, and fell off steeply as window size was increased, reaching 1/2 max with between 10° and 15° windows. Response vs. size curves for the same regions of the eye in adults were similar. There was no evident shift of these curves to smaller window widths.

It is concluded that through development, the spatial tuning of the MD system is not simply a constant function of the number of ommatidia stimulated. Compensatory changes must occur to ensure that stimuli of the same size elicit similar responses in different age animals; these may involve a re-distribution in the optic lobe of the connections of those inhibitory interneurons which mediate the discrimination against larger stimuli.

475 A GOLGI STUDY OF THE DEVELOPMENT OF NEURONS IN THE PONTINE NUCLEI OF THE RAT. Catherine Adams*, John G. Parnavelas, Gregory Mihailoff and Donald J. Woodward (SPON: D. J. Woodward). Dept. Cell Biology, Univ. of Tx. Hea. Sci. Ctr., Dallas, TX 75235.

The development of neurons in the basilar pons of the rat was studied using the Rapid Golgi, Golgi-Cox, and Golgi-Kopsch techniques in female albino rats of ages 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 24, 28 and 90 (young adult) days. This developmental study was undertaken as part of an ongoing effort to examine the development of the cortico-ponto-cerebellar projection and its involvement in visuo-motor processes.

In the adult, the preponderance of neurons are multipolar and exhibit a wide gradation in size and shape. Their dendrites branch extensively and at varying distances from the cell body. The proximal portions of the dendrites are usually smooth while the more distal portions give rise to appendages of varying morphology. A characteristic feature of many of these cells is the presence of conspicuous finger-like projections present at the terminal tips of the dendrites. The axons of these neurons arise from either the cell body or from the proximal portion of one of the dendrites and on rare occasions they give rise to collaterals. A scarce but distinctive type of cell observed in the basilar pons has a bipolar appearance with smooth, sparsely branched dendrites arising from the opposite poles of an elongated cell body.

At birth and for a few days thereafter, the perikarya of the pontine neurons are small and sometimes irregular in shape. Their dendrites are thin, short and beaded, and frequently display bulbous growth cones at the terminal tips. Growth cones are also present at dendritic branch points and along the course of the delicate axons. The cell perikarya are considerably larger at the end of the first postnatal week and continue to grow with time, as do the dendrites, which increase their branching and thickness and resemble those of mature neurons. At this stage a small number of thin appendages appear to arise from the dendrites and cell body surfaces, a feature quite different from the adult morphology. Dendrites continue to grow during the second postnatal week and at the end of this period they appear to achieve their mature arborization patterns. Dendritic thickness continues to increase during the third week and all neurons attain their mature morphological features by the end of the fourth postnatal week.

Supported by NSF BNS 77-01174 to D. J. Woodward and a grant from the Biological Humanics Foundation.

476 RESPONSE OF INFANT RAT BRAIN TO CHANGES IN PLASMA CONCENTRATION OF BICARBONATE. J.L. Allen*, C.D. Withrow* and C. E. Johanson* (SPON: S.A. Turkanis). Dept. of Pharmacology, Univ. of Utah College of Medicine, Salt Lake City, Utah 84132

There is a paucity of knowledge about acid-base regulation in the neonatal CNS. Recent evidence suggests regulation in the neonate may differ from that seen in the adult (Life Science 19:961, 1976); therefore it was of interest to carry out an ontogenetic study of the response of cerebrospinal fluid (CSF) and cerebral cortex (CC) to metabolic acid-base distortions.

Sprague-Dawley rats 1, 2 and 4 wks of age were bilaterally nephrectomized and injected i.p. with 0.3 mg NH_4Cl, 0.8 mg $NaHCO_3$ or 0.1 mg NaCl, per g body weight. For a given age and treatment, 7-9 animals were used. ^{14}C-DMO was used to indicate intracellular pH. After 2 hr rats were anesthetized with ketamine, arterial blood acid-base parameters were measured, and blood, cisternal CSF, and CC were sampled for analysis of radioactivity. CSF was analyzed for total CO_2.

Plasma HCO_3^- was 24 mM in 1-wk control rats, and 18 and 14 mM in 2-wk and adult animals, respectively. In control animals, blood pCO_2 was higher in 1- and 2-wk rats (57 and 47 torr, respectively) than in adult rats (29 torr). Blood pH was lowest (7.23) in 1-wk control rats and increased with age to 7.39 in adult animals.

Bicarbonate administration increased plasma HCO_3^- only 2 mM in 1-wk rats, and 9 and 13 mM in 2-wk and adult rats, respectively; the consequent metabolic alkalosis increased the CSF HCO_3^- 2 mM in 1-wk rats, and 4 and 2 mM in 2-wk and adult animals, respectively. Thus, although the blood-brain barrier to HCO_3^- is quite permeable in the 1-wk rat, some decrease in permeability is apparent at 2 wk. Metabolic acidosis caused greater changes in blood and CSF HCO_3^- in 1-wk rats than more mature animals.

CC intracellular pH was greater for control 1-wk rats (7.12) than for control adults (7.04). One and two weeks after birth there was regulation of cell pH in CC during both metabolic acidosis and alkalosis. This observation demonstrates substantial buffering capacity in cortical cells despite the leakiness of the blood-brain barrier to bicarbonate in young rats.

Elevated brain cell HCO_3^- has been noted (Am. J. Physiol. 206:521, 1964) in immature rats. This is in part related to the relatively high pCO_2 in the neonate. Despite the ability of HCO_3^- to enter the immature CNS, it appears that there is an adequate homeostasis of cellular pH in the brain in the face of wide fluctuations in plasma HCO_3^-. (Supported by NIH grants NS13988 and GM07579)

477 FETAL RAT BRAIN CATECHOLAMINE CONCENTRATIONS ARE INCREASED FOLLOWING MATERNAL TYROSINE INJECTIONS. Anthony Altar*, Mark F. Nelson*, Carla S. Whitacre*, Edwin Meyer, Jr.*, and Loy D. Lytle. Department of Psychology, University of California, Santa Barbara, CA 93106.

The biosynthesis of a variety of monoaminergic neurotransmitter compounds in adult rats depends to some extent on the relative availability of precursor compounds to brain or peripheral neurons. For example, dietary or pharmacological manipulations which increase the brain concentrations of L-tyrosine produce parallel increases in the rates of synthesis of the catecholaminergic neurotransmitters dopamine and norepinephrine [*C.J. Gibson and R.J. Wurtman, Biochem. Pharmacol. 26: 1137 (1977)*]. Similarly, injections of the essential amino acid L-tryptophan also produce dose- and time-related increases in the brain concentrations of the indoleaminergic neurotransmitter serotonin [*J.D. Fernstrom and R.J. Wurtman, Science 173: 149 (1971)*]. In the present set of experiments we were interested in determining the possible extent to which alterations in the availability of precursor amino acids might cause changes in the abilities of fetal animals to synthesize brain neurotransmitters.

To accomplish this goal, eighteen day post-conception pregnant albino rats were fasted overnight, and then injected subcutaneously with 0, 50, 100, or 200 mg/kg of L-tyrosine (10 ml/kg of 0.9% saline; pH 10.4). Animals were killed 30, 60, or 120 min post-injection and tyrosine concentrations were determined in the maternal plasma, placenta, and in bodies and brains of the fetuses using a fluorimetric assay. Dopamine and norepinephrine concentrations in fetal brain tissues were also measured using the radiometric-enzymatic assay of J.T. Coyle and D. Henry [*J. Neurochem. 21: 61 (1973)*]. Tyrosine concentrations were increased in a dose-related fashion in all tissues examined for up to 60 min post-injection, and returned toward vehicle control values by 120 min. Fetal brain concentrations of dopamine and norepinephrine were increased 30 min or 60 min, respectively, following the maternal tyrosine injections. These results indicate that the synthesis of some neurotransmitters in the brains of fetal animals may be regulated, at least in part, by the relative availability of precursor compounds. (Supported in part by a grant from NIMH).

478 THE FORM OF THE C.N.S ARBORIZATIONS OF SENSORY NEURONS IS GOVERNED BY THEIR EPIDERMIS OF ORIGIN. Hilary Anderson* and Jon Bacon* (SPON: K. Graubard). Dept. Zoology, Univ. Washington, Seattle, WA 98195 and Max-Planck-Institut für Verhaltensphysiologie, D-8131 Seewiesen, Germany.

The head of the locust, Schistocerca gregaria, bears a population of wind-sensitive hairs whose inputs are used for the initiation, maintenance, and control of flight. The hairs themselves are physiologically and morphologically indistinguishable, but hairs in different locations on the head form different neuronal projections within the c.n.s.: hairs on the side of the head send axons to the c.n.s via the ventral tegumentary nerve, and to a small extent the dorsal tegumentary nerve,and form entirely ipsilateral projections within the suboesophageal ganglion and thoracic ganglia; hairs on the top of the head send axons to the c.n.s. exclusively through the dorsal tegumentary nerve and form additional contralateral branches and arborizations in the suboesophageal ganglion and thoracic ganglia.

Are these patterns of projection determined by factors intrinsic to the sensory receptors themselves, or do they primarily depend upon later interactions between their axons and the environment through which they grow in the c.n.s.?

The hairs are formed throughout postembryonic life by the division and differentiation of single epidermal cells. We grafted pieces of epidermis between head locations at a stage when few wind-sensitive hairs had developed. We then examined the projections from hairs which subsequently developed from the grafts by cobalt-filling individual neurons and viewing them in wholemount preparations.

Hairs developing from the grafts were encircled by host hairs developing from the surrounding host epidermis. The graft axons now grew from the epidermis in association with the surrounding host axons and entered the c.n.s. at the host site via the host nerve. However, once the graft axons were in the c.n.s, in no case did they behave according to their altered environment. Rather they all formed projections which were entirely appropriate to their epidermis of origin.

We conclude that receptor cells are assigned, on the basis of early decisions taken by their epidermal cell precursors, an intrinsic developmental programme which specifies their projection patterns.

479 EFFECT OF ENVIRONMENT OF ACH SENSITIVITY OF DEVELOPING NODOSE NEURONS IN VITRO. Paola Baccaglini and Ellis Cooper. Dept. Neurobiol., Harvard Med. Sch., Boston, MA 02115.

Cholinergic synapses form between dissociated neurons from newborn rat nodose ganglia when grown in culture. The formation of these functional synapses as well as the presence of acetylcholine (ACh) receptors depends on the environmental conditions in which these cells are grown. Nodose ganglia (an autonomic sensory ganglion) were dissected from the neck region of newborn rats and mechanically dissociated. All neurons were grown in the presence of NGF; by 24 hrs they attached to the substrate and extended processes. We plated the neurons under 2 different conditions (1) on collagen and in the virtual absence of non-neuronal cells (2) on a preformed layer of skeletal myotubes or myocytes. We recorded from these neurons as early as 10 days after plating. In condition (1) many neurons had spontaneous excitatory postsynaptic potentials (epsps). In addition, in about 20% of randomly chosen neuron pairs in some cultures stimulation of one neuron evoked an epsp in the other. Both spontaneous and evoked epsp's were reversibly blocked by standard cholinergic antagonists indicating that the synapses are cholinergic. In contrast spontaneous or evoked epsp were much rarer events in sister cultures grown in condition (2); they were detected in less than 5% of pairs tested.

One factor that accounts for the failure of at least some neuron-pairs to form cholinergic synapses is the absence of cholinergic receptors. We tested neuronal sensitivity to acetylcholine (ACh) primarily by pressure ejection (similar results were obtained by either bath or iontophoretic application). For each culture dish, which usually contained approximately 500 neurons, we routinely tested 50 neurons chosen at random and scored each one as either sensitive or insensitive. With the concentrations of ACh used a sensitive neuron was one which depolarized by at least 5mV, although most neurons gave responses of 20-40mV; insensitive neurons gave no detectable response (less than 2mV). In experiments where sister cultures were grown either alone or on muscle, 70-90% (n = 130) of neurons grown alone and fed every 2 days were sensitive to ACh whereas only 20-30% (n = 250) of the neurons grown on muscle and fed every 4 days were sensitive. Feeding the 'neurons - alone' cultures every 4 days does not give as many sensitive neurons (50-70%, n = 500) compared to those fed every 2 days, however, the proportions are still significantly different from sister cultures grown on muscle.

We are investigating what factors may be involved in the expression of ACh receptors as well as the pharmacology of these receptors and whether the neurons are sensitive to other transmitter substances. (Supported by the Whitney Foundation, Muscular Dystrophy Association and American Heart Association 78364).

480 ENDURING MORPHOLOGICAL ALTERATION OF HIPPOCAMPUS AND CEREBELLUM IN RATS PRENATALLY-EXPOSED TO ETHANOL. David E. Barnes, Don W. Walker and Steven F. Zornetzer. Dept. of Neuroscience, Univ. of Florida College of Medicine and VA Medical Center, Gainesville, FL 32610.

Pregnant Long-Evans rats were maintained on an ethanol containing liquid diet (40% ethanol-derived calories; 9.7% v/v ethanol) during days 10-21 of gestation. Control groups were pair-fed a sucrose-containing liquid diet with sucrose substituted isocalorically for ethanol or given free access to pelleted laboratory food and water. At parturition, the offspring were culled to 8 males and fostered to additional control mothers. Offspring from the three groups were sacrificed at the time of weaning or 60 days of age. The brains were removed and coded to prevent experimenter bias and hemisected by a midsagittal cut. One half of each brain was embedded in paraffin, sectioned at 4 μm, and stained with cresyl violet. In midsagittal sections the total number of cerebellar Purkinje cells was determined as well as the granule cell population of lobule VIII (pyramis). The number of hippocampal pyramidal cells was determined in sagittal sections 1.5 mm from midline. The ethanol-exposed offspring were not significantly different from controls at birth or 21 days in body or brain weights. At sixty days of age there were large deficits in prenatally-formed neurons in the group prenatally-exposed to ethanol relative to controls. There was a 18% decrease in hippocampal pyramidal cells and a 27% decrease in cerebellar Purkinje cells. In addition, there was a decrease of cerebellar granule cells (9%) which may be a result of the loss of Purkinje cells rather than the exposure to ethanol per se. The results indicate that prenatal ethanol exposure results in an apparently permanent reduction in the number of prenatally-formed hippocampal and cerebellar neurons

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481 A POSTERIOR TO ANTERIOR GRADIENT IN VARIABILITY OF THE STRUCTURES OF TWO IDENTIFIED NEURONS IN CRAYFISH. Michael Bastiani and Brian Mulloney. Depts. of Physiology and Zoology, University of California at Davis, Davis, CA 95616.

The crayfish muscle receptor organs (MROs) monitor the position and movement of the abdomen. A pair of receptors, tonic (MRO_1) and phasic (MRO_2) is associated with each abdominal hemiganglion. The sensory neurons, SN_1 and SN_2 respectively, from these MRDs send axons via the second root into the segmental ganglia; these axons bifurcate and project to the brain and 6th abdominal ganglion.

The anatomy of the sensory neurons which originate in abdominal ganglia one through five was examined in the 6th abdominal ganglion. Microelectrode injection of Lucifer Yellow (Stewart, 1978) into neuropil processes of the SNs was used to determine their structures. Although all the SNs have a characteristic structure, those originating from the more anterior ganglia are much more variable and consistently make what appear to be errors.

Three types of errors commonly occur. The first is inappropriate termination, where the primary branch does not terminate as usual but continues part way back up the connective or into the 6th ganglion and ends in a large bleb. The second is unusual branching patterns. The third, which generally occurs along with the first two, is large (> 5μ) terminal blebs.

The physiology and connectivity of the SNs in the 6th abdominal ganglion has been examined by stimulating selectively SNs from all the abdominal ganglia while recording PSPs in a large number of motor neurons and interneurons. Although there is a very interesting anterior to posterior gradient in synaptic strength, there does not appear to be a correlation between the variability in the structure of the more anterior SNs and missing synapses.

Several hypotheses might account for this posterior to anterior gradient in structural variability. The SNs from the more posterior ganglia are closer to the 6th ganglion and their axons may reach the 6th ganglion earlier in development. This hypothesis predicts that the variability in the anatomy of an identified neuron will be related to its birthday or to the time the synapse forms.

Another hypothesis is that as axons get further from the cell body, there is some loss of control of structural growth. We are now trying to test these two hypotheses.

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482 DIFFERENTIAL EFFECTS OF D-AMPHETAMINE ON LOCOMOTOR ACTIVITY IN IMMATURE AND MATURE RATS. Richard H. Bauer, Department of Psychology, Kansas State University, Manhattan, KS. 66506.

In altrical species, such as the rat, biochemical and histochemical studies indicate that catecholamine containing neuronal cell bodies in the lower brain stem are nearly fully developed at birth. During development, axons from these norepinephrine (NE) and dopamine (DA) containing cell bodies grow in a rostral direction, such that successively higher structures are innervated. In the rat, innervation of cortical areas occurs at about 45 days of age.

Since ascending catecholamine neurons in the rat are developing from birth to about 45 days of age, drugs which alter behavior by acting on catecholamines would be expected to have different behavioral effects at different maturational stages. d-Amphetamine is thought to alter locomotor activity and induce stereotyped behaviors by presynaptically increasing the release and reducing the reuptake of NE and DA. Thus, d-amphetamine would be expected to have differential effects on locomotor activity of immature and mature rats. This hypothesis was tested in the present study by recording photo-cell crossings of 15-, 17-, 21-, 36-, 90-, and 275-day-old rats injected with either physiological saline or 0.5, 1.0, 4.0, 8.0, or 16.0 mg/kg of d-amphetamine sulfate. Photo-cell crossings were recorded starting immediately after the injection for a period of 4 hours. The two oldest groups were tested in a 45 x 45 x 45 cm Plexiglas chamber. All apparatus dimensions for younger groups were reduced according to the mean snout to rump length.

For each animal, the number of photo-cell crossing during 15-min intervals was determined. Statistical analyses showed that only the 0.5 mg/kg dose resulted in a slight increase in photo-cell crossings in the two youngest groups. In 21 and 36-day-old rats, there was a positive relationship between drug dose and photo-cell crossings. In the two oldest groups, lower doses increased photo-cell crossings, whereas higher doses reduced crossings. The temporal changes occurring during the 4-hour session also differed as a function of development.

In a second experiment, para-hydroxyamphetamine, which has only peripheral nervous system effects, did not alter photo-cell crossings of immature or mature rats.

The present findings are consistent with the hypothesis that, because of differences in maturation of catecholamine neurons, catecholaminergic drugs would produce differential behavioral effects in immature and mature rats.

483 TEMPERATURE DEPENDENCE OF EARLY INGESTIVE BEHAVIOR: PERCEIVED ENVIRONMENTAL TEMPERATURE VS. BODY TEMPERATURE. Ingrid J. Bennett and W. G. Hall. N.C. Div. of Mental Health, Raleigh, N.C. 27611.

When a food deprived neonatal rat receives a small infusion of milk in its mouth via an intraoral cannula, it ingests a substantial amount of the diet and also shows a dramatic behavioral activation. The feeding and activation only occur when the pup is maintained in a warm environment (Hall, 1978, in press). The present study determined whether body temperature or perceived ambient temperature was the basis for the temperature dependence of early ingestive responding.

Fine polyethylene intraoral cannulas, through which diet could be infused, were implanted in the mouths of six-day-old Charles River CD strain rats. Prior to testing, the body temperature of one pair of pups was adjusted to 33-34°C (for Warm Temperature pairs), while the temperature of another pair was adjusted to 28-29°C (for Cool Temperature pairs). One of the pups in each pair was tested at room temperature (26°C) and the other was tested in a warm (34°C) incubator. A pulse of milk was infused into the pup's mouth through the cannula once a minute for 3 min. Every 30 sec, the pup's activity was rated and a variety of behaviors, including probing and mouthing, were scored. Intake was determined by measuring the change in body weight from the beginning to the end of the test. Warm pups (n = 7 pairs) tested in a warm environment had significantly higher levels of activity, mouthing, probing and intake than warm pups tested in a cool environment. Similarly, cool pups (n = 7 pairs) tested in a warm environment were more active, showed more mouthing and probing, and ingested more diet than cool pups tested in a cool environment (see Table). These findings indicate a significant effect of perceived ambient temperature on the activation and feeding responses of infant rats

However, pup body temperature also had a significant effect, since warm pups tested in a warm environment were more active and ingested more diet than cool pups tested in a warm environment (see Table). Together, these results indicate that the effect of infused diet on the behavioral activation and intake of a deprived pup depends on both contextual cues (e.g. ambient temperature) and physiological conditions (e.g. body temperature).

	Warm Envt. Temp.		Cool Envt. Temp.	
	Warm Body Temp.	Cool Body Temp.	Warm Body Temp.	Cool Body Temp.
Intake (% of infused vol.)	93 ± 7	62 ± 9	56 ± 14	38 ± 7
Activity	11.1 ± .6	8.0 ± .9	6.4 ± .8	4.3 ± .8

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484 CELL DEATH IN THE DEVELOPING HAMSTER SUPERIOR COLLICULUS Anne T. Berg* and Barbara L. Finlay Department of Psychology Cornell University, Ithaca, NY 14853

Degenerating cells may be observed with light microscopy in the superior colliculus of the golden hamster during the first nine postnatal days. The number of degenerating cells observable at any point is small. In the period of maximum rate of cell death, postnatal days 3-5, 4-5 degenerating cells can be found per 1000 normal cells; by day 8, only 1-2 degenerating cells per 1000 are visible. As in chick (1) and rat (2), this period of cell death follows neuronal differentiation in the tectum and is coincident with the major period of axon ingrowth and outgrowth.

Within the tectum, the distribution of degenerating cells is fairly uniform. The rate and time course of cell death in tectum may be distinguished from the rate and time course in surrounding structures and other visual structures.

1. Cantino and Daneo, '72
2. Arees and Astrom, '77

Supported by NSF Grant BNS 77-07066

485 BIOLOGICAL CHARACTERIZATION OF A NEW NEURONAL GROWTH FACTOR. Emanuel M. Bloom, Michael D. Coughlin and Ira B. Black. Dept. of Neurol., Cornell Univ. Med. Col, New York, N.Y. 10021.

A new neuronal growth factor(s), derived from medium conditioned by mouse heart monolayer cultures, enhances survival and stimulates development of mammalian autonomic neurons. The factor caused a 2-3 fold increase in neurite outgrowth and tyrosine hydroxylase activity of embryonic mouse superior cervical ganglia (SCG), cultured without added nerve growth factor (NGF). Dose-response analysis revealed that these responses occurred as a saturable function of factor concentration. In addition, choline acetyltransferase activity was also increased in the cultured SCG by the factor.

Antiserum to NGF did not inhibit growth responses to the new factor, suggesting that it was separate and distinct from NGF. Moreover, the new factor enhanced survival of parasympathetic and sympathetic neurons in cell culture, but not sensory neurons, clearly distinguishing the factor from NGF. These results suggest that heart cells in culture are capable of elaborating a new growth factor which fosters survival and differentiation of sympathetic and parasympathetic neurons.

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486 GANGLION CELL AXONS MAINTAIN THEIR NEIGHBORS IN THE SELF ASSEMBLY OF THE EMBRYONIC OPTIC NERVE. Neil Bodick* and Cyrus Levinthal. (Spon Malvin Teich) Dept. Bio. Sci., Columbia University, New York, New York, 10027.

Morphological analysis of a developing visual system has been undertaken at the EM level in an attempt to elucidate cellular interactions operative in the formation of the retino-tectal projection. At maturity, the zebrafish (Brachydanio rerio) projects approximately 50,000 fibers from each eye to the contralateral optic tectum. Staged embryos, with relatively few (about 1800) fibers leaving the eye provide the opportunity to examine cells forming the connection to the tectum. Developing cells in the visual system have been reconstructed from serial section electron micrographs using the CARTOS computer graphics system. In the retina, small clusters of adjacent ganglion cell somata spin out axons which, over long distance in the optic nerve, maintain their contiguity. Growth cones of these cells confine their activity to neighboring axonal surfaces.

The retinal surface maps in a systematic fashion into the cross section of the optic nerve. Cell soma position in the retina can be defined by polar coordinates (r,θ), with the choroid fissure at $\theta=0$ and $r=0$ at the nerve head. In the optic nerve, radial position transforms into the Dorso-ventral axis, while angular position transforms into the nasal-temporal axis.

The present study suggests that the optic nerve develops as a highly ordered structure capable of carrying the projection back to the tectum as an organized array of fibers. Such organization is mediated by interactions between and among growing ganglion cell axons which originate from cell bodies which are adjacent to each other in the retina. Details of the ultra-structural interactions will also be discussed.

These studies were supported by NIH grant PCM 78-06636 and Computer Graphics Facility NIH grant 5 P 41 RR-00442-10.

487 PROLIFERATION OF GRANULE CELL PRECURSORS IN RAT HIPPOCAMPUS IS INHIBITED BY HYDROCORTISONE TREATMENT: AN AUTORADIOGRAPHIC STUDY. Martha C. Bohn* (SPON: Kent Morest).Dept. Biobehavioral Sciences, Univ. of Connecticut, Storrs, CT. 06268.

Postnatal genesis of microneurons in the rat brain occurs in both the cerebellum and hippocampus(HP). Neonatal treatment of rats with glucocorticoids has previously been reported to inhibit cell proliferation in neuronal precursor cells in the cerebellum resulting in a permanent deficit in cell number and cerebellar size (Bohn,M.C.and Lauder,J.M.,Develop.Neurosc. 1,250,1978; Cotterrell et al.,J.Neurochem.19,2151,1972).The present study investigated the possibility that postnatal neurogenesis in the HP might also be affected by glucocorticoid treatment.

Rat pups were injected with hydrocortisone acetate(HCA) on days 1-4(200µg/day), sacrificed by perfusion fixation and the brains embedded in paraffin.

For short-survival autoradiography, rats were sacrificed during the first two weeks after birth; each was injected with ^{3}H-thymidine (^{3}H-T)one hour before sacrifice and serial sections of the HP prepared for autoradiography.The total number of cells labeled by ^{3}H-T throughout the dentate hilus and stratum granulosum (sG) was decreased during HCA treatment and recovered to control values during the second week.Decreased labeling was already evident on day 2 in the ventral HP,but was not apparent until day 4 in the dorsal HP.It was concluded that proliferation of granule cell(GC)precursors was transiently inhibited by HCA treatment.

The effect of this inhibition on "birthdays" of GC was investigated by long-survival autoradiography by injecting with ^{3}H-T at various ages and sacrificing at 72 days.The time courses of GC birthdays at ventral and dorsal levels of the sG were determined by plotting the ratio of heavily labeled GC/total GC versus age. Dorsally, the time course for GC birthdays was unaffected. Ventrally, GC birthdays were significantly depressed on day 5 probably reflecting the prolonged inhibition of cell proliferation in this region. This fall was in marked contrast to GC genesis in controls which was maximal on day 5 and resulted in a late peak in GC birthdays at the ventral level in treated rats.

Integrated area measurements in serial sections showed that the total volume of the sG was reduced by 20% in treated rats at 7 days,but was control size at 60 days.Therefore,even though cell proliferation was reduced during the first postnatal week when most GC were formed in controls,this did not result in a permanent reduction in the size of the sG.

These observations of decreased cell proliferation and delay in the peak of GC birthdays during HCA treatment indicate that the rate and pattern of postnatal neurogenesis in the rat HP are altered by glucocorticoid treatment.

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488 DEVELOPMENT OF THE TROCHLEAR NERVE FOLLOWING COMPLETE REMOVAL OF THE PERIPHERY. William R. Boydston* and G. S. Sohal (SPON: S. D. Stoney). Dept. Anat., Sch. Med., Medical College of Georgia, Augusta GA 30912.

The aim of the present investigation was to study the development of the trochlear nerve with respect to sprouting in the complete absence of the superior oblique muscle in the white Peking duck embryo. The temporal coincidence between the onset of collateral sprouting in the developing trochlear nerve and the time at which neuromuscular contacts are established had previously suggested that sprouting may be triggered by the periphery. Unilateral optic vesicles along with the surrounding mesoderm were removed on Day 5 of incubation. To ensure complete removal of the periphery, an electric cautery was used to cauterize the extirpation site following removal of the optic vesicle. Embryos were sacrificed daily beginning on Day 11 and continuing through Day 18. Upon sacrifice, experimental and control nerves were fixed for electron microscopy with brains processed for routine paraffin histology. Axon counts were obtained from montages of the nerve while cell counts of the experimental and control trochlear nuclei were done by counting nucleoli in alternate sections. The preliminary results of this investigation based on limited samples indicate that sprouting is essentially absent in the peripherally deprived trochlear nerve. The average axon count on Day 12 of the experimental nerve yields 1639 fibers with an average cell count in the control trochlear nucleus of 1513 neurons. This presents a cell to fiber ratio of approximately 1:1 on this day. This ratio is observed to remain constant through Day 14 (1442 cells, 1827 axons). Subsequently, large numbers of cells and fibers are lost until Day 18 when the experimental nerve and contralateral nucleus are essentially absent. In contrast, cell and axon counts of the control nerves on Day 12 indicate that the trochlear fibers have sprouted. The cell to fiber ratio on this day is 1:4. The ratio is eventually reduced to 1:1 by Day 18 as a result of a loss of cells and fibers between Days 13 and 17. Whether the lack of sprouting in peripherally deprived nerve is due to lack of neuromuscular contacts or the absence of a trophic factor provided to the nerves by the muscle remains to be investigated.

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489 THE EMBRYOLOGIC APPEARANCE AND DISAPPEARANCE OF A LITTLE KNOWN TELENCEPHALIC NERVE IN THE BAT. Jerry William Brown. Dept. Anat., Med. Cent., Univ. Ala. in Birmingham, Birmingham, Ala. 35294

Within the few years spanning the turn of the century a ganglionated telencephalic nerve was described by Pinkus and named the nervus terminalis by Locy. In the early decades of this century numerous investigators described this nerve in representatives of all classes of vertebrates; however, in recent years it has received only casual mention. During a study of the development of the telencephalon of insectivorous bats ranging from 6 to 19 mm (near term) C-R length, the ganglionated nervus terminalis was noted to appear first in the 7 mm embryos. As the embryo grows there is a progressive enlargement of the nerve and an increase in the number of associated ganglion cells, reaching the largest size and cell number in embryos of 12 to 12.5 mm C-R length. In the 13 mm embryo the nerve is smaller and fewer ganglion cells are present, some showing degenerative changes. In all embryos older than that of 13 mm the nerve and ganglion cells are absent as in the adult. The origin of these ganglion cells, a matter of some dispute, is clearly a thickened region of the epithelium of the nasal septum of insectivorous bats. Clusters of these cells break away from the epithelium and migrate centrally towards the medial wall of the telencephalon. Even the few intrahemispheric ganglion cells associated with fascicles of the nervus terminalis are most likely of peripheral origin. Between 7 mm, when they first appear, and 12.5 mm, when only one small cluster arises from the epithelium, the cell clusters gradually increase in number, reaching a maximum in the 10.5 mm embryos and then decrease in number. No cells arise from the nasal septal epithelium in embryos of 13 mm and longer. Centrally the nervus terminalis penetrates the brain wall in the region of basal forebrain structures having secondary and tertiary olfactory connections. Evidence will be presented which suggests the functional type of the neurons of the nervus terminalis in insectivorous bats.

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490 CHANGES IN BRAIN MORPHOLOGY DUE TO THE EFFECTS OF MALNUTRITION. MB.T. Buschmann, E.M. Burns, L.E. Comerford*, T.W. Kruckeberg*, P.K. Gaetano*and J.M. Meyer*. Dept. Gen. Nurs., College of Nursing, University of Illinois at the Medical Center, Chicago, IL 60612

The effects of protein-calorie malnutrition on the brain is one of the major problems of our time. Malnutrition in animals during early life has been found to cause anomalies in the development of neuronal synapses, reduction in brain size, loss of neuronal cells and other abnormalities. Children who have endured severe malnutrition in early life manifest varying degrees of retarded learning ability. Because of the critical involvement of the dendrites and the synapses in neurological functioning and because of the impaired learning ability noted in severely malnourished children, synaptic morphology was investigated in malnourished and well nourished animals. Further, because of the important regulatory role of the cerebral capillary in the maintenance of an optimal microenvironment of brain cells, the ultrastructure of cerebral capillaries was also studied in these animals.

The purpose of this study therefore was to determine the effect of protein malnutrition on the morphology of brain structures. Dams of the experimental animals were fed isocaloric diets containing either 25% casein (well nourished) or 8% casein (malnourished) for two weeks prior to mating and throughout gestation and laction. Diet regimes were continued after weaning in the young and subsequently the male and female offspring were sacrificed at 35, 100, and 300 days after birth and the brains were fixed for electron microscopy.

Examination of whole brain weight showed a significant decrease in the malnourished animals. Concomitant with the decreased weight was a decrement in synaptic morphology as well as a significant decrease in the number of endothelial mitochondria per cerebral capillary profile. The latter of these implies decreased energy for active transport across the blood-brain barrier.

The results of these studies provide both qualitative and quantitative morphological evidence of a loss of function in the brain due to protein malnutrition. Decreased brain size, loss of mitochondria in the capillary endothelium and altered synaptic structure suggest a functional impairment at the cellular level.

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491 THE EFFECTS OF AMPHETAMINE ON ACETYLCHOLINE LEVELS AND SYNTHESIS IN VARIOUS REGIONS OF THE DEVELOPING KITTEN. S.H. Butcher, M.S. Levine, N. Buchwald and C.D. Hull. Mental Retardation Research Center, School of Medicine, UCLA, Los Angeles, CA. 90024.

The development of acetylcholine (ACh) levels and synthesis in the precruciate cortex, caudate nucleus and thalamus of the kitten were measured using stable isotope techniques and a gas chromatographic/mass spectrometric method developed by Jenden et al. (Anal. Biochem. 55: 438, 1973). Kittens were sacrificed using microwave irradiation following a pulsed i.v. injection of deuterium labeled choline ($^2H_9$-Ch). Within the first 24 hrs after birth endogenous levels of ACh were significantly increased when compared to the levels found on the 2nd postnatal day. Since many neuronal input connections are present at birth, this change from the first to second postnatal day may represent a reflection of increases in spontaneous neural activity during this time. The concentration of deuterium labeled ACh ($^2H_9$-ACh) reached adult levels by 40 days postpartum but the concentration of endogenous ACh had not yet attained adult levels within this 40 day period. In order to study the effect of an increased release of endogenous Dopamine (DA) on the developing cholinergic system, kittens were pretreated with amphetamine (5 mg/kg i.p. 30 min prior to sacrifice). Pretreatment with amphetamine produced an increase in the concentration of deuterium-labelled ACh while having no effect on endogenous levels of ACh at any of the early time points measured (5,8,15 and 20 days postpartum). However, on day 40, the synthesis of $^2H_9$-ACh was significantly reduced compared to control levels in all three brain regions studied, while the levels of ACh remained unchanged in the caudate nucleus and thalamus. This latter response is more characteristic of that found in the adult rat (unpublished data). It is possible that this apparent decrease in synthesis is a result of the reduced precursor availability due to the effect of amphetamine on blood flow to these brain regions, or that the newly synthesized $^2H_9$-ACh is being preferentially released.

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492 CENTRAL PROJECTIONS OF ANTENNAL SENSORY NEURONS IN MATURE AND DEVELOPING *MANDUCA SEXTA*. Scott M. Camazine* and John G. Hildebrand. Dept. of Neurobiology, Harvard Med. Sch., Boston, MA 02115.

As a prelude to studies of the organization, physiology, and development of the antennal centers in the brain of the moth *Manduca sexta*, we have examined the normal anatomy of antennal afferents by intracellular staining with cobalt sulfide followed by Timm's intensification of whole-mount or sectioned material.

Axons in the flagellar component of the antennal nerve (largely olfactory) project to the glomeruli of the ipsilateral antennal lobe (AL), to the ipsilateral "dorsal lobe" (DL), and to the ipsi- and contralateral sides of the subesophageal ganglion. Males exhibit a distinct flagellar projection to a macroglomerular complex that is found only in the male AL. This is the only sexual dimorphism that we have observed in the CNS. Cobalt-sulfide staining reveals no neural connections to the AL from the contralateral antenna, the ventral nerve cord, or the nerves of the subesophageal ganglion. Mechanosensory fibers from the Böhm's bristles on the basal segments of the antenna (pedicel and scape) project through and ramify in the DL and terminate in the thoracic ganglia. The projections of these axons are similar to those of the tegumentary nerve fibers, which originate at mechanosensory sensilla on the head. Johnston's Organ, a proprioceptive organ in the pedicel, sends mechanosensory axons to the lateral region of the DL in a distinctive pattern. These fibers terminate in the DL in close proximity to the dendritic arborizations of the antennal motor neurons (of which there are at least 13). This observation suggests that there may be synaptic connections between proprioceptive afferent axons and motor neurons, constituting an antennal-motor reflex pathway.

It thus appears that several classes of mechanoreceptive inputs from the antenna and the sensilla of the head project to the DL, while flagellar chemoreceptive inputs project to the glomeruli of the AL. Other antennal afferents whose sensillar origin and functions have not yet been ascertained terminate in a neuropil region between the DL and the AL and in the protocerebrum.

All of these classes of antennal afferent fibers have reached the CNS of developing adults as early as 2 days after the birth of the flagellar sensory neurons. Over the ensuing 10 days, the density of projections increases dramatically. These findings extend our earlier observation that sensory neurons throughout the antenna are born synchronously and immediately begin to elaborate axons (Devel. Biol. *51* 300, 1976).

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493 EFFECT OF LEAD EXPOSURE ON DEVELOPMENT OF HIPPOCAMPAL MOSSY FIBER SYNAPSES. Jerrolynn B. Campbell*, Vijaya K. Vijayan and Dorothy E. Woolley*. Depts. Human Anatomy and Animal Physiology, Univ. Calif., Davis, CA 95616

Postnatal exposure to lead has been shown to mimic traumatic damage to the hippocampal formation and to reduce the normally high levels of zinc in the hippocampal mossy fiber layer. At subclinical exposure levels, thermoregulatory and learning abilities are retarded, and seizure patterns in young animals exposed to maximal electroshock stimulation are altered. In order to correlate patterns of structural development of the hippocampal mossy fiber synapses with the physiologic and biochemical alterations observed following lead exposure, our laboratory has initiated a quantitative morphological study of the suprapyramidal mossy fiber layer in the hippocampal CA3 region of the rat. To minimize variations of development caused by factors other than exposure to lead, all litters were reduced at parturition to 6 pups, and a litter size of 4-6 was maintained throughout the experiment. Following parturition, 0.2% lead acetate was added to the drinking water of the dam. After fixation by intraaortic perfusion of aldehydes, slices from the center of the dorsal hippocampus were processed for quantitative electron microscopic evaluation. Random, non-overlapping fields throughout the suprapyramidal mossy fiber layer were photographed at a magnification of 4,800. A total of 1500 to 3000 u^2 of neuropil area was analyzed from each animal for number, size and structure of synaptic profiles. The typical mature mossy fiber ending appears as the Gray type I ending, with dendritic spines that are deeply embedded in invaginations of the pre-synaptic element. They exhibit dense populations of synaptic vesicles and multiple synaptic contacts, as well as punctata adherens. Gray type II profiles, which display dense vesicle populations and punctata adherens, but lack the deeply embedded dendritic spines, probably also represent mossy fiber endings in which the point of synaptic contact was excluded from the plane of section. The type II endings are interspersed with large bag-like endings and smaller endings with simple or multiple synaptic contacts. The bag-like endings are characteristic of young animals and seem to represent immature forms of the mossy fiber endings, while small, simple endings may be mossy fibers synapsing en passage, or may represent afferents of non-mossy fiber origin. Our initial results indicate that the ratio of type I (mature mossy fiber) endings to other synaptic profiles is directly proportional to the weight of age-matched animals. While there is considerable variation within treatment groups, there is also an average reduction in the number of type I endings per unit area neuropil in lead exposed animals regardless of weight.

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494 RETROGRADE TRANSPORT OF NERVE GROWTH FACTOR IN CULTURED RAT SYMPATHETIC NEURONS. Robert B. Campenot*, Edward Hawrot* and Paul H. Patterson. Dept. of Neurobiology, Harvard Medical School, Boston MA 02115.

Nerve growth factor (NGF) is essential for the survival of sympathetic neurons in vivo and in culture. The retrograde transport of NGF to the sympathetic ganglia has been demonstrated in vivo (Hendry et al., Br. Res. 1974). We have begun an examination of the retrograde transport of NGF in rat sympathetic neurons maintained in a 3-chamber culture dish devised by Campenot (PNAS, 1977). Neurons send their axons across a fluid-impermeable seal into 2 separate chambers located on either side of the cell bodies. It is quite common for a single neuron to have axons extending into both chambers. Upon incubating endings in one chamber with ^{125}I-NGF, radioactivity appears in the cell bodies at a time consistent with an axoplasmic transport rate of ~ 3 mm/hr. An apparent steady state was reached in ~10 hrs and was maintained for at least 34 hrs continued incubation. The cell body-associated radioactivity is not due to leakage of ^{125}I-NGF across the seal and direct uptake by the cell bodies since incubating the cell body chamber with excess unlabeled NGF has no effect on the appearance of ^{125}I-NGF in the cell bodies. On the other hand, incubation of nerve endings with ^{125}I-NGF in the presence of excess unlabeled NGF reduces the amount of retrogradely transported radioactivity by >90%. Furthermore, transport is largely inhibited by 20 µg/ml colchicine. SDS gel electrophoresis indicates that the cell body-associated radioactivity migrates as one band with the appropriate molecular weight of NGF. Thus, these findings with isolated neurons in culture confirm the results obtained in vivo. Although ^{125}I-NGF was transported retrogradely to the cell bodies, none of this radioactivity migrated further from the cell bodies in the orthograde direction into endings located in the opposite chamber. This is consistent with the observed local effects of NGF and the transport of NGF into spinal ganglia of the chick embryo (Brunso-Bechtold and Hamburger, PNAS, 1979).

Cultured sympathetic neurons are able to transport ^{125}I-NGF when exposed to concentrations as low as 0.5 ng/ml (2×10^{-11}M) suggesting the existence of a high affinity uptake mechanism in the nerve endings. Retrograde transport appears to be regulated by the external NGF concentration. Neurons starved of NGF for 24 hrs showed a 5-fold increase in the amount of ^{125}I-NGF retrogradely transported. Binding studies performed on dissociated neuronal cultures suggest that this regulation is not at the level of the surface receptor as detected by the low temperature specific binding of ^{125}I-NGF.

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495 AN ULTRASTRUCTURAL MORPHOMETRIC ANALYSIS OF THE ORGANIZATION OF THE DEVELOPING OPTIC NERVE OF XENOPUS LAEVIS. Charles Cima* (Spon: G. Hoyle). University of Oregon, Eugene, OR 97403.

A fine structural analysis of the developing optic nerve (ON) of Xenopus laevis was undertaken to determine if and how axons become organized. During early development of the ON there is a lineal addition of small size class axons (0.15-0.75um) from st. 28, when fibers first appear in the optic stalk, through st. 43-44. At this stage a large size class axon (1.0-4.5um) appears in the ON, which consists entirely of unmyelinated axons. At st. 47, the first myelinated axons (less than 100) are found in the ON. This is preceded by the first appearance of glial cells in the central portion of the ON. Myelination seems to begin in the middle of the ON and extend distally and proximally. Myelination is first apparent in the optic chiasma (OC) at st. 50 and the pigmented epithelium (PE) of the retina by st. 52. Myelinated fibers are not seen in the retinal portion of the ON until after metamorphosis. Myelinated axon distribution was analysed at the level of the retina, at the point of exit from the PE, at the midpoint and at the OC. Results show that the number of myelinated axons at the midpoint OC exit from PE retina. Further, this distribution remains constant from st. 47 through 3 months post-metamorphosis (PM). Assymetrical distribution has also been observed in large size class axons throughout the length of the ON during development. Large size class axons (myelinated and unmyelinated) are homogeneously distributed in the portion of the ON found in the retina. At the midpoint of the ON, between the retina and OC, there is a skewing of large size axons to the central portion of the ON. There is further reorganization at the OC which results in accumulation of large size axons dorsally and slightly rostrally. This organization is apparent at st. 44 and remains constant until at least 8 months PM.

The dorsal skewing of large size class axons is the first detectable event in the central pathway which seems to segregate visual fibers. The significance of this fiber shift in ordering connections in visual centers is not known. Several lines of physiological evidence however, suggest that visual centers in the diencephalon may be selectively innervated by large ganglion cells with dendritic arborizations which subtend 10°-15° of the visual field.

496 TECTAL CONNECTIVITY IN THE FROG FOLLOWING EMBRYONIC REMOVAL OF EYE PRIMORDIA. Martha Constantine-Paton and Patricia Ferrari-Eastman. Dept. Biol., Princeton Univ., Princeton, NJ 08544.

In order to determine the degree to which central visual circuitry is dependent upon retinal input we have raised Rana pipiens tadpoles through metamorphosis after either bilateral or unilateral removal of the eye primordia. The operation is performed at Shumway stage 17 before optic nerve axons have entered the brain.

Bilateral eye removals result in a ~70% decrease in post-metamorphic tectal volumes compared to unoperated controls. Tectal layers 1 through 6 remain distinct in these developmentally uninnervated tecta, but layers 7 through 9 are condensed. Volume and lamination changes are qualitatively similar but less pronounced in the contralateral tectum after unilateral eye primordia removals.

The nuclei projecting to these tecta have been identified in post-metamorphic animals using HRP histochemistry. A control analysis on normal animals agrees with those of previous authors (Wilczynski and Northcutt, 1977, JCN 173:219-229) in most respects. After bilateral eye primordia removal small pellets of HRP placed beneath the dorsal tectal surface label all ipsilateral nuclei that project to that region in normal animals. Contralaterally, cell groups in the diencephalon and mesencephalic tegmentum continue to project to these developmentally uninnervated tecta. However, the projection from the contralateral nucleus isthmi (Gruberg and Udin, 1978, JCN 179:487-500; Grobstein et al, 1978, Brain Res. 156:117-123) is absent in all bilateral removals examined. HRP application to the uninnervated tectal lobe in animals with unilateral removals successfully labels cells in both isthmic nuclei.

Axons in the crossed isthmio-tectal tract usually decussate as part of the post-optic commissural system (Gruberg and Udin, 1978; Grobstein et al, 1978). Thus the absence of a contralateral isthmio-tectal projection probably represents a failure of isthmio-tectal fibers to cross the midline when retinal axons are absent from the chiasm region.

The tecto-topic topography of the ipsilateral tecto-isthmi and isthmio-tectal projections (Gruberg and Udin, 1978) is also being examined in this material. Our results to date indicate that these projections are similar in both normal animals and animals that develop without eyes. Our observations are consistent with the idea that central synaptic connections and the gross topography of projections within the frog visual system develop independently of the retinotopic organization normally imposed on the tecta by the optic tracts.

497 EFFECTS OF β-BUNGAROTOXIN INJECTIONS ON THE DEVELOPING MOTOR NEURONS AND THEIR TARGET. Tony L. Creazzo* and G. S. Sohal (SPON: R. K. HOLT). Dept. Anat., Sch. Med., Medical College of Georgia, Augusta GA 30912.

β-Bungarotoxin (β-BTX), a presynaptic blocker, contains two polypeptide chains (M.W. A chain, 15000-17000; B chain, 7000) and has been shown to have phospholipase A_2 (PLA) activity. Chronic application of β-BTX into the yolk sac of avian embryos produces massive destruction of neurons and skeletal muscle. The destructive effects of β-BTX have been postulated to be due to PLA activity. In an attempt to deactivate PLA activity, β-BTX was heated for three minutes in a boiling water bath. About 80% of PLA activity was abolished and the protein consisted of only one subunit (M.W. about 15000). Effects of heated β-BTX on the development of the trochlear nucleus and superior oblique muscle were examined. Either 2 or 4 µg of toxin/day was applied to the vascularized chorioallantoic membrane of the white Peking duck embryo through an opening in the shell. Embryos injected daily just prior to and during the period of maximum embryonic cell death (days 11 to 18) indicate an increase of about 25% of neurons over the control in the trochlear nucleus. Injections of toxin prior to the onset of cell death (days 8-11) indicate that toxin has no stimulatory effect on cellular proliferation of the young trochlear neurons. Similarly, injections of toxin after the period of cell death (days 21-24) indicate that toxin does not cause a transient increase in the number of trochlear neurons. Thus, it appears that β-BTX increases the number of trochlear neurons when applied during the period of cell death, presumably by retarding the magnitude of cell death. β-BTX delays differentiation of the superior oblique muscle. For example, on day 18 the control muscle is composed of myotubes and myofibers and the motor endplates become morphologically identifiable by cholinesterase stain. The toxin-treated muscle on day 18 is composed of myoblasts and myotubes. The motor endplates do not appear until day 22.

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498 ONTOGENY OF CYCLIC AMP BINDING PROTEINS IN RAT BRAIN. Bruce Culver, Patricia B. Hoyer* and Boyd E. Haley*. Departments of Pharmacology and Biochemistry, University of Wyoming, Laramie, Wyoming 82071.

The cAMP photoaffinity probe [^{32}P]-8-N$_3$cAMP (8-azidoadenosine -3',5'-monophosphate) has been used to study cAMP binding proteins of rat brain. 8-N$_3$AMP specifically substitutes for cAMP and, on photolysis, forms a covalent bond with proteins that bind it. Partial characterization of brain cAMP binding proteins was performed in rats at various stages of development from the twelfth gestational day to 6 months of age. Four different proteins were photolabeled by [^{32}P]-8-N$_3$cAMP. The approximate molecular weights were 127,500; 58,000; 54,000; and 48,000 ± 5% as determined by SDS-polyacrylamide slab gel electrophoresis. Addition of cAMP protected all 4 proteins from photo-incorporating 8-N$_3$AMP. Cell fractionation procedures showed that the 127,500 MW protein (protein A) was found only in the particulate fraction. Protein A exhibited an increase with development, but the function of this protein is not known. The three smaller proteins in order of decreasing MW have been tentatively identified as R_{II}-phosphorylated (R_{II}-P), R_{II} and R_I where R is the regulatory protein of type II and type I protein kinases. These three proteins were found in both soluble and particulate fractions. The R_I protein appeared to increase during development. The R_{II} protein, however, appeared to decrease with age and there was an age-related change in the ratio of R_{II} to R_{II}-P.

The approach of using [^{32}P]-8-N$_3$cAMP as a photoaffinity probe provides a valuable tool to study cAMP-macromolecular interactions. It is proposed that the ontogenic patterns exhibited by different cAMP binding proteins reflects changing physiological roles of components of the cAMP system in the developing brain. The data also suggest that cAMP probably exerts its effects through proteins in addition to types I and II protein kinases.

499 EFFECTS OF PRENATAL AND POSTNATAL NERVE GROWTH FACTOR DEPRIVATION ON THE DEVELOPING MAMMALIAN NERVOUS SYSTEM IN AN AUTOIMMUNE MODEL. Pamela H. Dolkart* and Eugene M. Johnson* (SPON: Thomas Woolsey). Dept. Pharmacol., Washington Univ. Sch. Med., St. Louis, Missouri 63110.

We have previously described an autoimmune model of nerve growth factor (NGF) deprivation whereby adult rats immunized with 2.5S mouse NGF in complete Freund's adjuvant make antibodies which cross-react with rat NGF and which are transferred to offspring in utero and in milk. Anti-NGF activity in the serum of NGF-immunized adults and their offspring, measured in the chick embryo dorsal root ganglia bioassay, is retained in the IgG fraction after ammonium sulfate precipitation and Sephadex G-200 chromatography.

Cross-fostering experiments were used to separate the effects of in utero and in milk exposure to anti-NGF on peripheral sensory neurons, long adrenergic neurons, short adrenergic neurons and adrenal medullary cells. Dorsal root ganglia in 12 week old rats exposed to anti-NGF only in utero were unable to retrogradely transport ^{125}I-NGF injected in the forepaw and had a 20-30% reduction in protein content. Superior cervical ganglia had significant reductions in tyrosine hydroxylase, choline acetyltransferase and protein with either in utero or in milk exposure to anti-NGF. Heart, a tissue innervated by long adrenergic neurons, had greatly reduced levels of norepinephrine with either prenatal or early postnatal exposure to anti-NGF. In contrast, the vas deferens, a tissue innervated by short adrenergic neurons, had no reduction in norepinephrine, and the adrenal gland had no reduction in tyrosine hydroxylase activity. These experiments suggest that a subpopulation of sensory neurons in the rat is dependent on NGF during fetal development for survival. Long adrenergic neurons appear to be NGF-dependent during both prenatal and early postnatal development.

Similar experiments in rabbits indicate that higher titers of anti-NGF are achieved in the fetuses of NGF-immunized female rabbits and greater effects on the peripheral nervous system are observed. Supp. by Natl. Federation March of Dimes, NIH Grant HL-20604 and by NIH Training Grant HL-07275. E.M.J. is an Established Investigator of the AHA.

500 ACTH$_{4-10}$ ENHANCES RETENTION OF CONDITIONED TASTE AVERSION LEARNING IN INFANT RATS. Susan M. Dray & Anna N. Taylor. Depts.of Psychol. & Anat., Brain Research Inst., UCLA, Los Angeles,Ca 90024

Previous studies in our laboratory have indicated that neonatal treatment with ACTH$_{1-24}$ has prolonged effects on physiology and behavior. In view of the known effects of ACTH$_{4-10}$ on extinction, we were interested in whether similar organizational effects might accompany neonatal treatment with this peptide. 34 litters of Sprague-Dawley rats (N=288)were cross-fostered the day after birth (d.1) and injected sc on days 7-9 with either ACTH$_{4-10}$(10 ug in saline) or vehicle. On d.12, pups were maternally deprived and kept with littermates in a warm cage (37^{o}) for 6 hrs. Each pup was then stimulated to void, weighed, and given 0.5 ml apple juice by mouth with a syringe while hand held for 5 min, then weighed again. Wt gain was the index of amount drunk. Litters were divided into thirds for reinforcement, with 1/3 injected with LiCl ip (2 ml/100 g bw, .15M) immediately, 1/3 at 1 hr later, and 1/3 24 hr later. Following conditioning, pups were returned to their mothers. On d.15, pups were again 6 hr deprived, weighed, and given 0.5-0.6 ml apple juice. No conditioned taste aversion (CTA) would be expected with noncontingent pairing of taste and illness, and none was seen on d.15 in the 24 hr grp, regardless of ACTH treatment. At d.12, it is unclear whether infants can form aversions with a 1 hr delay, and, likewise, there was no clear CTA in either ACTH or saline pups in the 1 hr grp. Animals given LiCl immediately did learn to avoid apple juice, but again, no additional ACTH effects were seen. To rule out the possibility that the neonate simply is not able to respond to ACTH$_{4-10}$, 6 litters (N=69) were left undisturbed until d.12, at which time they were conditioned and tested as before. This time, however, half of each reinforcement group was injected sc with 10 ug ACTH$_{4-10}$ or saline 15 min prior to the start of the retention trial on d.15. Again, noncontingent reinforcement (24 hr grp) produced no CTA in either ACTH or saline groups. Saline-treated pups drank 184±19% (SEM, N=10) of their d.12 baseline, while ACTH-treated pups consumed 184±17% (N=11). Pups reinforced immediately showed clear aversions, and, likewise, ACTH made no further difference (Saline: 56±7%, N=11; ACTH: 55±9%, N=10). However, in animals given delayed LiCl (1 hr grp), ACTH made a striking difference. Vehicle pups were mildly averted (134±16% of d.12 baseline,N=12), while ACTH pups reduced their intake to 51±7% (N=12). This peptide, then, aided recall of CTA not seen without it. These results indicate that rat pups are sensitive to ACTH$_{4-10}$, and that they are able to learn a CTA with a 1 hr delay of reinforcement. Supported by NIH grant NS 09122, NSF grant PCM 76-80955 and a UCLA Patent Fund Dissertation Grant.

501 FAILURE OF NGF TO AFFECT FETAL BRAINSTEM CATECHOLAMINERGIC NEURONS IN CULTURE. Cheryl F. Dreyfus, Michael D. Gershon, Edith R. Peterson* and Stanley M. Crain. Dept. of Neuroscience, Albert Einstein Coll. Med., Bronx, New York, 10461 and Dept. of Anatomy, Columbia Univ., P&S, New York, New York 10032.

Catecholaminergic (CA) neurons of the fetal mouse brainstem, explanted from the region of the locus coeruleus, survive in organotypic tissue culture for up to 5 weeks. These neurons exhibit the glyoxylic acid induced histofluorescence (GIF) of CA and their axons become radioautographically labeled by ^{3}H-norepinephrine (^{3}H-NE; Dreyfus, Gershon and Crain, Br. Res. 161, 1979). In the present study we determined whether nerve growth factor (NGF) enhances neuritic outgrowth and/or is necessary for the survival of these central CA neurons. Portions of right and left brainstem, containing the locus coeruleus, were explanted from fetal mice of 13 or 18 days' gestation. Paired sides were grown for 1-3 weeks in either control nutrient media or in media containing NGF (1,10,100 or 1,000 U/ml) or antiserum to NGF (anti-NGF; 1%). The activity of NGF and anti-NGF was bioassayed on cultured dorsal root ganglia. No differences in neuritic outgrowth were detected between control brainstem cultures and their pairs grown with either NGF (up to 100 U/ml) or anti-NGF. Cultures were examined periodically while alive and then were exposed to glyoxylic acid and viewed for CA histofluorescence. A decrease in total neuritic outgrowth as well as the CA component of the outgrowth revealed by GIF, was found in cultures exposed to 1000 U/ml of NGF.

Uptake of ^{3}H-NE was used to evaluate quantitatively the CA neuritic outgrowth. Explants were incubated with ^{3}H-NE (0.5 uM) and nialamide (12.5 uM) for up to 60 min and then washed for 2 hr at 4^{o}C. Uptake was measured into the compartment represented by the slowest single exponential component of the washout curve. Radioautography shows this compartment to be axonal. The uptake process was saturable, temperature dependent, and inhibited by desmethylimipramine (5 uM). No significant differences in uptake of ^{3}H-NE were found between control cultures and their pairs exposed to NGF (100 U/ml) or anti-NGF. Therefore, under these conditions, we could not detect any effect of NGF on the growth or maintenance of cultured fetal central CA neurons. (Supported by the Dysautonomia Foundation and NIH grants NS14990 and NS12969. CFD was a fellow of the Pharmaceutical Manufacturers Association Foundation. Cultures were prepared in Dr. M. Bornstein's laboratory at Einstein.)

502 ALTERED NEURONAL DEVELOPMENT IN HAMSTER OFFSPRING FOLLOWING MATERNAL ETHANOL CONSUMPTION. C.R. Dunmire-Graff* and F.W. LaVelle* (SPON: A. LaVelle). Dept. Anat., Loyola Univ. Stritch Sch. Med., Maywood, IL 60153.

Previous work in this laboratory has described a developmental sequence for the structure of the nucleolus in maturing neurons of the hamster facial motor nucleus. A young neuron initially possesses multiple nucleoli but acquires a single nucleolus as it matures. In the adult hamster this single nucleolus is capped by nucleolar-associated chromatin (DNA) and often contains a central, RNA-rich intranucleolar body (INB) about 1.0 to 2.0 μm in diameter. Among facial motor neurons, many of the cells reach their adult nucleolar appearance by 20 days postnatal age.

In this study, facial motor neurons from 20-day-old hamsters born to control and to ethanol-consuming females were compared at the light microscopic level. The day following mating, female hamsters were either continued on their standard diet of water and laboratory chow (control) or were placed on a regimen of a 15% ethanol solution and laboratory chow (experimental). This regimen was maintained throughout pregnancy and the postnatal suckling period. After weaning, the pups ate and drank the same diet provided to their mother. Twenty-day-old offspring were subsequently weighed, anesthetized with sodium pentobarbital, and perfusion-fixed. The brains were double-embedded and serially sectioned at 4 μm; sections were stained with buffered thionin. Each facial motor neuron exhibiting some type of nucleolar structure was judged to possess either multiple nucleoli (Mult) or a single nucleolus containing no INB (A), a punctate INB (Pu), or a prominent INB (Pr).

Counts in 20-day-old controls yielded the following results: Mult = 14%, A = 9%, Pu = 27%, Pr = 50%. Twenty-day-old offspring of the ethanol-consuming mothers fell into two groups: (1) seemingly healthy animals having a mean body weight close to that of the controls (24 gms), and (2) "runts", which appeared weak, were unable to walk properly, and had a mean weight of 11 gms. Counts of nucleolar structure yielded the following: (Group 1) Mult = 27%, A = 14%, Pu = 20%, Pr = 39%; (Group 2) Mult = 36%, A = 11%, Pu = 25%, Pr = 28%. Group 1 and Group 2 percentages both reflected more immature neuron populations as compared with controls, thus suggesting that ethanol may play a role in retarding the developmental sequence of nucleolar structure in neurons. Central chromatolysis and extensive nuclear infolding were also evident in both experimental groups, indicating further alterations in the synthetic mechanism of developing neurons exposed to ethanol. How much of the increased retardation seen in runts was directly due to ethanol and how much indirectly to malnutrition is not as yet clear.

503 GROWTH AND DEVELOPMENT OF THE OPTIC NERVE IN JUVENILE GOLDFISH. Stephen S. Easter, Jr., Phillip E. Kish*, and Steven S. Scherer*. Div. Biol. Sci., U. Michigan, Ann Arbor, MI 48109.

Previous studies have shown that the retina of juvenile goldfish grows by adding new cells, including ganglion cells, whose axons make up the optic nerve. We describe the optic nerve in young and old goldfish and find structural changes which accompany retinal growth. Optic nerves and tecta were prefixed in situ with buffered aldehydes, osmicated, and embedded in Epon. Semithin sections were stained with toluidine blue and examined light microscopically. Thin sections were stained with U and Pb and examined electron microscopically.

The table gives a quantitative comparison of the optic nerves of young and old fish (ages: 1 and 5 yr; standard lengths: 4 and 12 cm; weights: 1.4 and 67 gm; lens diameters: 1.6 and 4.0 mm.)

Mean values in:	young (N=4)	old (N=3)
Nerve cross section (um^2)	56,780	362,155
Number of myelinated fibers	115,000	179,000

In the orbit, the optic nerve is accompanied by the retinal vessels and a small nerve, containing tens of fibers, which enters the choroid. Glial processes divide the optic nerve into fascicles, the number of which varies depending on the level of section. Small groups of fibers commonly cross between fascicles. In individual nerves, fiber diameters vary by more than tenfold. Within each fascicle, the fiber diameters and myelin sheath thicknesses are relatively homogeneous. Neighboring fascicles are generally more similar in these respects than fascicles widely separated. In small fish, there is always one fascicle which contains nearly exclusively non-myelinated fibers which number a few thousand and measure a few tenths of um in diameter. These are probably the fibers from the new ganglion cells because: 1) new fibers are expected to be non-myelinated and 2) these fibers segregate in the tectum to those regions innervated by peripheral retina, where the new ganglion cells are formed. In the large nerves, there are many fewer non-myelinated fibers, and they are not localized to a single fascicle.

These observations suggest that fibers of the same age fasciculate together in the nerve. The intrafascicular homogeneity of fiber size and myelin thickness probably results because these fibers have all grown and developed over similar periods. The similarity of nearby fascicles suggests that new fibers fasciculate near preexisting fascicles of slightly older fibers. The relative absence of non-myelinated fibers in nerves of large animals suggests that these animals' retinas have stopped adding ganglion cells.

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504 FUNCTIONALLY DISTINCT FACTORS SUPPORTING THE SURVIVAL OF CHICK SENSORY AND SYMPATHETIC NEURONS IN CULTURE. David Edgar*, Yves Barde* and Hans Thoenen. Dept. Neurochem. Max-Planck-Institut, D-8033 Martinsried, W. Germany.

Culture medium conditioned by C-6 glioma cells is able to support the survival of sensory neurons from dissociated chick embryo spinal ganglia. This property is also shared by a well characterised protein, the nerve growth factor (NGF), together with media conditioned by embryonic chick heart cells in culture, and extracts of adult rat central and peripheral nervous system. It has been demonstrated that the activities of these conditioned media and tissue extracts are not due to the presence of NGF, as specific anti-NGF antibodies, while able to inhibit the survival and neurite outgrowth induced by NGF, do not inhibit the effects of the conditioned media or tissue extracts. Here we show that the response of sensory neurons to glioma conditioned medium (GCM) differs from that to NGF: culturing neurons from chicks of embryonic ages ranging between day 7 and day 15 in ovo revealed that early in development, NGF is more active than GCM in supporting sensory neuronal survival. However as the sensory ganglia mature, NGF becomes less potent whereas GCM is able to support the survival of an increasing proportion of the neurons plated.

It is suggested that NGF is one of the factors necessary for the early survival of embryonic chick sensory neurons, which subsequently become dependent on factors such as that present in GCM. We further show that the responses of sympathetic neurons, dissociated from embryonic chick para-vertebral ganglionic chains, differ when supplied with NGF or GCM, thus adding support to the concept of functionally distinct factors. That NGF and GCM represent members of two families of factors, differentiated by the developmental stage during which they exert their activity, will be discussed with reference to those activities present in heart cell conditioned medium and extracts of nervous tissue.

505 LASER LESIONS OF EMBRYONIC CRICKET CERCI DISRUPT GUIDEPATH ROLE OF PIONEER FIBRES. John S. Edwards, Michael W. Berns* and SuWan Chen*. Dept. Zool., Univ. Wash., Seattle, WA 98195 and Devel. Cell. Biol., Univ. Calif., Irvine, CA 92717.

The normal development of neuronal connections between embryonic abdominal sensory appendages, the abdominal cerci, and the central nervous system occurs in two phases. The first of these, which occurs at about 50% elapsed time in the developmental sequence precedes elongation of the appendage. Apical bodies of these pioneer fibres underly the epidermis and are associated with glial cells situated along the dorsal and ventral midlines. It is along this pathway that axons from functional sensilla subsequently make their centripetal growth in the final 15% of the developmental sequence.

In order to assess the role of the pioneer fibres as a necessary pathway for the organisation of the cercal nerve within the cercus, embryonic cerci were lesioned in situ by means of a laser microbeam at several stages of development immediately before, during, and after projection of pioneer fibres. Treated embryos were allowed to develop to later stages when neuronal and glial cell populations were examined by transmission electron microscopy.

Apical lesions of cerci that had newly reached full length and had thus completed pioneer fibre formation did not cause significant alteration to the configuration of the dorsal and ventral bundles of functional afferents as sampled in hatchlings.

In contrast, the effect of apical lesions made before the development of pioneer fibre bundles and accompanying glial cells resulted in the subsequent development of multiple bundles of functional fibres within the cercus lumen.

We conclude that the organisation of the neuronal and glial components of the pioneer fibre pathway is necessary for the orderly projection of functional axons. However, once established, the glia alone, having survived the elimination of pioneer neurons appear to be sufficient to organise axon bundles in their normal position.

Passage of axons from the base of the cercus across the hemocoel to the CNS may present more stringent requirements, including intact pioneer fibres. Supported by NIH grants NB 07778 (JSE) and GM 23445 (MWB).

506 STUDY OF PROLIFERATIVE CELL COMPARTMENT IN DEVELOPING CNS BY COLONY CULTURE ASSAY. S. Fedoroff, L. Doering*, B.H.J. Juurlink* and C. Hall*. Department of Anatomy, University of Saskatchewan, Saskatoon, Saskatchewan, Canada. S7N OWO

When the cells of developing CNS are mechanically dissociated and planted in appropriate concentrations in cultures, cell colonies develop. The prerequisite for the formation of a colony is that a cell must adhere to the substratum (plastic) and proliferate. We investigated the possibility of using cell colony formation in cultures as an assay to monitor the proliferative cell compartment during CNS development.

The spinal cord of chick embryos, cerebral hemispheres of mouse embryos at different developmental stages, and the hemispheres of postnatal mice were isolated and the meninges were removed. The basal ganglia and hippocampus were separated from the hemispheres. The spinal cord and hemispheres were then dissociated into cell suspensions which were planted in various concentrations in Falcon plastic dishes. After 7, 10 and 14 days of culturing, the numbers of colonies formed as a function of the size of innocula were determined by direct counts under a light microscope.

The chick embryo spinal cords yielded only few colony forming cells until 4.5 days (E4.5) of development. Between E6.5 and E7.5 there was an exponential increase in the yield of colony forming cells; the peak yield was reached at E11.5.

The youngest mouse embryos used were E11. The hemispheres of such embryos yielded colony forming cells but the peak yield was reached at E15. Postnatally, the yield began to decrease gradually and at P30 only a few colony forming cells were present.

In another series of experiments the mouse cerebral hemispheres were cut into small portions which were divided into two fragments: An outer fragment containing the marginal zone, cortical plate and most of the intermediate zone, and an inner fragment containing a small portion of the intermediate zone, the subventricular zone and the ependymal layer. Such fragments were dissociated and planted in culture dishes. It was found that 75% of colony forming cells came from the subventricular region of the brain. The ultrastructure of the cells forming the epithelial type colonies was similar to that of the "pale" cells of the subventricular zone.

These studies indicate that the colony forming cells are derived primarily from the secondary proliferative zones such as the subventricular zone and that their formation in the CNS and their progression into postmitotic cells can be monitored with the aid of colony culture assay method. This work was supported by Grant MT4235 from MRC Canada.

507 POSTNATAL ONTOGENY OF HYPOTHALAMIC EXTRACELLULAR UNIT ACTIVITY IN THE RAT. Robin S. Fisher and C. Robert Almli. Dept. Psychol., Ohio Univ., Athens, OH 45701.

Extracellular single unit activity was obtained from the lateral hypothalamic area (LHA) and the ventromedial hypothalamic nucleus (VMH) in rats of 1-2, 5-6, 10-11, 25-26, and 90-110 days of age. The following unit activity characteristics were measure for comparison between age levels: spontaneous unit firing rate, unit response to external sensory stimulation (tactile, gustatory and olfactory stimuli), and unit response to variation of interna physiological parameters (hypertonic saline or glucose injection)

No clear or significant ontogenetic trends were found for any of these simple aspects of unit activity. Spontaneous unit firin rates were very low across age levels (X basal rates of 1 spike/sec with over 80% of all units having basal rates below .5 spikes, sec). By the most stringent criterion employed in this study, 69-80% of LHA units were responsive to external sensory stimulati across all age levels. Slightly lower proportions of VMH units were responsive to tactilem gustatory, or olfactory stimulation (54-72% across all age levels). Finally, hypertonic saline or glucose injection altered LHA and VMH unit activity in nearly all age levels. Unit responses to internal and external stimulation were determined by statistical criteria for single stimulus trial or averaged across stimulus trials. Such criteria were highly reliable and indicated that the employed forms of stimulation could either facilitate or diminish firing frequency in both LHA and VMH units. Thus, no typical forms of unit responses to stimulation were noted for particular neural regions or for particular age levels.

Significant age-related trends were found for more complex data interrelationships. VMH units decreased basal firing rate ranges as a function of age while LHA unit basal firing rate ranges reciprocally increased. Furthermore, both VMH and LHA units tended to become responsive to exclusively internal or external sources of stimulation as aging progressed. Therefore, the failure to find ontogenetic trends in the simple aspects of unit activity indicated a qualitative maturity throughout the postnatal life of the rat that correlated well with the age-constant aspects of ingestive behaviors recently elaborated by numerous investigators. The postnatal maturation of more complex characteristics of LHA and VMH unit activity represented a process that would only allow for postnatal "fine-tuning of quantitative aspects of ingestive behavior regulation.

508 IN VIVO AND IN VITRO ONTOGENY OF TORPEDO ELECTROCYTES. G.Q. Fox*, G.P. Richardson* and C.Kirk*(SPON: J.D. LANE). Max-Planck Inst. für biophy. Chemie. 3400 Göttingen, West Germany.

The electric organ of Torpedo marmorata is derived from branchial mesoderm. It is composed of several hundred columns each consisting of vertically oriented myotubes. The myotubes are characterized by myofibrils, diffusely distributed acetylcholine receptors and contractility. With development, the vertical myotubes are converted to horizontally flattened electrocytes. Concurrently myofibrils are broken down and contractility is lost. Acetylcholine receptor becomes localized along the ventral plasma membrane as does cholinesterase. The latter is also found on the ventral basal lamina which forms at this time. The mechanics of transformation can, in part, be explained by the appearance of filamentous structures with possible contractile properties. Synaptogenesis begins upon completion of this cellular transformation with axons from intercolumnar nerves invading the interelectrocyte spaces. The axons course along and form synapses exclusively along the ventral electrocyte membrane. Electrocytes continue to enlarge throughout life with increased multinucleation caused by fusion of satellite cells found along the dorsal surface of each electrocyte.

It is now possible to maintain viable explants of embryonic electric organ and our results suggest that the in vitro system closely parallels many events seen in vivo. For example, with myogenic electric organ explants, myoblasts migrate out, form myotubes and subsequently differentiate into electrocytes. Myofibrillogenesis and breakdown occurs, cells round up, and distinctive regions comparable to in vivo ventral and dorsal plasma membrane form. Acetylcholine receptor and basal lamina are found exclusively associated with ventral plasma membrane. Electric organ explants in which differentiation of electrocytes has occured similarly produce a migration of satellite cells that also form myotubes. Unlike those from myogenic explants these produce substantially more myofibrils. On contact with nerve(i.e. co-cultured electric lobe) contraction is initiated. Experiments are currently underway exploring possible reciprocal differential effects between electric organ and electric lobe explants.

509 NOVEL PERIPHERAL TARGETS OF SENSORY GANGLION CELLS CAN MODIFY CENTRAL PROJECTIONS IN THE SPINAL CORD. E. Frank and M. Westerfield* (spon: R. W. Baughman). Dept. Neurobiology, Harvard Medical School, Boston, MA 02115.

How do sensory neurons establish specific synaptic connections within the spinal cord which are appropriate to the particular muscle or patch of skin that they innervate? One possibility is that a target in the periphery may specify the central connections to be made by the sensory cell that innervates it. We have explored this possibility in developing bullfrog tadpoles by forcing sensory cells to innervate novel peripheral targets.

In normal adult animals, the arm is innervated almost entirely by the 2nd spinal nerve. The 3rd spinal nerve innervates muscle and skin of the thoracic body wall and only occasionally sends a few fibers into the nerve innervating the arm. However, sensory neurons in the 3rd dorsal root ganglion (DRG) will take over the territory of the 2nd DRG, if it is removed at early to middle limb bud stages and the tadpoles are allowed to mature into adults.

Functional innervation of the forelimb by 3rd nerve fibers was first established behaviorally; frogs withdrew the arm on the operated side when it was pinched, and this withdrawal was abolished by severing the 3rd nerve. Later anatomical studies showed that the 3rd dorsal root was often twice its normal diameter. The 2nd and 3rd spinal nerves formed a plexus on the operated side and fiber bundles could be traced from the 3rd spinal nerve into the arm nerve. Central projections of 3rd DRG cells on operated and control sides were traced by backfilling the spinal nerves with horseradish peroxidase (HRP). In most animals, sensory fibers on the operated side projected into the brachial region of the spinal cord (where motoneurons innervating the arm are located) much more extensively than on the control side, or than in unoperated animals. These unusual anatomical projections presumably underlie the arm withdrawal seen in operated animals.

Central rearrangements of sensory projections were not sufficient to produce completely normal function, however. Even though an operated animal could use his arm, the arm was often held in an abnormal position. In several of these animals, central projections of fibers from the 3rd DRG providing sensory innervation of the triceps muscles were traced by backfilling the muscle nerves with HRP. These fibers did not appear to reach the brachial region of the spinal cord where normal triceps sensory afferents (from the 2nd DRG) project. The behavioral and anatomical results therefore show that removal of a dorsal root ganglion can produce distinct but limited alterations in other reflex pathways. NS 00212 & NS 14451.

510 MATERNAL USE OF ALCOHOL, CIGARETTES AND/OR MARIHUANA DURING PREGNANCY: EFFECTS UPON THE OFFSPRING. Peter A. Fried* (SPON: W.G. Webster). Dept. Psychol., Carleton University, Ottawa, Ont. Canada K1S 5B6.

A sample of 200 predominantly middle class women were interviewed once during each trimester and questioned about their usage of alcohol, cigarettes and marihuana. The babies of a number of these women were examined at birth, 2,9 and 30 days of age on a variety of tests that included assessment of behavioral and neurological development. A reduction in birth weight was observed in babies born to mothers who smoked more than 10 cigarettes a day or drank an average of just over a drink or more a day or smoked more than 5 "joints" of marihuana per week. Cigarettes had the most marked effect and a combination of these soft drugs did not greatly potentiate the reduction in birth weight. Cigarettes, and to a lesser degree, social use of alcohol were related to smaller head circumferences in the newborn. A decrease in responsiveness to auditory stimuli was found in the offspring of smokers and habituation rates were slower among babies born to smokers and marihuana users. A very pronounced increase in irritability was observed in the offspring of marihuana users and, to a lesser degree, in the babies of heavy smokers. A large increase in tremors and startles was related to marihuana usage during pregnancy and to a slightly lesser extent were also found in the offspring of heavy social drinkers and/or smokers. The relationship between the social use of soft drugs and subtle neurological abnormalities in offspring are discussed.

511 AGE-DEPENDENCE OF TRANSNEURONAL DEGENERATION IN THE PIRIFORM CORTEX OF THE RAT. B. Friedman* and J.L. Price (SPON: M.B. Bunge). Dept. Anat. & Neurobiol., Washington Univ. Sch. Med., St. Louis, MO 63110.

Previous studies of the effect of deafferentation on the piriform cortex have demonstrated growth of the cortical association fibers into the denervated layer Ia following olfactory bulb removal in neonatal rats, and the absence of similar reactive fiber growth in adults. In neonates the expansion of the cortical projection is associated with the continued growth of layer I (composed of the apical dendrites of the cortical neurons) to nearly its normal mature width, while in adults the absence of reactive growth of the association fibers is correlated with shrinkage of layer I and atrophy of the distal portion of the apical dendrites (Caviness, Korde, and Williams, '77; Friedman and Price, '78). In addition, olfactory bulb removal in adult rats produces a severe and rapid transneuronal degeneration in the piriform cortex especially among the cells in the outer part of layer II (IIa). These cells become argyrophilic within 16 hours and subsequently are lost completely (Kalil and Heimer, '78). The present study has examined the response of layer IIa cells to olfactory bulb removal in neonatal rats.

In rats examined 24-48 hours after olfactory bulb ablation on the day of birth (P-1) with the de Olmos Cu-Ag method, no cellular argyrophilia was seen in the piriform cortex, although axonal degeneration was clearly evident in layer I. In contrast, rats examined at the same survival periods following olfactory bulb ablations at P-21 show conspicuous cellular argyrophilia concentrated in layer IIa.

Previous studies in adults using horseradish peroxidase (HRP) as a retrograde axonal marker have also shown that IIa cells can be characterized by distinct connections to the anterior olfactory nucleus (AON), olfactory tubercle (OT) and the lateral entorhinal cortex, and that these cells lack a centrifugal projection back to the olfactory bulb (Haberly and Price, '78). Therefore, other animals were allowed to survive into adulthood following olfactory bulb ablation on P-1, then injected with HRP in the AON or OT and their brains processed with tetramethyl benzidine. Although the cytoarchitectonic distinction of layer IIa is not clear in these animals, cells were demonstrated in the outer part of layer II which had connections typical of the IIa cells. These results indicate that following neonatal deafferentation, the IIa cells do not undergo transneuronal degeneration and survive into adulthood. This is in contrast to other systems where cellular degeneration following deafferentation is more severe in immature animals than in adults.

512 QUANTITATIVE ANALYSIS OF NEURONAL DEVELOPMENT IN THE GUINEA PIG RETINA. Keith R. Fry* and Arthur W. Spira* (SPON: M.A. Bisby) Lions Sight Centre,Division of Morphological Science,Faculty of Medicine,University of Calgary,Calgary,Alberta,Canada T2N 1N4

Differentiation of many of the main components of the guinea pig retina e.g. synapses, outer segments appear well advanced at birth compared to many other species (Spira,Anat.Embryol. 146: 279, 1975). How do populations of neurones change throughout development and when do these populations acheive full differentiation? Quantitative light microscopic techniques have been employed to study neuronal development in the guinea pig. Retinae from animals ranging in age from 35 days post-coitus to 100 days (gestation=65 days) postnatal were processed for light microscopy. Orientation in the retina was maintained throughout the study. Quantitative analysis was performed using a program developed from the Abercrombie method (Anat.Rec. 94:239, 1946) with correction factors for split profiles and underestimation of mean nuclear diameter (Wimer,Brain Res. 133:376,1977). Nuclei of the inner nuclear layer (inl) were classified by their nuclear morphology, location within the inl and the cytoplasmic:nuclear ratio to one of the following categories: (i) horizontal (ii)amacrine (iii) rod or cone bipolar (iv) undifferentiated and Müller. Number of each type of nuclei occupying a column through the retina 10 µm by 10 µm were determined for each age.

Numbers of photoreceptor cells decline throughout fetal life until birth at which time adult levels are attained. Similarly total cell number in the inl declines rapidly until 55 days post-coitus and then undergoes no further change. Analysis of individual neuron types demonstrated that some types do undergo changes in number after birth although total inl counts do not change. Amacrine cells undergo a rapid increase in number between 45 and 60 days post-coitus and continue to increase slowly in number after birth. Rod bipolar cells increase in number rapidly between 50 and 60 days post-coitus and also demonstrate a further gradual increase in number during postnatal life. Cone bipolar cells reach peak levels at 55 days post-coitus but decrease in number slightly until maturity. The appearance of new amacrine and rod bipolar cells must be due to differentiation of undifferentiated neurons. This is substantiated by the observation of significant numbers of undifferentiated neurons at birth which gradually decline until about 100 days postnatal.

The major period of neuronal differentiation in the retina occurs between 45 and 60 days post-coitus. Selected neurons in the inl undergo a second stage of differentiation which continues through the first 1-2 months of postnatal life.

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513 ONTOGENY OF THE RETINOTECTAL CONNECTION IN XENOPUS LAEVIS. Steven Glasser, Dept. Ophthal., N.Y. Med. Col., Valhalla, N.Y. 10595.

An ordered projection of retinal ganglion fibers can be found on the tectal surface throughout most of Xenopus larval life. The existence of this ordered retinotopic projection has been taken to imply that ordered retinotectal connections are formed during ontogeny. However, since there are asymmetries in the development of retina and tectum, Gaze, et.al. have proposed that connections between retinal and tectal cells are temporarily formed and later broken. Their hypothesis, further states that during the growth of the eye and tectum there is a continuous caudomedial shift of synaptic contacts between retinal ganglion fibers and tectal cells.

In the present study, I have isolated visually driven postsynaptic tectal unit activity in larval Xenopus laevis. Utilizing standard electrophysiological techniques, postsynaptic visually driven units were isolated and characterized first in the rostrolateral tectum of adult and juvenile Xenopus laevis. These cells, found in the deep tectal layers, responded to larger stimulus targets within larger visual receptive fields than did retinal ganglion terminals. Postsynaptic units, unlike presynaptic fibers, also habituated to the successive repetition of a visual stimulus. Furthermore, the postsynaptic response could be eliminated by the topical application of curare or sodium pentobarbital, whereas presynaptic activity was unaffected by these drugs.

Visually driven retinal fiber terminals were found in the rostrolateral tectum of the youngest animals studied (stage 49). However, on the basis of criteria established in the adult and juvenile preparations, postsynaptic units could be isolated no earlier than stage 52-53.

The implications of these findings will be discussed.

514 ORIGIN, TRANSFORMATION, AND DEATH OF NEURONS FROM AN IDENTIFIED PRECURSOR DURING GRASSHOPPER EMBRYOGENESIS. C.S. Goodman, M. Bate*, and N.C. Spitzer. Dept. of Biology, UCSD, La Jolla, CA 92093, and Max-Planck-Institut für Virusforschung, Tübingen, FDR

We are interested in the differentiation of identified neurons from their birth to their maturation during grasshopper embryogenesis. We have reported that an identified neuroblast gives rise to identified neurons, and described their morphological, physiological, and biochemical differentiation. Here we report on the differentiation of neurons that are the progeny of a different class of precursor cells. There are seven of these cells per segment, called midline precursors (MP 1-7), which lie anterior to the dorsal unpaired median (DUM) neuroblast whose progeny were the subjects of our previous study.

The progeny of the single division of cell MP3 were examined by serial section reconstructions of light microscopic sections, direct observation of cells with interference contrast optics, and intracellular dye injections with Lucifer Yellow. We find that the two progeny are initially bilaterally symmetric. Each sends a single process anterior in the contralateral longitudinal fiber tract which becomes a ventral nerve cord. These processes and those of the progeny of the other MP cells are the first to appear in the embryonic CNS, and have been implicated in the guidance of the axons from the progeny of neuroblasts.

Several days after initial process extension, when there are numerous axons in the longitudinal fiber tracts and commissures, one of the pair of progeny of MP3 in the metathoracic ganglion begins to grow a second process. This second process bifurcates at the posterior commissure, and bifurcates again in each of the longitudinal fiber tracts. These processes then extend in both nerve cords, to the brain and to the terminal abdominal ganglion. Within a few days thereafter the first unilateral process disappears and the cell fully differentiates into the unpaired and bilaterally symmetric identified "H" neuron. Its sibling often dies at this time; when it persists it retains only its original unilateral process.

During the course of embryogenesis, the soma of the "H" cell assumes a position immediately anterior to the oldest progeny of the DUM neuroblast. Thus we have been able to follow the origin and transformation of a single cell which seems to assume two different roles during embryogenesis. It first appears as one of a pair of putative "pioneer" fibers and second transforms into an unpaired identified neuron.

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515 TRANSLATION IN VITRO OF RAT BRAIN mRNA CODING FOR A VARIETY OF TUBULIN FORMS. Illana Gozes[1], Annie De Baetselier* and Uriel Z. Littauer*. Department of Neurobiology, The Weizmann Institute of Science, Rehovot, Israel. [1]Present address: Laboratory of Neuroendocrine Regulation, Department of Nutrition and Food Science, MIT, Cambridge, MA 02139.

Tubulin, the subunit protein of microtubules, is present in all eukaryotic cells and has been found to have a variety of structural and dynamic roles in cell shape, division, motility, transport and secretion. In the nervous tissue,neurite outgrowth and axoplasmic transport are thought to be dependent on microtubule integrity. The question therefore arises as to whether changes occur in the tubulin structure upon assumption of different roles within the nerve cell.

We have previously shown that extensive tubulin microheterogeneity is prominent in the brain, whereas tubulin isolated from other organs is less heterogeneous. Mature rat brain tubulin can be resolved by isoelectric focusing into nine distinct components. Different proportions of these nine isotubulins are displayed by various brain regions, resulting probably from the heterogeneity of the brain cell population. Brain tubulin microheterogeneity is developmentally determined, increasing from five to six components (isotubulins 1-6) in the prenatal rat brain to nine components in the mature brain (isotubulins 1-9). It was therefore of interest to determine whether the age-dependent increase in tubulin microheterogeneity is controlled at the RNA level or results from post-translational modifications.

In the present study, mature brain mRNA was translated in vitro in the reticulocyte lysate cell-free system, and was found to direct the synthesis of five tubulin forms, namely isotubulins 1,3,4(or 5),6 and 7. The mRNA species coding for isotubulins 3 and 7 could be partially separated on formamide-sucrose gradients while in the absence of formamide, the mRNA species directing the synthesis of isotubulins 1,4(or 5) and 6 showed differences in mobility. It therefore appears that brain mRNA may consist of five different species coding for distinct tubulin forms. Moreover, a marked age-dependent enhancement in the relative translation of the mRNA coding for isotubulin 7,which is not apparent among the translation products directed by the prenatal mRNA,was detected. Thus, some of the age-dependent increase in tubulin microheterogeneity might be controlled at the mRNA level.

516 EFFECT OF EYE ROTATION ON THE DEVELOPMENT OF OPTIC FIBER PATHWAYS IN XENOPUS LAEVIS. Philip Grant* and PoKay M. Ma* (SPON: D.P.Kimble). Dept. Biol., Univ. of Oregon, Eugene, OR 97403.

The rotation of embryonic eyes is a classic experimental procedure in studying retino-tectal connections in amphibia. The optic fiber (OF) pathways that developed after such operations, however, have not been adequately studied. Conceivably, the manner whereby OF return to the tectum may play an important role in reordering connectivity. To see whether the optic pathways that develop are dependent upon local environmental conditions encountered by growing OF as they emerge from a rotated eye or are determined by factors intrinsic to the eye, we rotated Xenopus eyes at embryonic stages 21/22 to 33 and traced the OF pathways by proline radioautography after mid tadpole stages. Several rotation paradigms were compared, including in situ rotations and isochronic or heterchronic transplantations (eyes transplanted and rotated between animals at the same or different stages).

90% of control animals developed normal optic pathways to the tectum. In all experimental groups however, the pathways that developed seemed to depend upon (1) the stage at which the operation was done, and (2) the local condition around the optic nerve head at the time of the operation. About 65% of eyes rotated at stage 21/22 (optic vesicle stage) developed normal optic pathways. The principal abnormal pathway is the trigeminal pathway. OF grew into and through the ipsilateral trigeminal ganglion to the trigeminal (Vth) root. Most fibers proceeded caudally along the descending tract of the trigeminus into the spinal cord. A high percentage of oculomotor pathway is also observed. OF followed the ipsilateral oculomotor nerve into the ventral tectum, where some crossed to the contralateral tectum. 50-85% of the eyes rotated at later stages developed abnormal pathway, less than 20% followed a normal route via the chiasma. Multiple as well as single (normal and/or abnormal) OF pathways were observed. The early stage operations gave a higher proportion of single pathways than later stage rotations. Most pathways involved the trigeminal and oculomotor pathways.

The high incidence of trigeminal pathways can be explained by the fact that the trigeminal ganglion anlage develops dorsocaudally over the eye. When OF emerge from rotated eyes, they are frequently intercepted by one or more of the several developing trigeminal projections, following these projections into the ganglion and subsequently the Vth root. Other aberrant pathways may develop in a similar fashion. OF display no preferences for specific pathways but tend to follow any accessible fiber pathway.

517 THE DEVELOPMENT OF AFFERENTS AND MOTONEURONS IN RAT SPINAL CORD. Carolyn Smith Grobstein* (Spon: M. Hollyday) Dept. Biology, Univ. Chicago, Chicago, IL 60637.

The morphology of specific populations of motoneurons and afferents in the thoracic spinal cord was examined in fetal and neonatal rats by exposing either the cut dorsal or ventral ramus of a peripheral nerve to horseradish peroxidase. In the adult, each ramus innervates several thoracic muscles and contains afferents for either dorsal or ventral skin. The morphology of the afferents and motoneurons from the 14-18th days of gestation (birth occurs about day 21) was compared with that at postnatal day 10.

At all ages studied, a centro-medial cluster of motoneurons was filled from the dorsal ramus, while the ventral ramus labelled a distinctive lateral pool as well as more medially located cells. This differential distribution of motoneurons was evident in animals in which endplate formation had not yet begun (Kelly and Zacks, J. Cell Biol. 42: 154, 1969). In fetuses a population of cells was also filled in a part of the spinal cord not occupied by motoneurons in older animals. These cells were located more dorsally and medially. They had axons in the ventral root, and, like some motoneurons, frequently had a process which terminated in the ventricular zone. The number of such cells decreased during the prenatal period, and few were seen by day 18. These cells may be migrating motoneurons. The fetal motoneurons appeared immature in that they often had dendrites extending into the ventral white matter and lacked the longitudinally directed dendrites characteristic of more mature motoneurons.

Some dorsal root ganglion cells were filled in fetuses at day 14, indicating the presence of afferent axons in the periphery. Afferent fibers with clearly identifiable growth cones were observed in fetal spinal cords from the 15-18th day. Afferent growth cones were in the vicinity of motoneurons on day 16, and by day 18, afferent contacts with stained motoneurons were frequent. When significant afferent branching in the dorsal horn is first observed, at day 18, both dorsal and ventral ramus afferents have their major arborizations in the restricted regions to which they project in rats 10 days after birth.

These observations indicate that: 1) axons of motoneurons form an adult-like pattern of peripheral innervation before the formation of synapses with muscle begins; 2) afferents contact motorneurons before the motoneurons have attained their mature morphologies and 3) the central processes of afferents in a given peripheral nerve grow preferentially into specific regions of the dorsal horn.

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518 THE DEVELOPMENT OF THE CORTICAL COLUMN: A (^{14}C)-2-DEOXYGLUCOSE STUDY IN THE RAT. P. Hand, M. Kossut*, U. Patel*, and C. Goochee*. Department of Animal Biology, School of Veterinary Medicine, and Institute of Neurological Sciences, University of Pennsylvania, Philadelphia, Pennsylvania, 19104; Department of Neurophysiology, Nencki Institute of Experimental Biology, Warsaw, Poland; and Laboratory of Cerebral Metabolism, NIMH, Bethesda, Maryland 20014.

Previous 2-deoxyglucose (2DG) studies of Hand et al., (Neurosci. Abst., 3,4:1977,78) demonstrated that stroking of a facial vibrissa increased glucose utilization in a single, spindle-shaped cortical column which extended from lamina I to lamina VI. The precision of this system makes it a useful model in which to investigate plasticity. Hand et al., produced significant alterations of labeling in the single column after unilateral ablation of all but one vibrissal follicle (C3), 2-4 days postnatally (P-2, P-4). The column activated by C3 stimulation was discernable only in lamina IV. In order to determine if the diffuse labeling of supra-and infragranular layers was the result of a reorganization of developing axonal terminations or that such diffuse connections were in existence at the time of follicle ablation and did not "retract" to the adult columnar configuration, the single whisker stroking paradigm was instituted in rats on P-2, 4, 6, 8 and 12 (PO=birthdate). Briefly, 5uCi of (^{14}C)-2DG was injected intraperitoneally in the unanesthetized, restrained baby rat, the C3 whisker stroked, and the rat sacrificed at 60 minutes and prepared according to Sokoloff et al., (J. Neurochem. 28, 1977). The results were as follows: (1) no increased cortical labeling was detectable in SI cortex on P-2; (2) the column, 250 um in diameter, was detectable on P-4; but was labeled lightly and involved only the superficial - half of the neocortex (presumptive layers I-IV); (3) on P-6 and P-8, the column was more densely labeled, with densest labeling in superficial layers and light labeling in deeper cortical layers; (4) on P-12, supra-and infragranular layers of the column were more equally labeled; (5) on P-4 to 12, the most densely labeled portion of the column was layer IV.

These findings suggest that results in our previous columnar alteration studies were probably due to a reorganization of ingrowing axonal terminations (P-2 rats) or of newly established connections (sprouting?) in P-4 rats. We postulate that lack of labeling in cortical laminae VI and V, containing neurons projecting to thalamus and brainstem-spinal cord, may be responsible (in part) for the lack of cortical control in neonates. (Supported by grants NS-06716 of USPHS and 76-10-9 of the Sloan Foundation).

519 THE EFFECTS OF TWO CENTRALLY ACTIVE DRUGS ON THE DIFFERENTIATING RAT CEREBELLUM. Richard S. Hannah*, Sheldon H. Roth and Arthur W. Spira*. Divisions of Morphological Science, and Pharmacology and Therapeutics, University of Calgary, Calgary, Canada.

It is currently believed that many therapeutic agents which can cross the placental barrier may produce subtle effects on the central nervous system of the developing fetus and during the lactation period postnatally, on the neonate. It is our hypothesis that these subtle effects may include a reduction in neuronal population resulting from the action of these drugs on the differentiating neuronal membranes, thus preventing the optimum number or types of synaptic contacts from forming. The two drugs chosen for this study were Chlorpromazine and Phenobarbital. Both agents are capable of crossing all membrane barriers and are frequently administered both pre- and postnatally. Chlorpromazine was chosen as a prototype of the phenothiazine tranquilizers and antiemetics and phenobarbital as a prototype of the barbiturates, sedative/hypnotics. Separate groups of time-pregnant Sprague-Dawley rats were given daily I.P. injections of either 0.6 mg/Kg Chlorpromazine or 0.6 mg/Kg Phenobarbital beginning on day 18 post coitus until they were sacrificed on postnatal days 13, 15, 18 and 21. The pups were anesthetized and perfused with a buffered aldehyde mixture. The cerebella were removed, the vermis dissected free and bisected in the mid-sagittal plane. The tissue was then processed for electron microscopy utilizing conventional techniques. Sections (0.5 µm in thickness) were photographed and quantitated for the number of Purkinje cells per mm^2. Both Chlorpromazine and Phenobarbital produced a statistically significant decrease in total numbers of Purkinje cells below control levels. Large numbers of pyknotic cells were observed in both treated groups. Ultrastructurally, Purkinje cell degeneration was marked by chromatin condensation, increased electron density of the cytoplasm and a paucity of axo-somatic synaptic contacts. The climbing fiber input appeared to be the most severely affected. These results indicate that the Purkinje cell population of the differentiating rat cerebellum is susceptable to the actions of centrally active agents during the period of neuronal differentiation, both pre- and postnatally. (Supported by Alberta Mental Health).

520 EFFECTS OF CELL-SUBSTRATUM INTERACTIONS ON THE SURVIVAL AND DEVELOPMENT OF CULTURED SYMPATHETIC NEURONS. Edward Hawrot* (SPON: Story Landis). Dept. Neurobiology, Harvard Medical School, Boston, MA 02115

The maintenance of low-density, dissociated rat sympathetic neurons for long times in culture requires strong adhesion of the extending neurites to the culture substratum. Several chemically-defined surfaces as well as some cell-derived substrata were examined for their effects on the survival, neurite outgrowth, and neurotransmitter development of sympathetic neurons. In comparison to dried collagen films, both three-dimensional hydrated collagen gels and surfaces coated with basic polymers provided a substratum highly adherent for developing neurons. Good survival was obtained and neurites formed a well-attached network in the absence of Methocel, a viscosity-increasing agent, normally added to the culture growth medium in order to mechanically stabilize the neuronal cultures. Surfaces simply coated with polylysine or polyornithine by adsorption did not support long-term culture (>2 weeks). Polylysine covalently linked with glutaraldehyde to an underlying layer of dried gelatin was, however, suitable for long-term culture in the absence of Methocel.

The neurons also attached strongly to a substratum of killed non-neuronal cells (eg. monolayers of rat cardiac myocytes and associated fibroblasts) that had been fixed with paraformaldehyde, heat, ethanol, or trichloroacetic acid. Such surfaces provided good long-term survival and promoted extensive neurite outgrowth under conditions where dried collagen films alone did not support neurite extension. In addition, an extracellular, substrate-associated material produced by certain kinds of nonneuronal cells promoted the long-term adhesion of growing neurites. The adhesive property of this microexudate was sensitive to trypsin, periodate, alkali, and SDS extraction; but resistant to hyaluronidase, chondroitinase, 0.5 M acetic acid, 8 M urea and mercaptoethanol. The glycoprotein, fibronectin, known to form fibrillar extracellular matrices, has been reported to have several of these characteristics.

All the highly adherent surfaces were examined for effects on development of neurotransmitter characteristics. The choice and development of neurotransmitter function was unaffected by the various substrata tested with one exception. A substratum of non-neuronal cells fixed with paraformaldehyde caused an induction of cholinergic properties similar to that seen with nonneuronal conditioned medium.

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521 CHANGES INDUCED BY X-IRRADIATION OF THE MID-THORACIC AND LUMBOSACRAL LEVELS OF NEONATAL RAT SPINAL CORD. Jeanne K. Heard* and Shirley A. Gilmore (Spon: E. A. Lucas). Dept. Anat., Univ. Arkansas Med. Sci., Little Rock, AR 72201.

The present report compares histopathological changes in three groups of rats irradiated at three days of age. The irradiated zone was limited to a 5-mm length of mid-thoracic spinal cord (T only) in one group, to a 5-mm length of lumbosacral spinal cord (L only) in a second group, and to 5-mm lengths of both mid-thoracic and lumbosacral spinal cord (T/L) in the third group. A single dose of 4000 R of soft x-rays was administered to each site. Groups of irradiated rats and their sham-irradiated littermates were killed by formalin perfusion from 9 through 60 days post-irradiation (P-I), and the spinal cords were prepared for light microscopic examination. The occurrence of intramedullary Schwann cells and peripheral-like myelin following irradiation of the same levels of neonatal rat spinal cord has been presented previously (J. K. Heard and S. A. Gilmore, 1978) and will not be included.

Irradiation of Mid-Thoracic Area (T only and T/L irradiated groups): The neuroglial population was decreased in the thoracic area from 9 to 13 days P-I. By 15 days this neuroglial decrease was no longer apparent. Scattered hemorrhages were seen in both gray and white matter from 11 to 17 days P-I. Luxol fast blue-periodic acid-Schiff staining revealed that all funiculi were transiently hypomyelinated in both groups. The dorsal funiculus was particularly interesting in that the fasciculus gracilis showed a greater degree of hypomyelination than did the fasciculus cuneatus from 9 through 15 days P-I. These two fasciculi could not be differentiated on this basis by 19 days P-I, and by 60 days the state of myelination in the irradiated thoracic areas appeared the same as that in controls.

Irradiation of Lumbosacral Area (L only and T/L irradiated groups): Lumbosacral spinal cord areas of L only and T/L irradiated animals showed a decrease in neuroglia at all post-irradiation intervals. This neuroglial decrease was most marked from 13 to 17 days P-I. Scattered hemorrhages were seen at 11 days P-I, and from 15 to 60 days some animals showed large areas of hemorrhagic necrosis and cystic degeneration. The dorsal funiculi were affected first, although the gray matter was also involved in a few animals. The lumbar spinal cords were hypomyelinated throughout the experimental period, with the dorsal funiculi being affected most severely. In general, the lumbar area of T/L irradiated spinal cords showed more necrosis, more dysmyelination, and a greater decrease in neuroglia when compared to the L only irradiated spinal cords. (Supported in part by USPHS Grant NS 04761.)

522 BRAIN CATECHOLAMINES AND BEHAVIORAL CHANGES FOLLOWING NEONATAL ASPHYXIA IN THE RAT. Thomas Hedner* and Per Lundborg* (SPON: N.H. Bass). Dept. Pharmacol. and Clin.Pharmacol., Univ. of Göteborg, S-400 33 Göteborg, Sweden.

In the rat brain, almost all the catecholamines-(CA)-containing nerve cell bodies present in the adult are also present at birth. The rate limiting step in the formation of the neurotransmitters dopamine (DA) and noradrenaline (NA), tyrosine hydroxylase, is markedly affected during oxygen deprivation, as molecular O_2 serves as a substrate for the synthesis of the intermediate substance dihydroxyphenylalanine (DOPA). Neonatal rats were exposed to a low oxygen environment (anoxia or 6% O_2) for various time intervals. DOPA accumulation was measured after NSD 1015 to estimate the rate of tyrosine hydroxylation in vivo and DA and NA levels were followed after α-methyltyrosine to study the nerve impulse activity in the CA neurons. During a short period of hypoxia (6% O_2, 30 min) the synthesis of DA and NA was impaired in the brain stem, "midbrain" and striatum regions of the neonatal rats. 6% O_2 over a 2 h interval also caused a reduced nerve impulse activity in the DA neurons but not in the NA neurons, as measured by DA and NA disappearance after α-methyltyrosine. The acquisition of a conditioned avoidance response (CAR) and the effects on CA neurotransmitter synthesis and levels were investigated in 28 days old rats after neonatal oxygen deprivation. A group of rats exposed to 20 min neonatal anoxia did not differ in avoidance responding compared to controls. However, neonatal rats subjected to 6% O_2 for 4.5 hours were markedly inferior in the CAR acquisition than the control group. CA synthesis, measured as DOPA accumulation after NSD 1015, was impaired in the rats exposed to 6% O_2 for 4.5 h but not in the animals exposed to 20 min neonatal anoxia. It is concluded that brief periods of acute asphyxia markedly affect CA synthesis and turnover in the neonatal rat brain. It is also suggested that the permanent behavioral deficits observed after prolonged neonatal asphyxia may be due to an impaired development of central catecholamine mechanisms.

523 SOME CHARACTERISTICS OF PRE- AND POST-SYNAPTIC MEMBRANES DURING THE DEVELOPMENT OF THE ADRENERGIC INNERVATION OF THE CHICK EMBRYO VENTRICLE. D. Higgins* and A. Pappano* (SPON: Y. Grimm-Jørgensen). Univ. of Conn. Health Ctr., Farmington, CT 06032.

Studies employing catecholamine histochemistry have shown that adrenergic axons first appear on the 11th incubation day in the chick embryo right ventricle (RV). We have studied the functional properties of the membranes of the pre-synaptic adrenergic axons and the post-synaptic cardiac muscle cells near the time of synapse formation. Terminals of developing adrenergic axons were characterized by their ability to transport and retain tritiated norepinephrine (^{3}H-NE) and to secrete previously stored ^{3}H-NE. RV were excised from chicks (9 days after fertilization to 1 week after hatching) and were incubated for 1 hour in Tyrode solution containing ^{3}H-NE (0.55μM). They were then transferred to a chamber where passive overflow of ^{3}H was monitored for 60 minutes. Subsequently release was evoked by elevated potassium (K^+, 140mM) or by 60 seconds of electrical excitation (ES) of intracardiac nerves (50V pulses, 5 msec, 30 Hz).

Cocaine-sensitive uptake and reserpine-sensitive retention of ^{3}H-NE were first seen during the third embryonic week. Release of ^{3}H by K^+ or ES was seen as early as the 14th embryonic day. The amount of ^{3}H released per mg tissue by either method increased more than 30-fold from the 14th to the 28th day. Release was blocked by omission of calcium. Blockade of sodium ion channels by tetrodotoxin inhibited release by ES at all ages tested (14, 18 and 28 days). (Preliminary results show the presence of α adrenergic receptors capable of modulating release on the 21st day, the youngest age tested.) It thus appears that membranes of developing adrenergic axons contain many proteins characteristic of mature axon terminals before prolonged contact with the target tissue has occurred. To test for functional β receptors on cardiac muscle cells, superfused RV were paced at 3 Hz and contractions were recorded before and during exposure to Isoproterenol (ISO). As early as the 5th day after fertilization ISO (1μM) augmented twitch tension. From the 8th to 28th day dose-response curves were obtained and the ED_{50} was used as a measure of sensitivity to ISO. A transient subsensitivity to ISO was seen during the 3rd embryonic week (ED_{50}'s for the 11, 18 and 28th day were 3.2±.3, 29±4.3, 2.3±.5nm respectively). Uptake II blockers did not increase the sensitivity of the RV of 18 day embryos, suggesting that the change in sensitivity was post-junctional. Reserpine (10μg/egg) delayed the onset of functional innervation without affecting the change in ISO ED_{50}. Although a subsensitivity coincides with cardiac innervation, they are probably not causually related. (Supported by HL-13339 and Pharm. Manu. Assoc. Fellowship.)

524 DEVELOPMENT OF SENSORY NEURON PROJECTION PATTERNS UNDER NORMAL AND EXPERIMENTAL CONDITIONS IN THE CHICK HINDLIMB. Marcia G. Honig* (SPON: C. Stevens). Dept. Biol., Yale Univ., New Haven, Ct. 06520.

It has frequently been suggested that before axonal outgrowth sensory neurons are unspecified and that they are later specified by contact with their peripheral targets. Alternatively they may somehow be intrinsically specified. The aim of these experiments has been to distinguish between these two possibilities. In addition the pattern of initial afferent outgrowth has been examined to determine whether it is selective or random.

Although sensory neurons projecting out particular cutaneous or muscle nerves in the chick hindlimb are scattered within individual dorsal root ganglia (DRG) (Honig, 1977, Neurosci. Abst. 3:108), they are predominantly localized in 2-3 adjacent DRGs. This segmental pattern of innervation has been used to determine whether afferents project to their appropriate targets initially during development and after experimental manipulations.

Early stages of axonal outgrowth into the limb have been examined by injection of HRP into DRGs, allowing visualization of labelled cell bodies and their axons. Retrograde transport of HRP and spinal nerve stimulation have been used. The segmental projection pattern for both cutaneous and muscle afferents found shortly after axonal outgrowth into the limb (St 27) prior to the period of DRG cell death and before sensory neurons establish their central connections is similar to that found later (St 38) after muscle cleavage and sensory cell death. Thus from the earliest stages, afferents in particular ganglia grow down the appropriate peripheral nerves, and muscle afferents do not show widespread branching throughout the undivided muscle masses.

After several segments of neural tube (prospective spinal cord and DRGs) have been removed at St 15-16, prior to the birth of sensory neurons, the cutaneous and muscle nerves which originate from this region are missing. The remaining ganglion cells grow down only the appropriate peripheral nerves. Furthermore before muscle cleavage, proprioceptive afferents seem to terminate in only appropriate regions of the muscle and do not innervate adjacent uninnervated areas. These results suggest that lumbosacral sensory neurons demonstrate regional differences before reaching their specific peripheral targets and that they do not need to compete with adjacent segments in order to form appropriate projections. In order to determine whether such regional differences are acquired prior to or upon initial outgrowth into the limb, segments LS 1-3 were reversed along the a-p axis prior to neural crest migration. This reverses both the prospective DRG cells and the spinal cord. Displaced motoneurons form appropriate connections in this situation (Lance Jones and Landmesser, 1978, Neurosci. Abst. 4:118). For sensory neurons the pattern of outgrowth is not completely random and projections down appropriate nerves can sometimes be found from displaced ganglia. These results although preliminary are consistent with the hypothesis that at least some sensory neurons may be prespecified to innervate their appropriate targets. (Supported by NIH NS10666).

525 ABERRANT DENDRITIC PROJECTIONS FROM AN IDENTIFIED AUDITORY INTERNEURON ARE INNERVATED BY CONTRALATERAL AUDITORY AFFERENTS. Ronald Hoy and Andrew Moiseff. Sect. of Neurobiology & Behavior Langmuir Laboratory, Cornell University, Ithaca, N.Y. 14850.

When auditory afferent innervation is withheld from an uniquely identified interneuron (interneuron-1) throughout postembryonic development in the cricket Teleogryllus oceanicus, its medial dendrites grow in a peculiar pattern. Normally, the medial dendrites project to the ipsilateral acoustic neuropile and never cross the midline. However, in unilaterally deprived animals the medial dendrites of the deprived int-1 cross the midline of the prothoracic (auditory) ganglion and project to the acoustic neuropile on the contralateral side. Moreover, morphological studies of cobalt-stained preparations suggest that in unilaterally deprived animals the intact auditory axons of the unoperated side innervate the medial dendrites of both the operated and unoperated interneuron-1s (Hoy, Casaday, & Rollins (1978) Soc. Neurosci. Abst. 4.).

Interneuron-1 has a unique response to acoustic stimulation. At sonic frequencies (5-10 kHz) spontaneous activity in the neuron is strongly suppressed; however at ultrasonic frequencies (20-70 kHz) the neuron is strongly and tonically excited. This unusual response serves as a "signature" by which to identify the neuron physiologically, and subsequent staining with cobalt confirms the identity of the unit as interneuron-1 (Moiseff and Hoy, in prep.).

Unilaterally deprived preparations were physiologically tested to determine whether the deprived interneuron-1 could be activated by acoustic stimulation of the intact ear on the contralateral side. We were able to record units from both the deprived and intact sides that responded to sonic and ultrasonic tones in a manner characteristic of interneuron-1. We conclude that deprived interneuron-1s establish functional synapses, through their medial dendrites, with the contralateral auditory afferents.

526 DEVELOPMENT OF CHICK SYMPATHETIC GANGLIA: MORPHOMETRIC AND BIOCHEMICAL ANALYSIS. K.A. Hruschak*, V.L. Friedrich, Jr., M. Marchi* and E. Giacobini (SPON: M. Wilson). Dept. of Biobehavioral Sciences, Univ. of Connecticut, Storrs, Ct. 06268.

Chicks at the four ages: 10 days in ovo (i.o.), 1 day and 30 days after hatching (a.h.) and adult (360+ days a.h.), were fixed by perfusion and the lumbar paravertebral ganglia, L1-L4, were prepared for electron microscopy. The volume of each whole ganglion was determined from area measurements of serial 20-40μm thick sections. Partial volumes of neuronal somas, neuropil, connectives and blood vessels were determined by point hit counting of 2μm thick sections taken at regular intervals. The absolute volume per ganglion of each compartment was determined as the product of the average volume per ganglion from serial sections and the average partial volume of the compartment. Preliminary results are presented here.

The partial volume of the neuropil increases with age from less than 10% at 10 days i.o. to 35% in the adult. The rate of increase is greatest in the interval before hatching and is substantially less, subsequently. By contrast, the partial volume of neuronal somas is greater than 60% at 10 days i.o. and decreases progressively to about 20% in the adult. Total ganglionic volume increases progressively from 0.01 mm^3 per ganglion to 0.8 mm^3 in the adult. Both neuropil and somas increase in their absolute volume per ganglion. The absolute volume of neuropil per ganglion increases during each of the three intervals studied. The ratio of the absolute volume of neuropil to absolute volume of somas also increases substantially during each interval, from 0.3 at 10 days i.o. to 2 in the adult.

Using the radiochemical micromethod of McCaman and Stetzler (J. Neurochem. 1976, 26:996) the levels of acetylcholine (ACh) and choline were also measured in chick lumbar sympathetic ganglia. Data are expressed as pmoles/ganglion. ACh increases over 90 fold (2 to 182 pmoles) from 10 days i.o. to adult. ACh levels are low (3.0) throughout embryonic and early posthatching stages and rise rapidly between 7 days a.h. and adult. Choline levels closely parallel ACh levels at each developmental stage.

Although a general correlation obtains between the amounts of neuropil and of ACh and choline at different ages, the patterns differ in detail. These differences may reflect changes with age in the ultrastructural composition of the neuropil, currently under analysis. Our results show that growth and development of these ganglia continues well into adulthood. This growth is evidenced by substantial increases (at least doubling) in the total ganglionic volume, the absolute volume of neuropil per ganglion, and in the amounts of ACh and choline per ganglion, in the period between 30 days and 360+ days a.h. (Supported by grants NIMH 5 F31 MH07326, NS09904 and UCONN Res. Found.)

528 BIRTH AND DIFFERENTIATION OF SPECIFIC NEURONS IN THE ABDOMINAL GANGLION OF APLYSIA. M. Jacob, S. Schacher* and V. Castellucci. Div. of Neurobiol. & Beh., Columbia U., P&S, New York, NY 10032

The egg of Aplysia is determinate; specific cells of the blastomere differentiate into definitive parts of the adult animal. As a result it should be possible to distinguish the exact part of the blastula which gives rise to precise regions of the central nervous system and thereby to explore the lineage of the identified cells in the various ganglia.

Embryonic development in Aplysia, from fertilization to hatching, takes approximately 12 days. The formation of the abdominal ganglion begins during this period and the anlage of the ganglion is already present at hatching. To examine the origin of the neuroblasts and their differentiation into specific neurons of the abdominal ganglion we exposed egg masses at various developmental stages to 10^{-5} M thymidine, (methyl-^{3}H)-(specific activity 20 mCi/mmol) and 10^{-5} M cytidine in sea water for two days. Following a 24 hour rinse in sea water alone, animals were sampled at definite time intervals relative to their developmental cycle and processed for light and electron microscopic autoradiography.

Exposing egg masses in the gastrula and segmented cavity stage (6&7 days after fertilization, respectively) resulted in the labeling of four or five neuroblasts in both the left and the right quadrants of the abdominal ganglion. At later larval (postembryonic) developmental stages these heavily labeled cells were recognizable as neurons.

Exposing egg masses in the late trocophore stage (8 days after fertilization) resulted in the labeling of fewer cells. At larval stage 1, two of these cells could be recognized as neurons in the left hemi-abdominal ganglion, one in the rostral and the other in the caudal segment. These two cells have been traced throughout the subsequent developmental phases up to adulthood. Preliminary morphological and electrophysiological characterization suggest that these neurons may be the identified cells L_{12} and L_{13}.

Thus, by combining ^{3}H-thymidine labeling with electron microscopic and electrophysiological techniques, it should be possible to study the pattern of differentiation of specific identified neurons of known behavioral function. We are also investigating 1) the origin of the precursor cells, 2) the signals that guide the migration of the neuroblasts and initiate their differentiation following final division and 3) the three-dimensional arrangement of the neurons within the ganglion.

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527 TRANSITION FROM A CONTINUOUS TO A DISCRETE DISTRIBUTION OF CALLOSAL PROJECTION NEURONS IN THE RAT PARIETAL CORTEX. Gwen O. Ivy* and Herbert P. Killackey (SPON: E.A. Davis). Dept. Psychobio., Univ. of Calif., Irvine, CA 92717.

In the adult rat, the two cerebral hemispheres are connected through the corpus callosum by neurons which are discretely located in areas of the neocortex. Retrograde tracing techniques have established that the perikarya of callosal projection neurons are found in distinct vertical arrays, with the majority of cells being distributed to layers III and V, and fewer to layers II and VI. During the first few postnatal days, however, the callosal projection neurons are distributed as continuous bands throughout the mediolateral extent of the cortex, as we have reported previously (Anat. Rec., '79, 193:573). The present study focuses on the events occurring during the transition from a continuous to a discrete adult distribution of callosal projection neurons.

Sixty rats of postnatal ages from 0 to 14 days and adults were injected unilaterally in parietal cortex with Sigma VI HRP. Most animals were sacrificed 24 hr post-injection, although some were allowed to survive for from 4 hr to 5 days. All brains were processed according to the technique of Mesulam (J. Hist. Cyt., '76).

From the day of birth, PND 0, through PND 4, two continuous bands of cells extend horizontally throughout the cortex beneath the upper dense cortical plate. These bands are in laminae destined to become layers V and VI in the adult. The Rapid Golgi method reveals that neurons in these laminae are immature pyramidal cells, while cells lying more superficially have not begun to differentiate noticeably. From PNDs 5 through 10, several changes occur. From PND 5 to PND 7, cells of the upper dense cortical plate become labeled in increasing numbers. These labeled cells appear initially as an extension of the band of label in layer V, and later, as a separate lamina above layer IV. Rapid Golgi preparations reveal that superficial pyramidal cells mature in form and acquire basal dendrites during this period. From PNDs 5 through 10, distinct aggregates and bands of cells which lack label become increasingly apparent in layer IV. Also during this period, the density of labeled cells in layer VI declines markedly. The greatest decline appears in areas destined not to be callosally connected in the adult, and in these same areas, labeled cells of the other cortical layers also become much less numerous. Thus, by PND 10, the trend toward the adult distribution of callosal projection neurons is firmly established. Finally, by PND 15, the pattern does not differ significantly from that of the adult.

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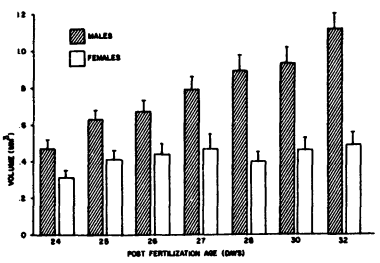
529 ONTOGENY OF THE SEXUALLY DIMORPHIC NUCLEUS OF THE PREOPTIC AREA. Carol D. Jacobson, James E. Shryne*, Fred Shapiro* and Roger A. Gorski. Dept. Anat., Sch. Med., UCLA, Los Angeles, CA 90024.

An area of increased cell density in the medial preoptic area of the adult rat brain which is markedly larger in volume in the male has been named the Sexually Dimorphic Nucleus of the Preoptic Area (SDN-POA). It has been further shown that the presence of the testes or exogenous androgen during the first week of postnatal life significantly increases the volume of the SDN-POA in the brain of the adult. The present study was conducted to describe the time course of the postnatal development of the SDN-POA in the intact male and female rat. Sprague-Dawley females were housed with males on proestrus. The presence of sperm in the vaginal smear on estrus was used to define day 1 of gestation. Male and female pups were sacrificed and perfused with 10% formalin on days 24, 25, 26, 27, 28, 30 or 32 post-fertilization. Following histological sectioning at 60 μ and staining with thionin, 3 investigators independently drew the boundaries of the SDN-POA on successive sections, using a microprojector at a magnification of 43.5. A fourth investigator averaged the 3 drawings; from this average, the nuclear volume was determined with a calibrated planimeter. All drawings and measurements were performed without knowledge of age or sex since the brains were coded according to a random number table. We found that the volume of the SDN-POA was significantly larger in males than in females on days 25 through 32, but no difference was found on day 24. Moreover, the volume of the SDN-POA increased significantly with age in the male, but there was no change in SDN-POA volume in the female (see figure). There was a significant increase in brain weight with increasing age in both males and females, but there was no consistent statistical sex difference in brain weight. Thus, the sex difference in volume of the SDN-POA cannot be accounted for by sex differences or age-related changes in brain weight per se. These data suggest that the development of the SDN-POA (as measured by volume) is itself sexually dimorphic. There are dramatic increases in the male, but not in the female, during a time period which is known to be critical for sexual differentiation of the brain. (Supported by NIH grant HD-01182, and the Ford, Grant and Kroc Foundations.)



530 DENDRITE BRANCH FORMATION AND GROWTH, AND ITS RELATIONSHIP TO SYNAPTOGENESIS ON THE DEVELOPING MAUTHNER CELL OF THE MEXICAN AXOLOTL (AMBYSTOMA MEXICANUM). Jean Jacoby* (SPON: Charles B. Kimmel), University of Oregon, Eugene, OR 97403.

It has been proposed that synaptic contacts during development are intimately involved in directing the course of dendrite growth and branching (Vaughn et al., 1974, JCB 60,664). I have examined the relationship between this growth and synaptogenesis in a case where the dendrite is uniquely identifiable.

The lateral dendrite and the adjoining perikaryon of the axolotl Mauthner cell was studied during a developmental period of about 4 days of the late embryo (Stages 39-43). During this time branch surface increased dramatically, with the cell acquiring its mature pattern of arborization by stage 43. A lesser amount of non-branch growth was also observed. Synapse density (number of synaptic profiles/100 micrometers cell surface) on the cell increased in parallel with the course of growth of the cell's surface, both branch and non-branch. This supports the model of Vaughn et al.

A class of profile termed "quasi-synaptic profiles", which contained features of synaptic profiles but lacking synaptic vesicles associated with the junction, were used to assay very early synaptic contacts. Quasi-synapse density was found to be significantly higher on branches than on non-branch surface at all stages. Quasi-synapse density was highest on branches at the early stages, and decreased with time, as expected for a feature transiently present on newly added surface. In addition quasi-synapse density was low on non-branch surface where growth was also low. Finally, the concentration of both quasi-synaptic and synaptic profiles at branch points was twice as high as that found on the adjacent surface, branch and non-branch. This suggests that a high concentration of afferent contacts stimulates dendrite branch outgrowth.

I conclude that spatiotemporal patterning of dendrite growth in this system is closely associated with patterning of input synaptogenesis. (Supported by NSF grant BNS 77-08685 and NIH grant NS 15001).

531 CHANGES IN RADIOSENSITIVITY ACCOMPANYING GRANULE CELL DIFFERENTIATION. Karl F. Jensen*. (SPON: Joseph Altman) Dept. Biol. Sci., Purdue Univ., W. Lafayette, IN 47907

The developing cerebellar cortex provides an ideal system to evaluate the change in radiosensitivity that occurs in the course of differentiation of granule cells. The proliferative, migratory, and maturational stages of granule cells are present concurrently in anatomically discrete layers and can be histologically quantified.

Ten day old Wistar rat pups were given a single dose of 300 KV whole head x-rays ranging from 25 to 7000 r. The pups were sacrificed 6 hrs after irradiation and the cerebella were embedded in glycol methacrylate. Coronal sections were cut at 3 microns and stained with hematoxylin. All cells were counted in samples of the external germinal layer (EGL), molecular layer (MOL), Purkinje cell layer (PCL), and internal granule cell layer (IGL) of the pyramis. The relative reduction of each cell type across dose was then determined.

Mitotic activity ceased above 250 r. The germinal cells of the upper portion of the EGL and the premigratory cells of the lower portion of the EGL were reduced by 80% at 300 r. The descending granule cells of the MOL and PCL were not significantly reduced until a dose of 1000 r. The basket cells of the MOL did not exhibit significant pyknosis until a dose of 7000 r. Purkinje, Golgi and Bergmann glial cells did not exhibit significant pyknosis at the doses applied. With the exception of the small contribution of the basket cells, the entire pyknotic population can be accounted for by the selective elimination of granule cells at different stages of their differentiation.

The decrease in radiosensitivity occuring with differentiation and associated migration may reflect functional metabolic changes accompanying increased specification. The selective elimination of undifferentiated granule cells can be used to determine their effect on the development of the cells with which they form connections as well as the circuitry in which they are normally involved.

532 THE GERMINAL ZONE IN GOLDFISH OPTIC TECTUM -- WHERE IT IS AND WHAT ITS CELLS LOOK LIKE. Pamela Raymond Johns and Stephen S. Easter. Depts. of Anat. and Zool., Univ. of Mich., Ann Arbor, MI 48109

In the brains of fishes, clusters of dividing cells are seen in several specific locations, called germinal or matrix zones. It is thought that new cells, both neurons and glia, produced at these sites contribute to normal brain growth and to regeneration of damaged tissue. We know from previous reports that the germinal zone of the optic tectum is restricted to the dorso-medial, ventrolateral and caudal edges. But its precise geometry has not been described, nor have the cells that are dividing been examined ultrastructurally. Here we identify the dividing germinal cells and chart their location in the tectum.

When goldfish (about 10 cm in length tip-to-tip) are injected with ^{3}H-thymidine, dividing cells incorporate the label. Labeled germinal cell nuclei are elongated, often lobulated, with prominent nucleoli; cytoplasm is sparse. The germinal cells are at the borders of the tectum, tightly packed between the edges of the periventricular layer and adjacent ependymal cells. A ventricular lumen always underlies the germinal zone. In electron micrographs the distinctive nuclei of the germinal cells are easily recognized.

In dorsal view (reconstructed from serial radioautographs of transverse paraffin sections) the germinal zone is a U-shaped ribbon, approximately 0.1 mm wide and 3.3 mm long, whose base is at the caudal tectal pole. One arm of the "U" lies along the dorsomedial tectal edge and the other arm is on the opposite, ventrolateral edge. The dorsomedial arm extends further rostrally and is 0.65 mm longer than the ventrolateral arm. The "U" opens at the rostrolateral tectal pole; this edge therefore lacks a germinal zone. The tectum receives a topographical projection from the retina, and the gap in the tectal germinal zone corresponds to a nasal segment of visual field subtending approximately 145°. (We compared our anatomical reconstruction with Schwassmann and Kruger's electrophysiological map: J. Comp. Neurol. 124: 113, 1965.) In goldfish retina the germinal zone is not "U" shaped, but rings the entire retinal circumferance. A similar mismatch in loci of cell production in retina and tectum of larval amphibians has led to the proposal that during growth there is a continual readjustment of topographically organized connections between optic fibers and tectal cells. Our results in growing goldfish support this hypothesis.

533 SELECTIVE LOSS OF NORADRENERGIC PHENOTYPIC CHARACTERS IN NEUROBLASTS OF THE RAT EMBRYO. G. Miller Jonakait, Judy Wolf*, Philippe Cochard*, Menek Goldstein and Ira B. Black. Dept. of Neurol., Cornell Univ. Med. Col., New York, N.Y. 10021 and Dept. of Psych., New York Univ. Med. Col., New York, N.Y. 10016.

Noradrenergic phenotypic characters are transiently expressed by neuroblasts in the gut mesenchyme of rat embryos. At 11.5 days of gestation, gut neuroblasts which contain tyrosine hydroxylase, dopamine-β-hydroxylase and endogenous catecholamine (CA) fluorescence, first appear (Cochard, P., Goldstein, M., Black, I.B.: Proc. Natl. Acad. Sci. USA, 75: 2986-2990, 1978; Teitleman, Baker, Joh, Reis: Proc. Natl. Acad. Sci. USA, 76: 509-513, 1979). They increase in number over the ensuing 24 hr. period, but by 13.5 days these noradrenergic characters have essentially disappeared. To define the fate of these cells, we studied formaldehyde-induced fluorescence following specific, high-affinity uptake of norepinephrine (NE). NE uptake, which was apparent in gut neuroblasts at 12.5 days, persisted at least through 17.5 days, long after the other endogenous noradrenergic characters had disappeared. Uptake was inhibited by incubation at 4°C or by inclusion of the highly specific uptake blocker, desmethylimipramine. These observations suggest that (a) high-affinity NE uptake develops as an additional noradrenergic characteristic in these gut cells, (b) the ability to take up NE persists even after the disappearance of other noradrenergic traits, and (c) the loss of noradrenergic characters is probably not simply due to death of the neuroblasts. Consequently, during embryonic development neuroblasts may transiently express transmitter phenotypes.

(This work was supported by the NIH, the Dysautonomia Fdn. Inc., the DGRST, France and the Hirschl Trust Fund.)

534 PATHWAY SELECTION BY EMBRYONIC CHICK LUMBOSACRAL MOTONEURONS. Cynthia Lance Jones* and Lynn T. Landmesser. Dept. Biol., Yale Univ., New Haven, Ct. 06520.

In order to examine the distribution of motoneuron axons in the chick hindlimb between the time of their initial outgrowth (St 23-24) and the establishment of specific functional connections (St 27), injections of HRP were made into individual segments of the lumbosacral lateral motor column. This procedure allowed the visualization of HRP reaction product in the motoneuron cell bodies as well as the outgrowing axons. At St 23-24, the spinal nerves projected only to the base of the limb bud, yet clearly were beginning to form distinct crural and sciatic trunks. Labelled axons, projecting from a specific segment of the cord, maintained their relative a-p axial position as far as the base of the limb. No evidence of diffuse outgrowth within the plexus region or limb tissue was found at this or subsequent stages. At St 26 1/2 - 27, prior to the cleavage of individual muscles and the motoneuron cell death period, labelled axons projected via specific anatomical pathways through the plexus to appropriate regions of the muscle mass. These results suggest that a period of diffuse or random axonal outgrowth followed by the death of inappropriately projecting neurons is not involved in the establishment of specific connectivity in the chick hindlimb. Similar characterization of axonal pathways at St 27 - 30 revealed that axons to an individual muscle tended to course in discrete tracts within the plexus and nerve trunks although they could change their topographical position with respect to other axons.

These results, although showing that axonal outgrowth is highly selective, did not allow us to distinguish between possible mechanisms involved in pathway selection. We therefore used similar techniques to trace axon pathways in embryos in which segments of neural tube were either deleted or reversed about the a-p axis at St 15-16.

In deletion experiments, axons from remaining segments followed their original pathways and did not alter their growth to compensate for deleted segments. These results rule out the possibility that axons divide up available peripheral territory on the basis of their relative a-p position, specific pathways being determined by competitive interactions with axons from adjacent segments.

When segments of cord were reversed about the a-p axis, motoneurons projected from the cord via adjacent spinal nerves. They established appropriate connections despite their incorrect order of exit from the cord by altering their original pathways within the plexus and nerve trunks. Labelled axons formed discrete groups as early as St 23 - 24 and never appeared to take random or inappropriate pathways. These results rule out simple mechanical guidance, and rather suggest that axons actively respond to positional cues on the basis of an early central specification. (Supported by NIH Grant NS 10666 and postdoctoral fellowship NS 05990)

535 A BASAL SUBSTRATE PATHWAY DEMONSTRATED BY TRANSPLANTED MAUTHNER AXONS. Michael J. Katz* and Raymond J. Lasek (SPON: H. Gluck) Anat. Dept., Case Western Reserve Univ., Cleve. OHIO 44106

A substrate pathway is a set of similar guidance cues aligned in a discrete route. Previously Katz and Lasek, 1979 (JCN 183:817) used transplanted optic axons to demonstrate a substrate pathway running through the alar region of the developing nervous system in Xenopus (Fig. 1). This alar substrate pathway has also been demonstrated in Rana by Constantine-Paton, 1978 (Br Res 158:31). Here we report that transplanted Mauthner axons have demonstrated a basal substrate pathway.Extra hindbrains were transplanted to various locations in the neural tubes of Xenopus embryos of the same stages.Tadpoles were examined in 10 um Bodian stained paraffin sections at st. 43-47. Among the tadpoles studied we found 10 donor Mauthner axons (M' axons) entering host CNS. 6 of these entered from donor hindbrains implanted in spinal cords in reverse rostro-caudal orientation -- 5 grew rostrally and 1 grew caudally. The other 4 M' axons entered and grew caudally from donor hindbrains attached to host diencephalon or forebrain. 9 of the 10 M' axons grew more than 100 um rostrocaudally in the host CNS, and all 9 followed an essentially identical ipsilateral ventral CNS route (Fig. 2), extending from the developing ventral thalamic region down the developing spinal cord. M' axons always grew along the same discrete route in the basal region, and thus the guidance cues which they followed must be aligned as a substrate pathway. Normally, the MLF may follow this basal substrate pathway.

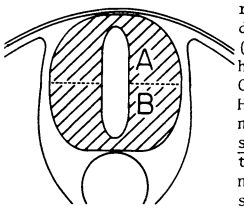


FIG. 1. Developing neural tube. A= alar plate B= basal plate.

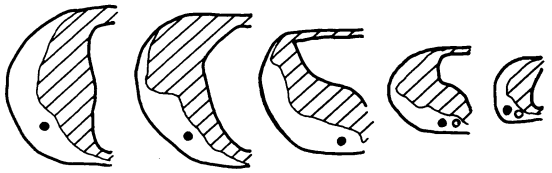


FIG. 2. CNS hemisections (st. 43 Xenopus) showing route of M'axon. L to R: diencephalon, midbrain, rostral hindbrain, caudal hindbrain, spinal cord. (●=donor M' axon, o=host M axon).

536 ORIGIN AND DIFFERENTIATION OF PIONEER NEURONS IN THE EMBRYONIC GRASSHOPPER. Haig Keshishian* (SPON: R.C. Van Sluyters). Neurobiology Group, Univ. Calif., Berkeley, Ca. 94720.

Pioneer neurons (Bate, '76, Nature 260: 54-56) are monopolar afferents found in the lumen of insect embryonic appendages. They may guide sensory neurons to the central nervous system.

The origin of these cells was determined in cultured embryos of the grasshopper Schistocerca nitens undergoing normal appendage morphogenesis. The development of the metathoracic leg and antenna was observed with time-lapse microphotography using differential interference contrast optics, at 30° C.

At the 25-30% stage of development (Bentley et al., J. Emb. Exp. Morph., In press), large (25-30μ) spherical cells can be distinguished in the lumen of the metathoracic leg. These cells give rise to pioneer neurons. The pioneer mother cells (PMCs) have been observed to remain in interphase for as long as six hours before undergoing cell division. During interphase the PMCs sometimes extend and retract 3-5μ cytoplasmic processes, and can undergo small rotations (10-20°). Mitosis of the PMCs is completed in approximately one hour.

PMC daughter cells were identified as pioneer neurons by (1) their location within the lumen, (2) their size, (3) their monopolar morphology, and (4) by a centrally coursing axon. They usually remain quiescent for 30 to 60 minutes following mitosis, after which they begin to sprout fine multiply digitated processes. Although these processes may extend tens of microns, they are often retracted abruptly, followed by a new extension in a different direction. Following this "exploration" of the immediate cellular environment, usually one daughter will migrate in an ameboid fashion along the lumen wall. Velocities of 1-2 microns / minute have been observed for daughter migrations. Axonal sprouting generally follows migration, with the axon spun out behind an elaborately digitated growth cone. Retractions and new extensions of the early axonal processes are sometimes seen. For example, a daughter cell in a 35% stage metathoracic leg was observed to send out cytoplasmic processes first towards the lumen wall, then towards the tip of the leg, and then into the middle of the lumen; finally the cell directed a projection towards the central nervous system which became an axon that grew at least 100-150μ in two hours.

From the 25-30% stage of development onwards some of the PMCs can be individually identified in the metathoracic leg: they occur in fixed number and position in the distal portion of the limb. The PMCs have not been observed to arise from the epidermis, as sensory neurons do, and some evidence suggests that they may be generated centrally and accompany the outgrowth of the appendage.

537 THE ONTOGENY OF EMBRYONIC NEUROBLASTS IS ALTERED BY NERVE GROWTH FACTOR. John A. Kessler*, Philippe Cochard* and Ira B. Black. Dept. of Neurol., Cornell Univ. Med. Coll., New York, N.Y. 10021.

The role of nerve growth factor (NGF) in neuronal phenotypic expression and selective survival was studied in embryonic autonomic neuroblasts in vivo. In the rat, the noradrenergic phenotype first appears in sympathetic ganglion primordia and in neuroblasts within the gut mesenchyme at 11.5 days of gestation (E 11.5), just after neural crest migration. Noradrenergic characters persist in ganglion neuroblasts throughout development, but disappear from the gut by E 14.5.

Transuterine injection of rat embryos with NGF on E 11.5 resulted in striking changes in the ontogeny of the gut cells; noradrenergic numbers were markedly increased. Moreover, injection of NGF caused persistence of catecholamine (CA) neuroblasts in the gut through E 15.5, whereas guts of controls were devoid of these cells at this stage. NGF treatment also increased the number of apparently ectopic neuroblasts in unusual locations such as the liver parenchyma, the somatopleural mesenchyme of the body wall, and the limb bud mesenchyme. In contrast to effects elsewhere, low doses of NGF had little apparent effect on neuroblasts in ganglion primordia. This suggested that the exogenous dose of NGF was too small, in comparison to local endogenous concentrations, to elicit changes in the ganglion primordia. To study the effects of high NGF doses on ganglion ontogeny, the smaller mouse embryo was used, allowing conservation of the protein. Transuterine treatment on E 12.5, during the stage of ganglion aggregation, resulted in a dramatic increase in neuroblast numbers and cell volume in the superior cervical ganglion. NGF treatment also significantly increased ganglionic levels of the CA-synthesizing enzyme, tyrosine hydroxylase.

These observations indicate that NGF can increase the number of noradrenergic neuroblasts in embryonic sympathetic ganglia, as well as the gut and ectopic locations. NGF treatment also caused persistence of noradrenergic neuroblasts in the gut and consequently, local concentrations of endogenous NGF in the embryo may normally determine which neuroblasts survive and/or continue to express noradrenergic characters.

(This work was supported by the NIH, the Dysautonomia Fdn. Inc and the DGRST, France. J.A.K. is the recipient of the Teacher Investigator Award NS 00351. I.B.B. is the recipient of the Irma T. Hirschl Career Scientist Award.)

538 A GRADIENT OF CALLOSAL FIBER DEVELOPMENT IN THE PARIETAL CORTEX OF THE RAT. Herbert P. Killackey and Rebecca M. Akers, Dept. Psychobio., Univ. of Calif., Irvine, CA 92717.

An autoradiographic study of callosal fiber development in the neonatal rat suggests that fibers connecting lateral areas of parietal cortex develop in advance of those directed towards medial parietal areas. Neonatal rats were injected unilaterally in parietal cortex with tritiated amino acids and allowed to survive for 24 hr. Extensive labeling of the entire corpus callosum is evident in animals sacrificed on postnatal day 1 (PND 1). At this stage of development, transported label is evident only in regions of parietal cortex lying adjacent to the rhinal fissure. By PND 2, transported label is evident in the deeper cortical layers of both medial and lateral parietal areas. In lateral areas label extends to the lower border of layer V, whereas in medial parietal cortex it is limited to the lower one third of layer VI. These differences in transport of label are maintained through the first five postnatal days. By PND 4, label in the lateral parietal cortex extends through the deeper cortical layers and reaches the lower border of layer III. In more medial transport sites, label is restricted in most cases to layers V and VI and does not reach the superficial cortical layers until the following day. By PND 6, label extends to the pial surface in both medial and lateral areas of parietal cortex, and regional differences in transport of label are no longer evident.

These findings indicate that the laterally directed callosal fibers may extend through the cortex and reach their superficial terminal fields in advance of fibers destined for the medial parietal cortex. This pattern of callosal fiber development mimics the lateral-to-medial gradient of cell production and migration which has been previously described for the cortex of the developing rat.

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539 A STUDY OF THE VITREAL-OPTIC NERVE BARRIER TO DIFFUSION OF HORSERADISH PEROXIDASE IN THE CHICK EMBRYO. Henry B. Kistler, Jr.*and Jennifer H. LaVail. Dept. Anat. UCSF, San Francisco, CA. 94143

Several investigators have described a region immediately beyond the lamina cribrosa of the optic nerve that limits the spread of horseradish peroxidase (HRP) from the vitreous into the extracellular spaces of the optic nerve. The apparent barrier might be explained by apposed membranes of glial cells or neuronal processes, or in the absence of tightly apposed membranes, by a physiological barrier determined by rapid endocytosis of HRP by glial and/or vascular and neuronal elements. As a basis for later study of retrograde transport in developing chick and to determine whether a barrier to vitreally secreted macromolecules might exist, we studied the spread of intravitreal HRP into the optic nerves of chicks ranging from embryonic(E) day 6 to 3 days posthatching. One hr after HRP in saline was injected, the animals were fixed and prepared for histochemical localization of HRP. Light microscopic (LM) examination of 8-10 µm thick longitudinal plastic sections of optic nerve and retina revealed dense reaction product throughout the retina and vitreal surface of the optic papilla. It extended into the proximal optic nerve but not beyond the level of the cartilagenous cuff surrounding the base of the optic stalk. HRP extended farther in the nerve within radially oriented glial septae. Three zones within the nerves of E5, E14 and P3 animals were examined by EM, 1) a proximal zone near the vitreal surface, 2) a zone in the lamina cribrosa where HRP could be detected by LM, and 3)a zone midway between zone 2 and the optic chiasm where HRP could not be detected by LM. In zone 1 there was abundant extracellular HRP and large labeled cytoplasmic organelles in the cell constituents (presumptive glioblasts, astrocytes, oligodendrocytes, and endothelial cells). In E14 and P3 nerves, HRP-filled organelles were seen rarely in axonal profiles. In zone 2, HRP was present extracellularly inbetween the astrocytic processes in radially oriented glial septae. The same HRP-containing cells were present in this zone, but the number, size and density of positive organelles in them were reduced. In zone 3, extracellular HRP was absent. The glial population, composed mainly of presumptive astrocytes, contained few HRP-positive inclusions. In this zone numerous punctate adhesions between apposed astrocytic processes were marked, but tight junctions were never observed. In no zone was there evidence of a transverse network of glial contacts that might form a physical barrier to HRP diffusion. We observed a gradient in the number of positive organelles per astrocytic profile that was inversely related to the distance from the vitreal surface. Our results suggest that the barrier to diffusion of HRP in fetal and early posthatching chicks is a function of uptake of HRP by glial cells that restrict and regulate the concentration of vitreally secreted macromolecules in the developing optic nerve.

540 HISTOGENESIS OF THE INNER NUCLEAR LAYER IN THE RETINA OF XENOPUS LAEVIS. George J. Kokoris and Leslie J. Fisher. Neuroscience Program and Dept. of Anatomy, University of Michigan, Ann Arbor, Michigan 48109

Qualitative and quantitative changes occur in the inner nuclear layer (INL) of the retina of Xenopus laevis as the larvae undergo metamorphosis. That the retina grows by the addition of cells to the periphery is not disputed, but conflicting evidence exists as to the presence of mitotic activity in the older, central retina. Previous investigators have employed paraffin histology, and their different observations may reflect the imprecision of this technique, rather than real differences in their results.

The question of mitotic activity in the central retina was re-examined using plastic histology, which allows more precise identification of nuclei. Xenopus laevis retinae were examined by means of radioautography over developmental stages 55 through 60. Larvae were injected intraperitoneally with ^{3}H-Thymidine and allowed to survive for 24 hours. Eyes were removed, and the tissue processed for radioautography. Under light microscopy, retinae were examined for labelled nuclei within 500um of the optic nerve exit. In addition, nuclear counts were taken over 500um intervals on either side of the optic nerve to ascertain changes in the nuclear density of the INL.

We verify that the INL exhibits a substantial increase in nuclear planimetric density at metamorphosis. The nuclear density of the INL increases significantly ($p<.01$, ANOVA) from stage 55 to stage 60. Furthermore, our experiments confirm Hollyfield's (1971) observation of ^{3}H-Thymidine uptake by nuclei in the differentiated central retina. Though few labelled cells are observed, the changes in nuclear density suggest that mitosis takes place in the central retina, late in larval life.

We present evidence that many of these labelled nuclei are differentiated glial elements (Müller fibers). The precise ultrastructural identification of these new cells is being investigated with electron microscopy.

541 INTRACEPHALIC IMPLANTS OF EMBRYONIC RHOMBIC LIP INTO ADULT RATS PROVIDE MODEL SYSTEMS FOR STUDYING CEREBELLAR DEVELOPMENT. Lawrence F. Kromer, Anders Björklund* and Ulf Stenevi*. Dept. of Neuroscience, UCSD, La Jolla, CA 92093 (L.F.K.) and Dept. of Histology, Univ. of Lund, S-223 62 Lund, Sweden (A.B. and U.S.).

Pieces of embryonic rhombic lip were taken from fetuses with crown-rump lengths (CRL) of 7-25 mm (approximately embryonic days 10-18) and implanted into a cavity in the parietal or occipital cortex in adult female albino rats (180-200 g). The implant was placed adjacent to the severed edge of the host hippocampal formation on the vessel-rich pia covering the thalamus or superior colliculus. Thus, the implant was not in contact with any regions of the host brain that normally have direct afferent or efferent connections with the cerebellum. After survival periods of 1-5 months the host animal was perfused with Bouin's or formalin fixative, and adjacent sections of the host brain containing the implant were stained with the cresyl violet and Klüver-Barrera stains and the Holm's silver method. Pieces of embryonic cerebellar anlage from all gestational stages survived and continued to differentiate within the ectopic site. Individual specimens were observed to contain isolated cerebellar cortex, non-cortical tissue, or both. All implants which developed a cerebellar cortex exhibited a high degree of cytoarchitectonic differentiation. The cortical region was easily identified from adjacent regions of the implant or the host CNS by its characteristic foliation and laminated structure composed of molecular, Purkinje cell and granule cell layers. Since most implants at the time of transplantation contained neuroepithelium with few or no postmitotic neuroblasts, the observations indicate, first, that all different neuron types present in the normal cortex can be generated in the implant, and, secondly, that these cells are able to interact in the isolated implant to produce a highly organized structure. This requires an orderly migration of the cell types involved, especially the granule cells. In addition to the differentiated cortical structure most implants also contain masses of more unstructured neuronal tissue, probably representing brain stem or deep cerebellar structures. This may signify that some of the normal afferent and efferent connections of the cortex can be established within the implant. That such connections are not necessary for cortical differentiation is, however, indicated by cases in which the implant contains only well-differentiated cortex and no or very little brainstem-like tissue.

Thus, it appears that the cell interactions in the fetal brain prior to the dissection and the intrinsic cell interactions occurring within the implant after transplantation are sufficient to produce the characteristic lamination and foliation of the cerebellar cortex. (Supported by UPS grant 5 F32 NS 05528-02).

542 LOSS OF GROWTH HORMONE SENSITIVITY IN BRAIN AND LIVER DURING MATERNAL DEPRIVATION IN RATS. C. M. Kuhn and S. M. Schanberg*. Duke University Medical Center, Durham, N. C. U.S.A.

We have shown previously that maternal deprivation (MD) results in a decrease in brain and heart ornithine decarboxylase (ODC) activity which may be mediated in part by a specific suppression of growth hormone (GH) secretion. To investigate further the relationship between the effects of maternal deprivation and the regulation of tissue development by GH, we tested the ability of GH to stimulate ODC activity in maternally deprived rat pups. Pups were removed from the mother and placed in an incubator for 2 hours, injected with GH (100 µg, s.c.) and liver ODC activity was determined 4 hours later. Littermate controls left with the mother were similarly treated with GH or vehicle. In a separate experiment, maternally deprived and control pups were injected intracisternally with GH (100 µg) and brain ODC activity was determined 4 hours later. ODC activity was significantly lower in liver and brain of deprived pups and was not stimulated by GH, while ODC activity in both brain and liver of control pups increased significantly after administration of GH. When pups that had been maternally deprived for 2 hours were returned to the mother for 2 hours and then injected with GH, significant stimulation of ODC activity in liver was observed. ODC activity did not increase following GH administration in livers of pups placed with a urethane-anesthetized mother although GH did stimulate ODC activity in livers of pups placed with a mother whose nipples had been ligated.

To determine if the loss of tissue response to GH resulted from a specific suppression of GH action, maternally deprived pups were treated with several other inducers of ODC activity. Ovine placental lactogen (oPL, 100 µg), did not stimulate ODC activity in livers of deprived pups, but PGE-1 (50 µg), dibutyryl cAMP (.3 mg) and dexamethasone (0.2 mg) significantly stimulated ODC activity in both maternally deprived and control pups. ODC activity in brains of maternally deprived pups increased significantly following intracisternal administration of dibutyryl cAMP (50 µg).

These findings suggest that maternal deprivation is associated with a specific suppression of tissue response to the growth promoting peptide hormones GH and oPL. This suppression is reversed rapidly when pups are returned to the mother, and appears to be triggered by the removal of active mothering behavior, not by nutritional deprivation or the removal of passive sensory stimuli associated with the mother. (Supported by NIMH grants MH-13683 and MH-06489).

543 RESPONSE OF THE CAT DORSAL LATERAL GENICULATE NUCLEUS TO INFANT VISUAL CORTEX LESIONS. Douglas R. Labar*, Nancy Berman, E. Hazel Murphy. Department of Anatomy and Department of Physiology/Biochemistry, Medical College of Pennsylvania, Philadelphia, PA 19129.

Previous observations indicate that following infant visual cortex lesions in cats, considerable shrinkage of the dorsal lateral geniculate nucleus (LGD) occurs, and the severely shrunken nucleus contains a sparse population of surviving isolated large cells. We looked at the time course of the occurence of these phenomena during the first few post-operative days. Eight kittens were given unilateral visual cortex lesions within 24 hours after birth. Following post-operative intervals ranging from 1 to 9 days, subjects were given a unilateral intraocular injection at H_3-proline, and brains were processed for autoradiography.

Volumes of lesioned and control LGD's were determined from projection drawings. Volumetric changes that occurred in a lesioned LGD were determined relative to the volume of the control LGD of the contralateral thalamus. At two days post-operative, the ratio of lesioned LGD volume to control LGD volume was .92; at four days post-operative, this ratio was .82; at six days post-operative, this ratio was .51; at eight days post-operative, this ratio was .43. These ratios indicate a linear shrinkage of the LGD with increasing survival intervals over the time period studied. Comparisons of volumes of individual autoradiographically demarcated laminae in lesioned and control LGD's showed that shrinkage is not selective for a specific lamina, but that all laminae undergo equal shrinkage, in parallel with the shrinkage at the entire LGD.

Isolated large cells are first seen in the lesioned LGD four days after the lesion. At this time they are located in the A/A_1 and A_1/C interlaminar zones; at the same time, similar appearing large cells emerge adjacent to the interlaminar zones in the control LGD. Isolated large cells appear within laminae A, A_1 and C of the lesioned LGD at 6 to 8 post-operative days; similar large cells are first seen within the cellular laminae of the control LGD at this same time.

The similarity in time of appearance and location of large cells in the normal cat LGD and the isolated large cells present in that nucleus after infant visual cortex lesions suggests that these two populations represent a single class of geniculate neuron. Perhaps the normally large Y cells are able to survive infant cortex lesions.

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544 DOES CELL POSITION DETERMINE RECEPTIVE FIELD PROPERTIES OF NEURONS IN THE MOUSE VISUAL CORTEX? Vance Lemmon* and Alan L. Pearlman. Dept. of Physiology and Biophysics, and Neurology, Washington Univ. Med. Sch., St. Louis, MO 63110

During the normal development of the neocortex, the first cells to be produced take positions in deep cortical layers, and later cells are positioned more superficially. Many studies have shown that afferents to the cortex from different sites terminate in specific laminae, that individual laminae have specific efferent targets, and that neurons in the visual cortex within particular laminae have specific receptive field (RF) properties. How important, then, is the laminar position of the various cortical neurons in determining their afferent and efferent connections? This problem can be studied in the reeler mutant mouse, where the positions of cortical cells are grossly disturbed; neurons that are normally located in deep cortical laminae are primarily located superficially in reeler, and vice versa.

We studied the receptive field properties of cortico-tectal (CT) cells because they are located in a single cortical lamina (layer V) in normal mice but are distributed widely in the superficial aspect of reeler cortex. In addition, CT cells have distinctive receptive field properties and can be positively identified by antidromic stimulation with electrodes in the superior colliculus. The properties of CT cells that we studied quantitatively in normal mice and reeler mutants included rates of spontaneous activity, RF size, velocity sensitivity and spatial summation. Statistical analysis of these properties demonstrated no significant differences between CT cells in normal and reeler mice. This finding indicates that the detailed connections underlying the RF properties of CT cells are properly established despite their abnormal cortical locations. (Supported by NIH Grants RO1-EY00621 and T01-EY00092).

545 THE BEHAVIORAL TOXICOLOGY OF LOW CHRONIC DOSES OF HALOTHANE DURING DEVELOPMENT IN RATS. Edward D. Levin * and Robert E. Bowman. University of Wisconsin, Dept. of Psychology, Primate Lab, 22 N. Charter St., Madison, WI 53706.

Chronic, low level exposure of rats during development to the anesthetic halothane has been shown to cause deficits in shock-motivated spatial learning, food-motivated spatial learning and shock sensitivity. (Quimby, Katz and Bowman, Anesthesia and Analgesia, V54(5),p628-33, 1975). We exposed Sprague-Dawley female rats to 12.5 parts per million of halothane in air for 8 hours/day, 5 days/week, from concention through parturition. Mothers and litters, which were pared to 8 pups, were exposed on the same schedule until day 25 when the pups were weaned. The pups alone were exposed until day 30. Male offspring were tested on day 55 for locomotor exploration of a novel T-maze. The halothane-exposed rats (N=12 litters) showed significantly slower speeds than control rats (N=12 litters), in locomoting from the start box at the bottom of the stem into either of the arms. These data indicate that the effects of halothane are descriminable with exposure to only 30 days after birth and that the behavioral effects are not confined to high motivation tasks. Low motivation tasks like locomotor exploration also appear to be sensitive indicators of behavioral toxicology.

MECHANISMS OF AFFERENT LAMINATION IN DEVELOPING HIPPOCAMPUS REVEALED BY OUTGROWTH OF FIBERS FROM SEPTAL IMPLANTS. E.R. Lewis* and C.W. Cotman (SPON: P.T. Kelly). Dept. of Psychobiology, U.C. Irvine, CA 92717

Pieces of embryonic septal tissue were implanted into the entorhinal or occipital cortices of neonatal rat hosts (2-3 days old). The initial outgrowth of fibers originating from the graft and their pattern of termination within the host hippocampal formation was examined using a histochemical stain for acetylcholinesterase (AChE). Fimbrial transections performed in conjunction with the implant surgery eliminated native septohippocampal fibers. When placed in the occipital cortex, septal implants innervate specific laminar zones within the host hippocampus and dentate gyrus; these are the same laminar zones that receive septal efferents in the normal animal. Septal implants placed in the entorhinal cortex also produce a pattern of AChE staining which is similar to that seen in the normal hippocampus. In the dentate gyrus ipsilateral to the implant however, the unstained commissural/associational (C/A) zone is expanded.

Temporal characteristics of the outgrowth of fibers from the implant were studied in animals sacrificed 6, 8, 10, and 12 days after the implant surgery. AChE activity indicative of innervation from the implant is present in 50% of the animals sacrificed 10 days post-implant; by 12 days post-implant all but one animal displayed AChE reaction product in the dentate molecular layer. Processes emanating from cells in the implant were oriented in the direction of the hippocampus throughout the developmental period. In one group of animals, the fimbria was left intact for 27 days after the implant surgery in order to examine competitive interactions between fibers from the implant and native septohippocampal efferents. Extremely light AChE staining is present in the hippocampus and dentate gyrus of animals in this group sacrificed 7, 14, and 30 days after the septohippocampal fibers were severed. When tissues from the striatal region of the embryonic brain were implanted into the entorhinal cortex, AChE reaction product in the hippocampus is restricted to the outer third of the dentate molecular layer.

The position of the afferent source and the time of arrival of the afferent fibers in the target area do not seem to be critical to the mechanisms underlying the development of specific connection patterns in this system. Temporal competition between homologous afferents and a hierarchical scheme of growth possibilities appear to play a role in developmental specificity. In addition, we have proposed that contact between a "critical afferent" (the C/A system in this case) and the target cell might "set" the dendritic membrane for the appropriate patterns of connectivity. (Grants NS 08597 and MH 19691).

A GOLGI STUDY OF DEVELOPING CHICK SYMPATHETIC NEURONS. L. Luckenbill-Edds. Dept. Zoology & Microbiology and College of Osteopathic Medicine, Ohio University, Athens, Ohio 45701

Developing sympathetic neurons in a series of chick embryos were examined in Nissl stained and Golgi impregnated (Stensaas, 1967) preparations. Retroperitoneal tissue blocks containing paravertebral ganglia were prepared from embryos at 7, 11 and 15 days (E7, E11, E15) of development. The general shape and orientation of the neurons, as well as the appearance of dendritic specializations such as end bulbs and claws were recorded.

At E7 when paravertebral ganglia have begun to develop in the mesenchyme, multipolar neurons of two basic shapes are observed. One type has several short thick dendrites that radiate from the cell body and give off fine branches proximally. Growth cones at the ends of the stout dendrites appear as flattened expansions with filaments extending from them. The second type of neuron at E7 has fewer short thick dendrites, some of which terminate in bulbs or claws. This type of cell has other processes which taper into extremely long thin extensions that run parallel to the long axis of the embryo. Occasionally these long thin extensions bifurcate to give rise to thicker processes which terminate in expansions. The long thin extensions do not branch along their course.

At E11 multipolar neurons assume several distinct shapes. One type is a large multipolar neuron with a cell body that is irregular in outline and has stout dendrites radiating from all sides. Some of the dendrites taper and extend distances that are many times the diameter of the cell body, while others remain short and end in claws or bulbs. A second type of neuron has a small oval cell body with fine caliber processes that are restricted to opposite poles of the cell and oriented parallel to the long axis of the embryo. A third type of neuron has a pear-shaped cell body with thick dendrites emanating from the narrower side. After these dendrites branch close to their origin, they turn to run parallel to the long axis of the embryo.

At E15 cell bodies are nearly twice the diameter they were at earlier stages and dendrites have thickened proximally and become more profusely branched distally. The three basic patterns of shapes seen at E11 are maintained in neurons at E15.

These results suggest that when chick sympathetic neurons develop, differences are established in the size and shape of their cell bodies and in their dendritic patterns. Further development involves growth and elaboration of these differences.

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547 EFFECTS OF EXPOSURE TO HORIZONTAL OR VERTICAL STRIPES DURING REARING ON EVOKED POTENTIALS TO THESE STIMULI AT MATURITY IN RATS. Jill London*, Howard Rowley*, Gregory McCarthy*, and William T. Greenough (SPON: E. Donchin). Depts. Physiol. and Psychol. and Neural & Behav. Biol. Prog., Univ. of Ill., Urbana, IL 61801.

Studies in cats (e.g., Blakemore & Cooper, Nature, 1970,228: 477) indicate that orientation specificity of receptive fields of visual cortex units may be biased toward the orientation of a striped visual field if the cats are exposed exclusively to a single orientation during development. We have examined this phenonmenon in rats, using evoked potentials to stripes in the exposed and orthogonal orientations.

Hooded rats of both sexes were reared from birth in the dark. Beginning at eye opening (approx. 13 d.) and continuing to day 52 rats were exposed for 1 h. per day 5 d. per week to a field of alternating black and white stripes (of variable thickness) oriented either vertically or horizontally. To maintain exposure orientation, the limbs were bound and the animals were wrapped in gauze and placed in boxes with their heads exposed. The boxes were supported by ring stands in (25 cm dia.) drums. Except for these exposure periods, animals were kept in darkness (or dim red light during brief daily maintenance) throughout the experiment.

Stainless steel screw electrodes were implanted unilaterally or bilaterally over areas 17-18 of the cortex on day 52. After 2-7 days, unanesthetized animals were secured to a board facing a vertical or horizontal stimulus field in the dark. Evoked potentials to strobiscopic illumination of the field at 1 sec. intervals were averaged with a Fabritek signal averager and additionally in some cases with a computer averaging program.

N1-P2 peak to peak amplitude was measured from visual evoked potentials to 64 presentations of vertical and horizontal fields from each electrode. For horizontally-exposed animals, 7 of 8 comparisons indicated greater amplitude to the horizontal stimulus with a mean difference of 17% ($p < .01$). For vertically exposed animals, 6 of 9 comparisons favored the vertical stimulus ($\bar{x}$ diff = 10%; $.05 < p < 0.1$). Preliminary work indicates no consistent differences in evoked potential amplitude to the two oriculations in dark and cyclic light reared animals. Studies of evoked potentials to square wave grating reversals at various orientations are in progress.

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549 DEVELOPMENT OF ACETYLCHOLINE BIOSYNTHESIS IN CHICK CILIARY GANGLION AND IRIS. Mario Marchi*, Ezio Giacobini, and Douglas W. Hoffman* Lab. of Neuropsychopharmacology, Dept. of Biobehavioral Sci., Univ. of Connecticut, Storrs, Ct. 06268 (Spon: G. Gianutsos)

The ciliary ganglion-iris complex of the chick has been used as an in vivo model system for studying age-related changes in development and aging in the peripheral nervous system. Previous work in our laboratory has described the developmental pattern of acetylcholinesterase (AChE) and cholineacetyltransferase (ChAc) activity as well as α-bungarotoxin binding in the ciliary ganglion and iris (e. Giacobini, in Maturation of Neurotransmission, S. Karger, Basel, 1978; 41-64). The present investigation follows the appearance and variation of levels of acetylcholine (ACh), choline (Ch), and Ch uptake in the same organs during development. The radioenzymatic method of McCaman and Stetzler (J. Neurochem. 26: 669, 1976) was used for determination of ACh and Ch, which are expressed as pmoles/ganglion or iris. ACh was found to be present starting at 5 days of incubation (d.i.) both in the ciliary ganglion (<1) and iris (13.3 ± 2.4). ACh levels per ganglion increase between 7 (1.05 ± 0.1) and 14 (10.7 ± 2.6) d.i. From 14 d.i. to 1 day after hatching (a.h.) this level remains constant but increases again from day 1 a.h. (8.5 ± 1.0) up to 1 yr. (161.2 ± 40.3). In the iris, between 5 and 7 d.i. the ACh level is low and constant (13.3 ± 2.4 to 14.0 ± 4.8). This is followed by a rapid increase up to 14 d.i. (93.2 ± 12.2). Following a small decrease from 14 d.i. to 3 d.a.h. (51.8 ± 10.0) there is a further increase in ACh up to 1 yr. (402.0 ± 42.6). Ch levels closely parallel ACh pattern both in ciliary ganglion and iris (M. Marchi et al., Dev. Neurosci., 1979, in press). Ch uptake is present in the iris starting at 4½ d.i.; and at 5 d.i. the K_m = 1.83 μM and V_{max} = 1.30 pmoles/min/iris. The V_{max} values follow the pattern of Ch and ACh content from 5 d.i. up to 3 months. Recent morphological studies by Aloisi and Mussini (Int. Meet. on Brain Dev. S. di Fasano, 1979, abst.) have confirmed the presence of innervation at 5 d.i. In conclusion our results demonstrate that: 1) ACh and Ch are present both in the ciliary ganglion and iris as early as 5 d.i. Increases in their levels seem to be related to both innervation and synaptogenesis; 2) cholinergic neurons are capable of taking up Ch and synthesizing ACh in their terminals starting at the time of penetration of the fibers into the iris; and 3) Ch levels correlate closely with ACh levels and in addition the changes in V_{max} of Ch uptake during development follow the pattern of Ch and ACh levels. As the V_{max} is determined by the density of transport sites this supports a regulatory role for Ch uptake in ACh synthesis from the earliest stages of development in the peripheral nervous system.

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550 PHYSIOLOGY AND MORPHOLOGY OF NEURONS IN THE ANTENNAL LOBES OF MATURE AND DEVELOPING *MANDUCA SEXTA*. Steven G. Matsumoto. Dept. of Neurobiology, Harvard Med. Sch., Boston, MA 02115.

The sensory neurons in the antenna of the moth *Manduca sexta* differentiate and send their axons into the CNS early in the 18 days of adult development. Interneurons in the antennal lobe (AL) of the brain also develop at this time. This study examines the morphological and electrophysiological development of the AL interneurons and their interactions with antennal primary afferents.

Using intracellular recording and dye-injection techniques, we have classified neurons in the AL according to their morphological and physiological properties. Cells in mature moths were tested for their responses to antennal stimulation with female sex pheromone, general odorant (*trans*-2-hexenal), and mechanical stimuli. At least 2 broad categories of interneurons can be discerned morphologically: "local" interneurons with multiglomerular arborizations and no axons, and "output" interneurons with arborizations in single glomeruli and axons projecting out of the AL to the corpora pedunculata. Both of these classes can be subdivided in males with respect to responses to pheromone; no female neurons respond to pheromone. Pheromone-responsive local and output interneurons arborize in a macroglomerular complex found only in the male AL.

Intracellular recordings from AL neurons in developing moths first detect synaptic transmission about day 9 (midway through adult development). The incidence and strength of synaptic transmission increase sharply over the following 4 days, coinciding with the final developmental increase in levels of acetylcholine and choline acetyltransferase activity in the AL (Brain Res. *119* 389, 1977) and with the appearance of mature synaptic profiles in electron micrographs of the glomerular neuropil (L.P. Tolbert, this volume). By day 13 the development of synaptic transmission, as monitored by intrasomatal recordings and electrical stimulation of afferents, is nearly complete. The morphological maturation of the AL interneurons appears to coincide with their electrophysiological maturation. The multiglomerular local interneurons develop by progressive elaboration of their arborizations and the appearance of spine-like structures. The output interneurons initially develop multiglomerular arborizations that subsequently retract between days 12 and 14 to leave a single-glomerular dendritic tuft that continues to increase in density.

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551 EFFECT OF RE-INNERVATION, DEVELOPMENT AND DEGENERATION ON MEPP AMPLITUDE DISTRIBUTIONS IN THE MOUSE DIAPHRAGM. C.G. Muniak* and C.G. Carlson* (SPON: N. West) Dept. Physiol., Upstate Medical Center, Syracuse, NY 13210

It has been established that there are two classes of MEPPs in the frog (Kriebel & Gross, 1974, J. Gen. Physiol.) and in the mouse (Kriebel, Llados & Matteson, 1976, J. Physiol.). These two classes of MEPPs have also been seen in the re-innervating cutaneous pectoris of the frog. (Dennis & Miledi, 1974, J. Physiol.).

We have found in the re-innervating mouse diaphragm that the number of small mode MEPPs relative to major mode MEPPs is often greatly accentuated. Some cells which are newly re-innervated show a clear normal distribution of small mode MEPPs and a few with a mean 10-12 times the size of the small mode MEPPs. As the process of re-innervation progresses the percentage of major mode MEPPs increases with respect to the small MEPPs. A gradient is often seen in a single preparation in which the fibers near the point of nerve entry into the muscle have many major mode MEPPs and relatively few small mode MEPPs, while penetrations far from nerve entry give predominately small mode MEPPs.

In preparations partially blocked with cobalt it was found that the unitary evoked response was always greater than the major mode of the MEPPs.

After cutting or crushing the phrenic nerve spontaneous activity ceased within 18 hours. Changes in MEPP distributions in these preparations are rare.

Some MEPP distributions of the newborn mouse diaphragm, show a similarity to the re-innervating preparations described above.

These results indicate that there are two types of release processes at the neuromuscular junction that develop at different rates. (Supported by NIH Grant 11-1524D)

552 DEVELOPMENT OF DOPAMINE AND NEUROLEPTIC RECEPTORS IN THE CNS: *IN VIVO* STUDIES. L. Charles Murrin, Dept. of Pharmacology, Univ. of Nebraska Medical Center, Omaha, NE, 68105

A number of laboratories have demonstrated the feasibility of studying dopamine (DA) and neuroleptic receptors in vivo in adult rat brain by the use of radiolabelled DA antagonists, such as ^{3}H-spiperone (^{3}H-SP)(e.g. Life Sciences 22: 203, 1978). These studies have shown a regional distribution of stereospecifically bound ^{3}H-SP that was consistent with in vitro studies and with known DA innervation of brain regions. The in vivo binding was saturable and was blocked by DA agonists and antagonists. We here report the adaptation of these in vivo binding techniques to the study of dopamine and neuroleptic receptors in the central nervous system of the neonatal rat. Rat pups 5 and 15 days old were administered 250μCi/kg of ^{3}H-SP intraperitoneally. At various times after the injection they were decapitated and the brain was rapidly dissected and frozen. Brain regions were weighed, dissolved in Protosol and analyzed for radioactivity. In 5 day old rat pups there was a regional distribution of ^{3}H-SP binding. The striatum demonstrated the highest level of specific binding followed by the olfactory bulbs. At this age no other brain region (cortex, hippocampus, hypothalamus, cerebellum, thalamus, brain stem) exhibited any specific binding under the conditions used here. Specific binding was defined as radioactivity displaced by (+)-butaclamol (5 mg/kg, i.p., 30 min. before ^{3}H-SP). (-)-Butaclamol had no effect on levels of ^{3}H-SP bound in any region. In the striatum binding reached maximum levels at 1 hour and remained at this level for 4 hours. At 4 hours the striatal/cerebellar ratio was 4:1. In 15 day rats similar results were obtained with striatum again demonstrating the greatest level of binding. By day 15 specific binding could also be demonstrated in olfactory bulbs, cortex, hypothalamus, thalamus and brain stem. Striatal binding reached its maximum at 1 hour and remained at these levels for 2 hours, at which time the striatal/cerebellar ratio was 8:1. Binding in all brain regions at 15 days was reduced to cerebellar levels by (+)-butaclamol and unaffected by (-)-butaclamol. These studies demonstrate the usefulness of i.p. injection for in vivo study of dopamine and neuroleptic receptors in neonatal rats. They are consistent with the general posterior to anterior pattern of dopaminergic development as demonstrated by fluorescence histochemistry. They are also in good agreement with in vitro studies of the ontogeny of DA receptors in the striatum (Brain Res. 125: 376, 1977) showing a large increase in the level of DA receptor binding between day 5 and day 15. The applicability of these data to autoradiographic studies will be discussed. Supported by a seed grant from the Univ. of Nebraska.

553 EMERGENCE OF COORDINATED ACTIVITY BETWEEN FORELIMB AND MOUTH IN RAT FETUSES. C.H. Narayanan and Y. Narayanan*. Dept. Anat., Sch. Med., LSU, New Orleans, LA 70119.

In a previous study, we described three distinct periods in the development of spontaneous oral area activity in rat fetuses: (1) an early phase, beginning from day 17; (2) an acceleration phase, reaching a peak of activity on day 18 through day 19; and (3) a deceleration phase, from late day 19 of gestation age through term. In the present study, we have investigated the sequential appearance of coordinated activity between mouth and forelimb during development as a basis for the analysis of interrelations and control of neural networks involved in this early appearing pattern of behavior. Fetuses ranging from 14 to 20 days gestation age were used in this study. Spontaneous and evoked activity were recorded both quantitatively and qualitatively.

Forelimb movements first appeared as part of a total pattern with head movements until day 16. This was followed by local forelimb movements from day 17 of gestation age. A distinct synchronization of forelimb and mouth activity was observed in fetuses of 18 days gestation age. These movements consisted of alternate flexion and extension of the forelimbs, and face wiping. The forelimb movements were accompanied by mouth opening each time the forelimb was extended in the direction of the head. After day 18, fine digit movements were noticeable with an increasing frequency of synchronized movements of forelimbs, mouth opening and head movements. Evoked responses produced localization of the stimulus when applied to areas innervated by the trigeminal nerves. The results indicate that local actions or units probably represent a minimal unit of centrally initiated motor output during midgestational period. Components of such units presumably provide the substrate upon which complex functions such as suckling, mastication etc., are based.

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554 ABNORMAL DIFFERENTIATION OF CERTAIN NUCLEAR CENTERS IN THE BRAIN OF A DUCK EMBRYO ASSOCIATED WITH PARTIAL DUPLICATION OF THE PRIMITIVE STREAK. Y. Narayanan* and C.H. Narayanan (SPON: L.T. Happel), Dept. Anat., Sch. Med., LSU, New Orleans, LA 70119.

Conjoint twins occur occasionally during development in avian species due to duplication of the primitive streak. In a batch of duck embryos raised from incubating eggs, we found a duck embryo of 16 days incubation age with two bills as the only outward sign of duplication. The two bills were of equal size, each with fully developed upper and lower beaks. No beak movements were observed as in normal embryos at corresponding stages of development. The abnormal embryo had two eyes which were normal in outward appearance and were symmetrically placed one on either side of the head. Dissection of the head revealed, however, duplication of the telencephalon, optic lobes and the rhombencephalon. The inner (=medial) half of each of the duplicated embryonic brain division was greatly reduced. Histologic examination of sections through the head revealed the presence of two additional optic primordia each consisting of an undifferentiated mass of tissue representing lens and a much folded epithelium. The orbital muscles except for those associated with the pair of normal eyes were greatly disorganized. Abnormal differentiation of nuclear centers such as the ciliary ganglia, trochlear and accessory oculomotor nuclei and mesencephalic nucleus of the trigeminal nerve was determined by cell counts, and for the non-nervous structures such as the superior oblique and masseter muscles by area measurements. Cell numbers for the four ciliary ganglia, four accessory oculomotor nuclei and two trochlear nuclei appeared to be remarkably proportional to the extent of the peripheral field of innervation. The number of cells in the mesencephalic nucleus was slightly less than normal in spite of the duplication affecting the bill. The masseter muscles did not show a corresponding duplication which could account for the absence of beak movements and for the slight decrease in the number of cells in the mesencephalic nucleus of the abnormal embryo.

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555 DEVELOPMENT OF GLOBULAR CELLS OF RAT ANTEROVENTRAL COCHLEAR NUCLEUS FOLLOWING SOUND DEPRIVATION. P. O'Connor and J. Coleman. Depts. Psychol. and Physiol., Univ. of South Carolina, Columbia, SC 29208.

Binaural sound deprivation in mice has been reported to cause an attenuation of growth of globular cells in the ventral cochlear nucleus (Webster and Webster, Arch. Otolaryngol., 1977, 103, 392). The purpose of the present study was to confirm these findings in the albino rat as well as to establish a critical period of sensitivity to monaural sound deprivation. Auditory input was attenuated by surgical ligation of the external auditory meatus. Nine animals from one litter were binaurally deprived at 8 days after birth; 4 control pups taken from 3 other litters were nonoperates and all these subjects survived for a total of 50 days. The monaurally-deprived animals underwent surgery at various ages: 8, 10, 13, 16, 20, 24, 28, 32 or 36 days of age; either the right or left meatus was tied off, and 6 normal animals which were littermates of the operates were used as control subjects. Rats in this series were sacrificed at 70 days after birth. Animals were discarded if the ear canal was not completely closed. Transverse sections were cut and stained with cresyl violet. Globular cells were systematically located throughout the range of the anteroventral cochlear nucleus and measurements of length and width were recorded for each of 6900 cells (50/ear). Results from a repeated measures analysis of variance demonstrate that the binaurally-deprived experimental group had significantly smaller globular cells than the normal control subjects ($p < 0.0003$). The results for the monaural group indicate that age of the subject at the time of surgery was not a significant variable and there was no significant difference between ears. However, when the monaurally-deprived groups were collapsed the effect was significant with operated animals having smaller globular cells in both ears. These findings suggest that surgery per se may account for the observed results; the operation may somehow have interfered with the feeding process and the discrepancy in cell size was perhaps the result of nutritional deficits or some other unaccounted variable occuring during development. The monaural control animals were littermates of the operates and, as such, were perhaps better able to compete for food. In contrast the binaural controls were from 3 different litters, while another entire litter comprised the experimental group. Therefore, all the binaurally-deprived pups had relatively better nutritional opportunities than monaurally-operated subjects. In any case, sham operates should be included in future experiments of this type.

556 INTERACTION EFFECTS OF PRENATAL Co^{60} IRRADIATION AND POSTNATAL NURSERY OR MOTHER REARING ON GROWTH, BEHAVIOR, AND PERSISTENCE OF IMPAIRMENT IN SQUIRREL MONKEY OFFSPRING. J. M. Ordy, K. R. Brizzee, T. Beavers and P. Medart* Tulane University, Delta Regional Primate Research Center, Covington, La. 70433.

For a variety of phylogenetic and logistical reasons, the squirrel monkey has rapidly become one of the more widely used primate species for studying the effects of environmental hazards on brain mechanisms and behavior. Two major complicating factors in the use of this diurnal primate model include: (1) segregation of main effects of hazards from possible interaction effects due to postnatal rearing, and (2) failure to identify one of four possible squirrel monkey phenotypes. The specific aims of this study were to identify and segregate the main effects of prenatal 0, 10, 100 and 200R Co^{60} irradiation from interaction effects of rearing and phenotype, on postnatal growth rates, and behavior in relation to neuronal alterations and/or plasticity in the visual cortex and hippocampus of Colombian and Bolivian squirrel monkey offspring. Differences among nursery and mother reared, as well as Colombian and Bolivian offspring were observed primarily in test adaptation, emotionality and stereotypic behavior rather than sensory, learning, memory, and motor performance. Prenatal 100R Co^{60} irradiation resulted in adverse effects on reversal of original visual discrimination learning at 90, 365, and 730 days after birth. Prenatal 200R Co^{60} irradiation resulted in reductions of neuronal number and dendritic spine counts in the hippocampus and to a lesser extent in the visual cortex. The prenatal 100 and 200R Co^{60} irradiation effects on growth, behavior and brain morphology occurred independent of offspring nursery rearing and phenotype. (Supported by grants NIH HD09942 and NIH RR00164-17).

557 ACETYLCHOLINE RECEPTOR MOBILITY AND LOCALIZATION IN THE DEVELOPING MUSCLE MEMBRANE: MOLECULAR MECHANISMS. Norman Orida and Mu-ming Poo*. Dept. Physiol., Univ. Calif., Irvine, CA 92717

We are studying the movement and localization of acetylcholine receptors(AChRs) in the Xenopus embryonic muscle membrane using intracellular recording and ACh microiontophoresis mapping methods. In previous studies on these spherical, cultured muscle cells plated on clean glass surfaces, we have shown that within 30 min, a small, externally applied electric field(10V/cm) will cause an accumulation of AChRs towards the sides of the cells facing the negative pole of the field as indicated by a post-field asymmetric distribution of sensitivity to iontophoretically applied ACh (Orida and Poo. 1978. Nature 275: 31).

In the present study, the degree of field-induced asymmetric ACh sensitivity was used as an indicator of AChR mobility in the developing muscle membrane between 1 and 8 days in culture. Between 1 and 4 d, exposure to a standard 30 min, 10V/cm field resulted in a ratio of ACh sensitivities at the two poles of the cells of 2.3:1(negative:positive pole). On day 5, the standard field produced less asymmetry in the ratio of the ACh sensitivities and, therefore, AChR distribution on the two sides of the cells. The decline in the side to side sensitivity ratio leveled off and remained constant from day 6 through 8 at about 1.3:1. Pre-treating the cultures with Ca-Mg free saline(CMF) prior to the field (applied in normal Ca-Mg medium) significantly increased the AChR asymmetry over values normally observed when tested on days 2,6, and 8 indicating an increased AChR mobility. CMF treatment is known to remove glycosaminoglycans and other extracellular constituents of the cell coat on cultured cells. In contrast, pretreatment with cytochalasin B and colchicine did not increase the AChR asymmetry on day 2 but significantly increased it on day 6. These results demonstrate a developmental decrease in AChR mobility in the muscle membrane and suggest two possible mechanisms for its basis: (1) An early CMF-sensitive restriction observed from day 2 onwards possibly mediated by the cell coat; and (2) A later-developing constraint mediated by the cytoskeleton.

Localization by the field leads to the formation of stable AChR clusters which persist for hours and are resistant to disruption by various pharmacological treatments. CMF or cytochalasin B and colchicine applied to the cultures after the field failed to disperse the clusters as determined by maintained asymmetric ACh sensitivities at the two poles of the cells throughout the entire period of post-field mapping(up to 5 hrs). These findings indicate that the maintenance of the AChR clusters may involve other mechanisms than suggested above.

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558 QUANTITATIVE ANALYSIS OF ULTRASTRUCTURAL FEATURES IN THE EARLY DEVELOPMENT OF THE CUNEATE NUCLEUS. E.-Michael Ostapoff*, John Irwin Johnson, and Charles D. Tweedle. (SPON:G. I. Hatton) Neuroscience Program and Zoology, Psychology and Biophysics Depts., Michigan State Univ., E. Lansing, MI 48824.

Electron micrographs of 500 μm^2 systematic samples of the dorsal cuneate nucleus near the obex, from pouch young opossums, in the period prior to and during initial synaptogenesis at 8 and 9 days after birth, were analyzed planimetrically. At 3 days postnatal, during the period of epithelial cell division, perikaryal cytoplasm (P) and nuclei (N) accounted for 93% of the identified area in the sample; profiles containing a floccular appearing substance and an electron-lucent background (ELF), possibly dendritic growth cones,were 2%; profiles of processes containing microtubules and ribosomes (MTR), possibly neurites, made up 4%; and profiles containing microfilaments (MF) made up the remaining 1%. At 5 days, when cell migration is under way, P and N made up 39% and 35% respectively, ELF 4%, MTR 11%, microtubule containing processes with no ribosomes (MT) 3%, MF 8%. By 7 days, P and N each accounted for 30%, electron-dense profiles (ED) possibly axonal growth cones and/or degenerating processes were 6%, ELF 18%, MTR 5%, MT 3%, AT less than 1%, ED 6%, and MF 2%. Axonal endings with round vesicles (AT) appeared in appreciable numbers on the 8th day, and large profiles containing a floccular appearing matrix and sparse or clumped synaptic-sized vesicles (ATL) were identified in the 8 and 9 day samples. At 8 days P were 23%, N 19%, ELF 23%, MTR 18%, MT 7%, AT 3%, ATL 2%, ED 4%, MF 1%. At 9 days P were 26%, N 18%, ELF 14%, MTR 11%, MT 12%, AT 4%, ATL 4%, ED 4%, and MF 5%. These findings are consistent with a sequence of events wherein dendritic growth has started on the third day and peaks on the 8th day; axonal growth cones appear on the 5th day and peak on the 7th day; both of these peaks are just prior to synaptogenesis on the 8th and 9th days. Postsynaptic profiles then include MTR, MT, ELF and P; presynaptic profiles are ED, AT and ATL.

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559 THE REGULATION OF PLAY: NEUROCHEMICAL CONTROLS Jaak Panksepp. Dept. of Psych., Bowling Green State University, Bowling Green, Ohio 43403

Although there is a vast descriptive literature on the play of animals, the systematic experimental and psychobiological analysis of this behavior remains rudimentary. In this work, we determined that play (as indexed by the frequency of pinning) could easily be measured in laboratory rats and found that through play stable dominance hierarchies were established between animals. Vigorous play started at approximately 18 days of age, increased to 24 days of age, and thereupon remained stable for about 20 days, gradually declining following puberty. The play appeared to be homeostatically controlled in that amount of play increased with social deprivation and decreased with social satiation. For instance, in 28 day old animals, 8 hrs of social isolation increased play by 125% and 24 hrs of isolation increased it by more than 300%. To evaluate the participation of various brain neurochemical systems in the control of play, we studied the effects of a large number of psychoactive drugs. Facilitation of brain serotonin activity with fenfluramine (2.5-10 mg/kg) or quipaaine (5-10 mg/kg) markedly reduced play, while methysergide (5 mg/kg) slightly increased play. Both reduction and facilitation of catecholamine activity (with 1 mg/kg of chlorpromazine and d-amphetamine respectively) reduced play. All of these effects were present in both socially housed and socially isolated animals. The opiate system was the only one of those tested which exhibited opposite effects with respect to social housing conditions. Whereas opiate blockade with naloxone (1-5 mg/kg) reduced play marginally in both isolated and grouped animals, morphine at low doses (.5-1.0 mg/kg) increased play of socially isolated animals while reducing the play of socially housed animals. In general, the results indicated that a systematic analysis of play is a feasible enterprise in rats. The deprivation and satiety functions were as systematic as those obtained with other basic homeostatic motivations such as hunger and thirst. Not only do brain neurochemicals regulate play, but we suspect that activity in certain brain circuits is, in turn, regulated by play.

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560 MORPHOMETRICAL ANALYSIS OF DEVELOPING NON-PYRAMIDAL NEURONS IN THE VISUAL CORTEX OF THE RAT. John G. Parnavelas and H.B.M. Uylings*. Dept. Cell Biology, Univ. Tx. Health Sci. Ctr., Dallas, Tx. 75235 and The Netherlands Inst. Brain Res., Amsterdam, The Netherlands.

In this study we sought to investigate with the aid of a computer system the 3-dimensional geometry and development of non-pyramidal neurons in the visual cortex of the rat.

Two to three brains of female albino rats from each of the following ages were treated with the Golgi-Cox technique: 4, 6, 8, 10, 12, 14, 16, 18, 20, 24 and 90 (young adult) days. 617 cells of layer IV were measured with a semi-automated dendrite tracking system[1] at X1,250.

The results show that the cell body size of non-pyramidal neurons, estimated by the projection of the surface area onto the plane of sectioning, increases conspicuously during the early stages of development and reaches adult dimensions at day 16 of postnatal life. This is also the time that the number of primary dendrites and total number of dendritic segments per neuron achieve adult values. However, the total dendritic length per neuron and the radial distance of terminal tips from the center of the cell body increase until day 18. As for the curvilinear lengths of dendritic segments, the analysis shows that they increase markedly until day 18. After this day the lengths of the intermediate segments decline slightly while those of the terminal segments continue to increase at least until day 90. From the morphometrical analysis it is concluded that the dendritic fields of non-pyramidal neurons of layer IV show a preferential orientation in the coronal plane.

1. Overdijk, J. et al. (1978) J. Microsc. 114: 271-284.

561 ORIENTATION OF AXONAL GROWTH OF EMBRYONIC XENOPUS NEURONS BY SMALL EXTRACELLULAR ELECTRIC FIELD. N. B. Patel* and M-m. Poo* (SPON: J. E. Swett). Department of Physiology, University of California, Irvine, CA 92717.

Small extracellular electric fields have been found to influence the neurite growth from chick medulla and dorsal root ganglia explants (Marsh and Beams, J. Cell. Comp. Physiol. 27: 139, 1946; Jaffe and Poo, J. Exp. Zool., 1979, in press). In the present study, we found that an extracellular electric field of about 6V/cm greatly enhanced the axonal growth of dissociated spinal cord neurons towards the cathode within a few hours. Single neural tube cells of 1-day old Xenopus embryos (Stage 17-19) were cultured on a clean, glass surface by previously reported method (Orida and Poo, Nature, 275: 31, 1978). After the cells adhered to the glass substratum, a uniform, steady electric field was applied to the culture via a pair of agar bridges filled with Steinberg's saline. The position of the growth cones with respect to the soma was recorded in polar co-ordinates after the field was removed.

In the absence of an electric field (control), there was a radially symmetrical growth of axons with an average growth rate of 11.7 $\pm$ 0.9 µm/hr ($\pm$ S.E.M., N = 53). When an electric field was applied for 6 hrs, in 3 separate experiments, there was a marked asymmetric growth. The average axonal growth rate towards the cathode was 20.6 $\pm$ 1.5 µm/hr (N = 85); while towards the anode it was 12.6 $\pm$ 1.1 µm/hr (N = 53). It was observed that after the onset of the field, the growth cones of axons previously extending perpendicularly to the field axis gradually changed their direction of growth towards the cathode. In addition, neurons with no previous axonal growth now grew their neurites predominantly from the cathodal pole of the soma. This indicates that the electric field produces its orienting effects both in the rate and direction of axonal growth, and in the initial point of origin of the axon from the soma. Since the electric field used was too small to act directly on the intracellular components, we hypothesize that its effects are mediated through the lateral electrophoresis of membrane-bound growth-controlling components along the plasma membrane.

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562 PURKINJE CELL DENDRITIC DEVELOPMENT IN LEAD-TREATED CATS AND RATS. George W. Patrick, William J. Anderson, and Patrick D. Brophy*. Indiana Univ. Sch. of Med., Terre Haute Ctr. for Med. Ed., Terre Haute, IN 47809

Beginning on day 1 of pregnancy (verified by the presence of sperm in a vaginal wash) female rats were given food ad lib mixed with 2% or 5% lead acetate by weight. Control females were also maintained. At birth the litter size was reduced to 8 and half the pups were transferred to a mother of the opposite design yielding 4 experimental groups: control (C), prenatal only (LC), postnatal only (CL), and pre- and postnatally treated (LL). Rats were then sacrificed at 30 days of age and prepared for Golgi analysis. Kittens were obtained from our cat colony. On postnatal day 1, the kittens were given 2 mg/100 g body weight lead acetate dissolved in distilled water at a concentration of 4 mg/ml or distilled water by esophageal intubation for 35 days. They were then sacrificed and prepared for Golgi analysis.

The body weights of rats were (% of control): LL - 31%, LC - 77%, and CL - 54% for the 5% study and LL - 57%, LC - 91%, and CL - 84% for the 2% study. The body weights of cats were reduced by 17% of control. The brain weights of rats were reduced as follows: (% of control) LL - 79%, LC - 86%, and CL - 78% for the 5% study and LL - 62%, LC - 87%, and CL - 75% for the 2% study. Cat brain weights showed no significant differences. The rats in the LL group of the 5% study showed hind-limb paralysis as described by Pentschew and Garro (1966). The other groups of rats showed no observable differences. Some kittens showed hind-limb ataxia and exopthalmia and occasionally displayed cerebral hydrocephalus and herniation of the cerebellum through the foramen magnum. The Purkinje cells in the kittens treated with lead were significantly different in the dendritic arborization. There were fewer tertiary dendrites and they seldom reached the surface of the molecular layer. The LL and CL rats of the 5% study showed significantly reduced dendritic arborization of the Purkinje cells also. The LC rats showed very little reduction.

563 EFFECTS OF AMINO ACID DEPLETION DURING EARLY ONTOGENY ON SUBSEQUENT LOCOMOTOR ACTIVITY AND LEARNING IN RATS. Jim H. Patton*, Richard R. Fritz*, Ernest S. Barratt, and Creed W. Abell*. University of Texas Medical Branch, Galveston, Texas 77550.

The effects of rapid and specific depletion of plasma levels of tyrosine (TYR) and phenylalanine (PHE) between 4 and 8 days of age on subsequent behavior were studied in rats. Phenylalanine ammonia-lyase (PAL) (E.C.4.3.1.3), an enzyme purified from yeast, catalyzes the deamination of PHE and TYR to cinnamic acid and coumaric acid, respectively. PAL rapidly, reversibly, and selectively depletes plasma of these amino acid precursors of catecholamine (CA) synthesis (Abell, et al., 1973; Barratt, et al., 1976). As a result brain levels of CA's are reduced (Barratt, et al., 1976).

Daily injections of 100 units PAL per kilogram body weight (Experimental) and buffer vehicle (Control) were administered I.P. to neonatal rats between 4 and 8 days of age (inclusive). The animals were weaned at 22 days of age and housed multiply thereafter. Locomotor activity testing at 39 days of age revealed that PAL-treated animals were less active in a novel environment initially (first five minute period) but were no different than controls during two subsequent five minute periods. This difference was statistically reliable. PAL rats also defecated less in the test apparatus than controls, a finding which also was statistically reliable. Differences in shuttle avoidance acquisition were marked at 65 days of age. Animals were given five blocks of 10 trials each (50 trials total). The PAL rats made more avoidances responses within each block of 10 trials than controls, although only avoidance differences during the last two blocks of trials were statistically significant. In addition, the PAL group made a significantly greater number of total inter-trial shuttle crossings than controls. No weight differences were seen at time of testing between the groups.

564 EFFECTS OF LITTER COMPOSITION ON BODY WEIGHT, ACTIVITY PATTERNS AND AVOIDANCE PERFORMANCE IN NORMAL AND 6-HYDROXYDOPAMINE (6-OHDA) TREATED RAT PUPS. David E. Pearson*, Martin H. Teicher* and Bennett A. Shaywitz. Dept. of Ped. and Neuro., Yale Univ. Sch. Med., New Haven, CT 06510

We have examined the effects of both environmental alteration and biochemical manipulation on the ontogeny of activity and cognitive performance in normal developing rat pups and littermates administered 6-OHDA at 5 days of age. Such treatment results in a permanent and selective destruction of brain dopaminergic pathways, and behavioral manifestations with many similarities to the childhood disorder known as Attention Deficit Disorder (ADD) with hyperactivity. Litter composition was arranged to examine four different rearing conditions: Homogeneous litters of all 6-OHDA treated pups (T-Homo); homogeneous litters of sham controls (V-Homo); and mixed litters of half 6-OHDA treated and half sham (T-Hetero, V-Hetero). Body weights for V-Hetero were found to be significantly greater than all other groups at all ages, but weights for V-Homo, T-Homo and T-Hetero were not significantly different from each other until 27 days. Activity levels at 12 days of age were lower in 6-OHDA pups, equal at 15 days and 6-OHDA animals demonstrated higher levels of activity thereafter. V-Homo was more active than V-Hetero at 15 days of age, ($p < .001$) and T-Homo more active than T-Hetero at 23 and 27 days of age, ($p < .001$). Over the hour long observation period, V-Hetero exhibited a more rapid reduction of activity (habituation) than any other group. 6-OHDA pups displayed a significantly greater escape latency (indicating an impaired performance) on avoidance tasks at 20 days (T-maze) and 28 days (one-way shuttle box). In the shuttle box task T-Homo exhibited an increased escape latency compared to T-Hetero ($p < 0.001$) indicating that 6-OHDA animals reared with normals performed better than 6-OHDA pups reared with only other damaged pups for littermates. Brain dopamine concentrations in 6-OHDA treated animals averaged 14% of controls levels, compared to norepinephrine levels which averaged 89% of controls. Within a similar treatment (either 6-OHDA or control), no differences in brain catecholamines were found, regardless of assignment to homogeneous or heterogeneous litter type. Our findings that rat pups reared in heterogeneous litters exhibit significantly different patterns of activity and avoidance performance from animals reared in homogeneous litter compositions supports the notion that both environmental and biological factors act to influence the behavioral repertoire of the developing organism. By demonstrating that the effects of 6-OHDA on activity and performance are unrelated to body weight, these results confirm the importance of brain dopaminergic pathways in the ontogeny of behavioral arousal.

565 SYNAPTIC ENDINGS IN THE CHICK TANGENTIAL NUCLEUS: A MORPHOGENETIC STUDY WITH THE ELECTRON MICROSCOPE. Kenna D. Peusner* (SPON: N. Moskowitz). Dept. of Anat., Jefferson Medical College, Philadelphia, Pa. 19107.

This study focuses on the morphogenesis of "spoon" endings of the chick tangential nucleus, part of the lateral vestibular complex. The principal cells selectively receive spoon endings from a special class of primary vestibular afferents, the colossal fibers. Previous studies with the rapid Golgi method showed that one spoon covers 1/4 to 1/3 of the cell's soma in 18 day embryos and that spoon endings undergo a rigid sequence of structural changes in development (Peusner). It was thus of interest to study the ultrastructure of spoon endings in embryos, perfusion-fixed, at ages preceding (8-9 days), during (10-13 days), and following (14-16 days) spoon formation. At 8-9 days colossal fibers may flank the principal cell soma with frequent attachment plaques and occasional vesicular synapses joining them. At the contact site, the colossal fiber contains the ultrastructural components found in developing spoons, namely vesicles, neurofilaments, mitochondria, and microtubules. Boutons with vesicular synapses on the principal cell soma are infrequent at 8-9 days and increase slightly in numbers during spoon formation. At 8-9 days each colossal fiber develops a swelling en passant which contains a dense thicket of neurofilaments, microtubules, and a plethora of mitochondria, with many degenerative profiles, perhaps forewarning the disappearance of the swellings by 11 days. The swelling surface has few if any synaptic contacts. Conceivably, spoon development depends on the formation of the swelling precursor. For at 10 days the swelling is joined near its medial edge by an expansion which becomes a young spoon by 13 days. From 11 to 13 days, the principal cell sprouts lush somatic spines where contact is made with the expansion, which stays in close parallel, for the junction has present attachment plaques and vesicular synapses. At 14 days, this junction contains a new specialization, which has symmetrical cytoplasmic densities and a cleft reduced to about 50 A; its identity needs to be explored. For at 15 and 16 days, the cleft at the junction of the spoon-principal cell shows dilatations, often bordered by gap junctions. The reduced-cleft specializations, attachment plaques, and vesicular synapses are found at the junction as well. In short, the junction of the developing spoon and principal cell is the site of mixed synapses, with vesicular synapses appearing well in advance of gap junctions. Furthermore, it is evident that the spoon ending is the predominant and, moreover, a quite powerful source of synaptic input to the developing principal cell. The morphology and consequent influence of spoon endings in the vestibular system of the mature animal remains to be established.

566 IMMUNOCYTOCHEMICAL LOCALIZATION OF ENKEPHALINE AND SUBSTANCE P IN RAT BRAIN DURING DEVELOPMENT. V.M. Pickel, K.K. Sumal*, R.J. Miller and D.J. Reis, Dept. Neurol., Cornell Univ. Med. College, New York, N.Y. 10021.

Using immunocytochemistry, we sought to determine the presence and regional distribution of neurons containing enkephalin and substance P (SP) at embryonic (E) days 15, 18 and 20, and postnatal (PN) day 1. Adjacent sections of brains were incubated with specific antiserum to enkephalin, SP or control serum and then processed by the peroxidase-antiperoxidase method. The reaction product for both peptides was absent at E15 and present in certain groups of perikarya and fibers at E18. At the latter age, enkephalin-like immunoreactivity (ELI) was localized to perikarya in many regions of the brain which did not contain SP labeled neurons. These include substantia gelatinosa of n. trigeminal, n. tractus solitarius, n. lateral lemniscus, and n. parabrachialis. Other regions such as the n. stria terminals, caudate-putamen, and n. central amygdala contained perikarya for both SP and enkephalin; whereas the n. habenulae lateralis contained only SP labeled perikarya. Fibers, but not varicose terminal fields, showed enkephalin and SP reaction product at the E18 stage. Terminal regions showing both enkephalin and SP were present by E20. The location of terminals and perikarya containing ELI at PN1 was similar to that of adult colchicine treated rat brains (Sar et al., J. Comp. Neurol. 182: 17, 1978). Although the nuclear groups contained more perikarya selectively labeled for the peptides at PN1, their distribution was essentially the same as E20. Both ELI and SP were present in well defined fiber bundles. ELI was noted in numerous pathways, some of which were also common to SP including the lateral lemniscus, medial forebrain, and stria terminals. Other bundles such as the mammillothalamic contained only ELI, while certain fiber systems such as the habenulointerpenuncular contained only SP. There were likewise similarities and differences in the terminal fields labeled with SP and/or enkephalin at PN1. We conclude that neurons containing SP and ENK can be detected immunocytochemically by E18 and that tnese peptides are more evident in perikarya and pathways during development than in untreated adult animals.

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567 ONTOGENY OF β-1 AND β-2 ADRENERGIC RECEPTORS IN RAT CEREBELLUM AND CEREBRAL CORTEX. R.N. Pittman*, K.P. Minneman*, and P.B. Molinoff. (SPON: D. Whitlock). Dept. of Pharm., Univ. of Colo. Med. Ctr., Denver, CO.

The β-adrenergic receptor antagonist ^{125}I-iodohydroxybenzylpindolol (IHYP) has the same affinity for β-1 and β-2 adrenergic receptors. Graphical analysis of the inhibition of specific IHYP binding by a variety of drugs selective for β-1 or β-2 adrenergic receptors results in curvilinear Hofstee plots. An iterative computer based method was used to determine the densities of the two receptor subtypes in homogenates of rat cerebral cortex and cerebellum at various times during development. In the cerebral cortex, which contains mainly β-1 receptors, total β-adrenergic receptor density increased sharply between postnatal days 10 and 21. The density of receptors remained fairly constant through six weeks of age though it subsequently declined. The relative proportions of β-1 and β-2 receptors were fairly constant throughout development of the cerebral cortex. Therefore, the development of the density of the two receptor subtypes closely paralleled the development of total β-receptors in the cerebral cortex.

The ontogeny of β-adrenergic receptors in the cerebellum, which contains mainly β-2 receptors, was strikingly different from that observed in the cerebral cortex. Total cerebellar β-adrenergic receptor density steadily increase from postnatal day 5 through day 42. At this time the density of receptors plateaued and remained constant for up to six months. The relative proportions of β-1 and β-2 receptors in the cerebellum changed markedly during development. Between days 8 and 13 approximately 18% of the receptors were of the β-1 subtype. This proportion steadily decreased with age, and in 3 to 6 month old animals only 2% of the receptors were of the β-1 subtype. Therefore, although the density of cerebellar β-2 adrenergic receptors showed an increase similar to that observed for total β-adrenergic receptors, the density of cerebellar β-1 receptors steadily declined between days 20 and 90. The relationship between the development of β-1 and β-2 receptors and various developmental events in the cerebral cortex and cerebellum will be discussed. (NS 13289 and NS 09199).

568 DEVELOPMENT OF WING-FLAPPING AND FLIGHT IN NORMAL AND FLAP-DEPRIVED CHICKS (Gallus domesticus): A STROBOPHOTOGRAPHIC ANALYSIS. Robert R. Provine. Dept. of Psych., Univ. Md. Balto. Co., Catonsville, MD 21228.

A previous study of spontaneous embryonic motility showed that there is a dramatic increase in bilateral wing coordination during the last days of incubation. By the time of hatching, the wings produce low amplitude, bilaterally synchronized, smooth wing movements (Provine, Dev. Psychobiol., in press). The present study extends the developmental analysis of wing movements to postnatal stages by investigating the emergence of drop-evoked wing-flapping and horizontal flight in 1 to 21 day chicks. Wing-flapping movements evoked by dropping tethered chicks 1.9 M were studied using strobophotography (100 Hz). This technique provides information concerning the position of the rapidly moving wings every 10 msec. The capacity for horizontal flight was evaluated by measuring the lateral progress made by untethered chicks when dropped 1.7 M.

Drop-evoked, bilaterally symmetrical wing extension and slow low amplitude flapping were found by the day after hatching. However, flapping rate and amplitude increased up to 13 days after which they leveled off. These developmental changes in wing-flapping behavior were positively correlated with the maturation of drop-evoked horizontal flight between 7-13 days and the development of larger, well feathered wings.

The influence of post-hatching wing movement experience on the development of wing-flapping and flight was evaluated by immobilizing both wings on the day after hatching and testing for drop-evoked wing-flapping and horizontal flight on day 13 immediately after freeing the wings. Day 13 was chosen for testing because adult levels of wing-flapping and horizontal flight are present in control chicks at this stage. The wings were immobilized by binding them to the thorax with elastic bandages. At 13 days, the movement deprived chicks showed amounts of drop-evoked, bilaterally synchronous wing-flapping and horizontal flight similar to unbound 13-day control chicks indicating that wing movement and practice are not necessary for the emergence of wing-flapping and flight. This result is consistent with the earlier finding that muscular correlates of wing-flapping were present in 13-day chicks that had both wings amputated on the day after hatching (Provine, Behav. Neur. Biol. in press).

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569 DEVELOPMENT OF β-ADRENERGIC RECEPTORS AND ADENYLATE CYCLASE ACTIVITY IN THE CHICK SPINAL CORD. Walter Prozialeck*, Arlene Pylypiw* and Leonard L. Ross. Department of Anatomy, Medical College of Pennsylvania, Philadelphia, PA 19129.

The chick spinal cord receives a substantial descending adrenergic input from supraspinal levels. It has been recently reported that during development these descending adrenergic axons contain norepinephrine and its synthesizing enzymes, and that they establish their first synaptic contacts with spinal cord neurons at 10-12 days in ovo (Caserta and Ross, Brain Res. 144:241, 1978). In order to further characterize the functional development of these synapses, we studied the development of β-adrenergic receptors and adenylate cyclase activity in homogenates of chick thoraco-lumbar spinal cord. β-adrenergic receptors were measured by the specific binding of ^{3}H dihydroalprenolol (DHA). Receptor density (Bmax) and the apparent dissociation constant (Kd) for DHA binding were calculated by Scatchard analysis. Adenylate cyclase activity was determined by the method of Krishna et al. (JPET 163:379, 1968). The binding of DHA in mature spinal cord homogenates was characterized by a high affinity (Kd = 6.5 nM), saturability (Bmax = 20 fmole/mg tissue), and was 100 times more sensitive to inhibition by l-propranolol than by d-propranolol.

β-receptors were first demonstrated at 14 days in ovo (Bmax = 4.3 fmoles/mg tissue). Few if any receptors were demonstrable in younger embryos. From day 14 to day 20 in ovo the number of receptors increased to 14 fmoles/mg tissue. Receptor density then increased dramatically over the next few days and by the second day after hatching reached a peak of 45 fmoles/mg tissue. Between the second and ninth post hatching days the receptor density declined to adult levels (∿20 fmoles/mg tissue). The affinity of DHA for the β-receptor did not change throughout development, with the Kd remaining ∿6.5 nM. The development of adenylate cyclase activity closely paralleled that of β-receptors with peak activity being reached on the second day after hatching.

Since β-adrenergic receptors are not detectable until 4 days after the formation of the first adrenergic synapses in the chick spinal cord, it appears that the early development of adrenergic synapses might not be dependent on the presence of β-adrenergic receptors on the spinal cord neurons. The role of the adrenergic input in the development and maintenance of β-adrenergic receptors is under investigation.

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570 THE ELIMINATION OF REDUNDANT INNERVATION IN NEONATAL SYMPATHETIC GANGLIA. Dale Purves and Jeff W. Lichtman. Dept. of Physiol. and Biophys., Washington Univ. Sch. of Med., St. Louis, MO 63110

Neurons in superior cervical ganglia from neonatal (0-7 day) hamsters are innervated by an average of 11-12 axons arising from 4 different spinal cord segments. Ganglion cells from adult animals, however, are innervated by only 6-7 axons arising from about 3 spinal cord segments. Thus many of the synaptic contacts initially present appear to be lost during development. This elimination of redundant innervation probably occurs during the first few weeks of postnatal life since ganglion cells in 2-3 week-old animals are innervated to an intermediate degree. The overall innervation of the superior cervical ganglion in adult hamsters arises from thoracic segments T1-T5; in spite of the loss of about half of the innervating axon terminals, no additional spinal segments contribute significantly to the innervation of ganglia in neonatal animals. Thus synapse elimination does not occur because some initial innervation arises from inappropriate spinal levels. As in other mammals (see for example Njå and Purves, 1977) the pattern of adult ganglion cell innervation in the hamster is selective in that each neuron tends to receive innervation from a preferred subset of the segments that innervate the ganglion as a whole. This selective tendency is less pronounced in neonatal animals. We conclude that a) the elimination of redundant innervation in early life is not limited to target cells innervated by a single axon in maturity, and b) the loss of redundant contacts refines the selective pattern of synapses made on individual ganglion cells.

Njå, A. and Purves, D. J. Physiol. (Lond.) 264: 565-583, 1977.

571 THE EFFECT OF CORDOTOMY ON DENDRITE BUNDLES AND TREADMILL WALKING IN KITTENS. Patricia Reback* (SPON: R.D. Lindsay). Dept. Kinesiology, UCLA, Los Angeles, CA. 90024.

Dendritic bundles of α motoneurons are present in the spinal segments innervating the forelimbs, but not the hindlimbs of the cat at birth. Since bundles mature in the lumbosacral area coeval with the ability to walk, Scheibel and Scheibel (Exptl. Neurol. 23:106 1970) have suggested that dendritic bundles might be a substation for spinal activity that helped to shape motor control, especially in the coordination of locomotion.

The present study addresses the problem of dendrite bundle formation and maintenance in relationship to treadmill walking of cats, with spinal cord transections at T-12 made during 2 stages of development: 2 weeks postnatal, at which time dendritic bundles and alternating flexor/extensor movements are rudimentary and 12 weeks postnatal, a time when dendritic bundles and walking ability approach that of the mature cat. Each age group was separated into 2 subgroups: those exercised daily for 20 minutes of treadmill walking and those left to spontaneous activity in a limited environment. Kittens were sacrificed 6 weeks or more post-cordotomy. Prior to sacrifice, both nonexercised and exercised groups were rated on a scale of 1-7 for ability to walk on a motorized treadmill at several speeds. At sacrifice the animals were perfused and the cords stained using the rapid Golgi technique. Horizontal cuts were made and the degree of bundling was observed by light microscopy. Spinal segments on coded slides were rated on a scale of 0-5 for presence and complexity of bundles. This data was then compared to the treadmill rating.

Preliminary results suggest that CNS integrity is not always a prerequisite for bundle formation and that dendritic bundle formation and/or maintenance in the lumbar cord are not necessary requirements for treadmill walking. Although there was evidence of bundle formation in 6 out of 8 of the animals cordotomized at 2 weeks (ratings ranged from 1-4), this formation was not correlated to ability to treadmill walk or to spontaneous weight bearing. There was also no correlation between bundle formation and treadmill exercised animals. Cordotomy on the 12 week animal had a detrimental effect on maintenance of the bundles. These animals were all sacrificed after 6 months of age, at which time, ordinarily, bundle formation is within the 4-5 classification (mature). None of the sections examined were rated higher than a 3 on the numerical scale, and signs of degeneration were readily apparent in all sections. Again, no correlation between the treadmill rating and degree of bundling was observed. Initial evidence, however, suggested that daily exercise on the treadmill may slow degeneration of the bundles.
Supported by a grant from Easter Seal Research Foundation R7712

572 BEHAVIORAL COMPONENTS OF THE CLONIDINE RESPONSE IN THE DEVELOPING RAT. Daniel K. Reinstein* and Robert L. Isaacson, Dept. of Psychology, State University of New York, Binghamton, NY 13901.

Clonidine (2, 1 and .5 mg/kg) elicits a response in the 7 and 14 day old rat which is characterized by increases in locomotor, wall climbing and head raising motor patterns. At 21 and 28 days the response to the same dose range is characterized by a form of catalepsy (see Reinstein and Isaacson, 1977. Brain Research).

Increases in open-field activity are observed in 10-20 day old normal rat pups when tested in isolation. One component contributing to this response could be hypothermia. An increase in locomotor activity has been proposed as one thermo-regulatory mechanism available to the infant rat, and clonidine produces hypothermia in the adult rat at room temperature. In the first experiment we undertook to determine if the locomotor response to clonidine before 21 days of age was related to its hypothermic effect. In an open field the behavioral response to clonidine was found to increase with increasing ambient temperatures. At the two lower temperatures (25° and 30°C) the 10 day old animals experienced a 2°C drug-induced drop in colonic temperature. At the high temperature 36°C the animal's temperature rose 1°C after the drug. Control animals at 25° and 30° did not drop core temperature after the pre-injection adaptation period but did experience a 1°C rise in temperature in the 36° environment. This indicates that clonidine-induced locomotor changes in activity are not due to a fall in core body temperature.

In the second experiment the open field response to clonidine at 25°C was observed in 10 day old animals pretreated with phentolamine (2 and 15 mg/kg), phenoxybenzamine (2 and 15 mg/kg) or naloxone (.2 and 2 mg/kg). Phentolamine pretreatment at 15 mg/kg reduced the initiation of wall climbing bouts after clonidine and, at both doses, attenuated the clonidine-induced change in core temperature. Phenoxybenzamine at 15 mg/kg attenuated the decreases in body temperature only. Naloxone had no effects on any of the clonidine-induced behaviors. There were no effects of any of the drugs on locomotion, duration of wall climbing bouts, once initiated, or head raises.

These findings indicate that the temperature reduction produced by clonidine is α-adrenergically mediated but that the locomotor changes found after the drug are not. The reduction in the initiation of clonidine-induced wall climbing seen after pretreatment with phentolamine may reflect actions on a developing adrenergic regulatory mechanism related to spinal cord organization and coordination. The behavioral effects of this adrenergic system may depend on the maturational state of the corticospinal tract.

573 THE DEVELOPMENTAL PATTERN AND LOCALIZATION OF A RAT BRAIN SPECIFIC ANTIGEN (G5) USING MONOCLONAL ANTIBODY. Jane Somsel Rodman* and Richard Akeson, Children's Hospital Research Fdn., Cincinnati, Ohio 45229.

G5 is a monoclonal antibody developed from the fusion of a myeloma cell line (P3-X63-Ag8) and spleen cells from a mouse immunized with rat pheochromocytoma cell line (PC12). G5 reacts strongly with adult rat brain particulate protein and to a lesser extent with rat spleen and bone marrow but not with eleven other cells or tissues as has been previously described (Akeson and Graham, submitted). Using a direct binding assay with antibody excess and ^{125}I-protein A, the presence of G5 antigen in the developing rat brain has been studied. The specific binding activity, as well as, total amount of G5 antigen is very low in the newborn rat brain (30 ng IgG bound/mg brain particulate protein). G5 binding increases rapidly with age until day 28 to levels of 300-400 ng IgG bound/mg brain particulate protein. After this time, G5 specific binding activity increases slowly to adult levels of 400-500 ng IgG bound/mg brain particulate protein. The total G5 binding to brain increases 150-200 fold from birth to age 60 days.

The macroscopic localization of G5 antigen has been studied using the direct binding assay on homogenates of regions of the dissected adult rat brain. It has been found that G5 antigen is concentrated in the cerebral cortex gray matter which contains ten-fold more G5 antigen than cerebellum, cerebral cortex white matter, brain stem or spinal cord.

Additional studies to determine the cell type specificity and molecular composition of G5 antigen will be reported.

574 A MORPHOMETRIC STUDY OF SYNAPSE FORMATION IN THE CHICK SYMPATHETIC GANGLION FOLLOWING SPINAL CORD TRANSECTION. Leonard L. Ross and Leo Cosio*, Department of Anatomy, Medical College of Pennsylvania, Philadelphia, PA, 19129.

We have previously described by morphometric analysis (Ross et al. 1978) that few synapses are found in the chick lumbar sympathetic ganglia prior to hatching. Beginning at two days post-hatching, there is a rapid proliferation of synapses and adult levels are attained by 21 days. We have also reported (Ross, Smolen and Cosio, 1977) that spinal cord transection in the chick embryo at the presynaptogenic stage (8 days in ovo), while reducing the afferent input to the preganglionic nucleus (of Terni) by 40%, does not affect either the number or the morphological development of the neurons of this nucleus.

To determine the effects of supraspinal input to the preganglionic neurons on the development of peripheral ganglionic synapse formation, the spinal cords of two days old chicks were transected at cord level T3 and the animals were allowed to survive until 21 days. At this time the chicks were perfusion fixed and the lower thoracic spinal cords and the lumbar ganglia were prepared for ultrastructural morphometric study.

In the spinal cord transected chicks, the synaptic density in the ganglia was reduced by 60% when compared with controls of the same age. Although the ganglia of the spinal cord transected animals were considerably smaller than those of the controls, the density of neurons was not significantly different. These observations provide a morphometric extension of the biochemical observations of Hamill, Bloom and Black (1977) and confirm the existence of a transynaptic influence on the formation of ganglionic synapses. That is, a neuron may require an intact afferent input for the formation of a normal complement of its own axonal terminals. The quantitative aspects of this phenomenon in the spinal cord are being determined by a morphometric analysis of the synaptic input and neuronal population of the nucleus of Terni of these post-hatch animals.

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575 DIFFERENTIATION OF ENTERIC NEURONS FROM UNRECOGNIZABLE PRECURSORS WITHIN THE MICROENVIRONMENT OF CULTURED FETAL MOUSE GUT. Taube P. Rothman, Cheryl F. Dreyfus and Michael D. Gershon. Dept. of Anatomy, College of Physicians & Surgeons, Columbia University, New York, New York, 10032.

In the developing mouse gut neurons cannot be detected morphologically by light or electron microscopy prior to day 12 of gestation. Before this time the gut consists of an apparent mesenchymal tube surrounding a layer of stratified mucosal epithelium and encompassed by a single layer of serosal epithelial cells. The gut shows no neuronal properties. It fails to synthesize ^{3}H-acetylcholine (^{3}H-ACh) from ^{3}H-choline; it fails to specifically take up ^{3}H-5-hydroxytryptamine (^{3}H-5-HT), and neither 5-HT nor norepinephrine (NE) can be shown by formaldehyde-induced histofluorescence. Neurons first appear morphologically in the small intestine at 12 days' gestation, at 13 days in the stomach and proximal colon, and at 14 days in the distal colon. Coincidentally, with the appearance of neurons, synthesis of ^{3}H-ACh from ^{3}H-choline appears and uptake of ^{3}H-5-HT becomes radioautographically demonstrable in the primordial myenteric plexus. When any region of the gut is explanted in organotypic tissue culture prior to neuron formation, neurons subsequently develop in the cultures. This can be shown in gut explanted as early as 10 days' gestation but also occurs at later ages. In the cultures the epithelium degenerates but two layers of smooth muscle form, properly oriented into an inner circular and an outer longitudinal layer. A myenteric plexus forms between the two layers of smooth muscle and exhibits typical neuronal perikarya and a neuropil. Within the neuropil of the myenteric plexus there are axons that specifically take up ^{3}H-5-HT and can be shown by radioautography. (Uptake is blocked by fluoxetine.) There are also abundant cell bodies that show the typical yellow 5-HT fluorescence when the cultures are treated with glyoxylic acid or formaldehyde gas. Therefore, neuronal precursor cells invade and colonize the gut substantially before neurons can be recognized chemically or morphologically. This indicates that differentiation of enteric neurons occurs within, and is probably dependent upon, the enteric microenvironment. (Supported by a Basil O'Connor Starter Research Grant, National Foundation - March of Dimes and NIH Grant #NS12969).

576 ORDER IN THE OPTIC NERVE OF GOLDFISH. Anne C. Rusoff* and Stephen S. Easter, Jr. (SPON: M. W. Dubin). University of Michigan, Ann Arbor, Michigan 48109

We have demonstrated that retinal ganglion cell axons are distributed non-randomly in the optic nerve of goldfish, and that one of the rules for their ordering is: axons from ganglion cells of the same age, that is, located the same distance from the optic disc, are together. The non-random distribution of axons in the optic nerve was demonstrated with small injections of HRP into the retina. Injections were made peripherally so that axons from a localized region, rather than axons of passage, were filled. Fish survived 2-8 days, depending on their size; retinas were prepared as whole mounts, and optic nerves and tecta were sectioned longitudinally; the tissue was reacted with H_2O_2 and o-dianisidine. Stained ganglion cell axons were traced from the injection site across the retina, into the optic disc and throughout the length of the nerve and tract. Stained axons remained in a bundle throughout this length. In one example, the same axons were followed for 0.8mm, and the distance between the most peripheral axons was 63μm ± 11μm (mean ± S.D.). Thus, axons from one location on the retina are together in the optic nerve.

The converse experiment--making a small injection of HRP into the optic nerve--was also done. HRP was injected into the optic nerve just behind the eye. Fish survived 20 hr; then the tissue was prepared as described above. When HRP injections were localized to a single bundle in the nerve, an annulus of ganglion cell bodies was stained in the retina. The annulus was centered on the optic disc. Stained axons radiated from the disc, but did not extend peripherally beyond the annulus, and scattered stained ganglion cells were found neither more peripheral nor more central than the annulus. Injections in separate fish, presumably filling different bundles of axons in the nerve, produced annuli at different distances from the disc. In goldfish the retina grows by addition of cells at the peripheral margin so all the ganglion cells in an annulus are the same age. Therefore, bundles within the optic nerve are composed of axons from ganglion cells of the same age. (Supported by EY05294 to A.C.R. and EY00168 to S.S.E.)

577 STEREOTAXIC PLACEMENT IN THE BRAIN OF NEONATAL SWINE (SUS SCROFA). M.-E. Salinas-Zeballos* and P.M. Gootman, Dept. of Physiology, Downstate Medical Center, SUNY, Brooklyn, N.Y. 11203.

The use of neonatal swine in a variety of maturational studies, especially in cardiovascular regulation (Gootman et al., Chapt. 4, In "Fetal and Newborn Cardiovascular Physiology", Vol. 1, edited by L.D. Longo and D.R. Reneau, Garland STPM Press, pp. 93-152, 1978), has made necessary the development of an atlas correlating the growth of the skull with the underlying structures of the maturing brain. 33 piglets aged 3 hr-12 days (0.74-3.65 kg) were used for this study.

The major modification of the Kopf piglet stereotaxic apparatus (Gootman et al., Am. J. Physiol., 222: 994, 1972) was the use of straight ear bars directly into the ear canal. These ear bars permitted the head to be held in a consistently reproducible position.

The position of the brain was standardized by keeping Lambda and Bregma at the same vertical level from stereotaxic zero by lowering or raising the palate-nose piece, in this way the surface of the brain remained parallel to the horizontal zero of the stereotaxic instrument.

Growth of the skull and brain was measured as distances between Lambda, Bregma and Nasal sutures to the stereotaxic zero. Lambda corresponded to the caudal tip of the occipital lobe, Bregma to the level of the medial thalamus and the Nasal suture to the anterior tip of the frontal lobe.

Four major groups were found to have significant differences in brain size:

Ages (days)	Weight (kg)	Lambda (mm)	Bregma (mm)	Nasal (mm)
≤ 1	1.4 ±0.09	-10.95±0.62	+8.35±0.52	+43.28±0.72
2-5	1.19±0.07	-10.77±0.37	+11.47±1.39	+44.37±1.29
6-9	1.8 ±0.18	-10.11±0.93	+13.53±0.91	+49.51±1.61
10-12	2.95±0.31	-14.47±1.64	+12.68±1.35	+49.72±2.34

The map containing these values will facilitate stereotaxic localization of brain structures in piglets of different ages. (Supported by NIH Research Grant HL-20864).

578 NICOTINE AFFECTS THE FETAL NIGROSTRIATAL DOPAMINE (DA) SYSTEM OF THE RAT. M. Schlumpf, R. Maire * and W. Lichtensteiger, Inst. Pharmacol., Univ. Zürich, Zürich, Switzerland.

Various types of interactions between cholinergic neurons and the nigrostriatal DA system exist in the adult rat. The prenatal development of cholinergic influences on these DA neurons was first investigated with nicotine. This drug increases the firing rate of nigral DA neurons in adult rats in doses of 0.25-1 mg per kg. DA nerve cells become detectable by fluorescence histochemistry in the mesencephalic flexure of rat fetuses at embryonic day (ED) 13 (ED 1 = 24 h after mating). Their projections reach the neostriatum at ED 15-16.

Nicotine (0.4 mg/kg s.c.) was administered to the mother rat at ED 18 3/4 or 19 3/4. At this developmental stage the catecholamine fiber innervation of the neostriatum is fairly dense but shows the immature patchy pattern. The fetal brains were dissected 10-40 min after drug injection. DA was determined in midbrain and caudate-putamen by radioenzymatic assay. DA concentrations were markedly elevated in both regions after nicotine. Preliminary experiments revealed an influence of the drug also after direct injection into the fetus. These observations demonstrate that fetal nigrostriatal DA neurons are responsive to nicotine. However, the biochemical characteristics of the reaction differ from the adult state where DA concentrations remained unchanged in the somatodendritic and terminal areas. First experiments on neonatal rats point to differences between pre- and postnatal animals in the nigral response to nicotine. Biochemical and histochemical observations on fetuses in the last third of gestation indicate that the nigrostriatal DA system is also influenced by chronic nicotine treatment during pregnancy. The biochemical aspects of these drug effects are further investigated.

Our results show that nicotine affects a prenatal central catecholamine system, the nigrostriatal DA neurons. A direct assessment of drug effects in the fetal period may contribute to a better understanding of the relationship between prenatal influences and their possible consequences for postnatal life.

579 DEVELOPMENT OF THE ANTENNAL SYSTEM OF *MANDUCA SEXTA* FOLLOWING TRANSPLANTATION OF IMAGINAL ANTENNAL DISCS. Anne Schneiderman. Dept. of Neurobiol., Harvard Med. Sch., Boston, MA 02115.

Each antenna of the moth *Manduca sexta* develops during the 18 days of metamorphic adult development from an imaginal disc that has grown but not differentiated within the cranium of the larva. At pupation the disc everts, and with the initiation of adult development, antennal sensory neurons arise mitotically and send axons into the head along pre-existing "pupal antennal nerves" that run from cells near the tip of the extended, everted disc to the brain (Roux' Archiv *178* 71, 1975; Devel. Biol. *51* 300, 1976). As the antennae and antennal lobes of the brain develop, sexual differences become apparent in their structure (Camazine and Hildebrand, this volume; Matsumoto, this volume). Male antennae possess sensilla and associated olfactory neurons that are specialized to detect the female sex pheromone and are not present in female antennae. Male antennal lobes contain a macroglomerular complex that is missing from females and receives arborizations from interneurons that respond to pheromonal stimulation of the antenna. The present experiments begin efforts to explore the role of the pupal antennal nerves in the centripetal growth of adult antennal axons and the role of appropriate neural interactions in the ontogeny of the sexually dimorphic components of the antennal lobe.

We have surgically interchanged antennal imaginal discs between late-instar larvae of the same age and opposite sexes. In many cases the transplanted disc develops into a functional, partial or complete adult antenna with morphology characteristic of the donor's sex. Sensory neurons in the transplanted antenna differentiate and grow into appropriate regions of the brain, although pre-existing neural connections between the disc and the brain have been unambiguously severed. The macroglomerular complex in the male antennal lobe develops irrespective of the "sex" of the antenna associated with it. Histological, electrophysiological, neurochemical, and intracellular staining studies of these surgically chimeric antennal systems are in progress and will be described.

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80 DEVELOPMENT OF ADRENAL STRESS RESPONSIVENESS IN THE RAT. Nancy M. Schoenfeld*, Jamshid Rabii and James H. Leathem. Dept. of Physiology, Rutgers University, Piscataway, NJ 08854.

The concept of a refractory period of responsiveness to ether stress in the neonatal rat has been questioned. Since the original determination of this stress non-responsive period had been based on the relatively insensitive fluorometric assay of corticosterone (B), we decided to re-evaluate this phenomenon using the more sensitive technique of competitive protein binding assay. Serum and adrenal B were measured 15 min after exposure to a 1-min ether (E) stress, as well as to subcutaneous injections of ACTH (A, 20 I.U./100 gm b.w.) and saline (S) in rats 5, 7, 9, 11, 13, 15, 20, and 25 days of age. Furthermore, the time course of the response to E was determined in 9- and 15-day old rats, at 0, 15, 30, and 60 min following the exposure to E. Significant elevations in serum and adrenal concentrations of B were measured at all ages following the treatments; the serum B values (µg/100 ml) are shown in the table:

AGE:	5	7	9	11	13	15	20	25
B	.33 ±.05	.11 ±.02	.42 ±.03	.24 ±.04	.60 ±.07	1.50 ±.27	2.23 ±.33	3.11 ±1.11
E	1.91 ±.56	1.10 ±.20	1.72 ±.13	2.66 ±.35	3.93 ±.21	11.58 ±.67	16.48 ±.92	40.04 ±3.76
A	3.92 ±.20	1.98 ±.16	1.95 ±.14	3.11 ±.23	3.75 ±.22	9.79 ±.31	16.35 ±1.17	39.56 ±4.56
S	2.81 ±.24	.29 ±.05	1.04 ±.12	1.08 ±.17	2.69 ±.26	4.72 ±.69	7.88 ±.59	21.90 ±4.16

The serum and adrenal response to E was maximal by 15 min in the 9-day old animal. In the 15-day old rat serum response continued to rise thru 60 min, whereas the adrenal response became maximal at 15 min. We concluded that the neonatal rat is capable of a significant response to ether as well as to ACTH and saline at all ages studied. Furthermore, the 9-day old rat does not show a delayed response to stress as evidenced by the significant rise in corticosterone levels observed 15 min after ether.

581 MATURATION OF WALLERIAN DEGENERATION: AN EM STUDY IN THE DEVELOPING OLFACTORY TUBERCLE. Thomas A. Schoenfeld, Christine K. Street* and Christiana M. Leonard. Dept. Neurosci., Univ. Fla. Coll. Med., Gainesville, FL 32610

Previous work has demonstrated that Wallerian degeneration argyrophilia (DA) shows qualitative and quantitative changes with age. In immature systems, DA develops between 8 and 16 hours of axon transection and, in many systems, has disappeared by 72 hours. With age, DA becomes long-lasting, increasing in density and extent during the first few days after a lesion. We have investigated the ultrastructural correlates of short-latency and long-lasting degeneration, using the olfactory tubercle of the golden hamster as a model system. Unilateral olfactory bulbectomies were performed in 5 and 19 day old pups, which were then sacrificed at 24 and 72 hour survivals and the brains removed and processed for routine electron microscopy.

Dark (electron-dense) profiles apposed to postsynaptic densities, typical of degeneration in the adult, were abundant in the superficial plexiform layer both 24 and 72 hours following bulbectomy at Day 19. The width of the plexiform layer was the same on both control and lesioned sides. At 24 hours after bulbectomy at Day 5, by contrast, the plexiform layer had shrunk by 50% and contained only a few dark or cystic profiles. By 72 hours, the layer had regained normal size and had a normal synaptic density (about 5/100 μ^2), suggesting that non-olfactory bulb afferents had come to occupy the vacated terminal space. The paucity of degeneration found after Day 5 lesions corresponds to the lack of degeneration argyrophilia seen at the light microscope level. It is possible that the rapid clearing of degenerative debris is associated with glial hypertrophy or proliferation. Numerous large dark, lysosome-containing processes occupied a substantial portion of the neuropil at 24 but not 72 hours after a Day 5 lesion.

Curiously, the density of synapses after a lesion at Day 19 was the same as after a lesion at Day 5, even though half of the synapses in the older pups were represented by degenerating synaptic profiles. The innervation of deafferented regions by the process of collateral sprouting may be limited by the presence of degenerating terminals, which, though non-viable, contribute to the total volume of terminal space. Such conservation of synaptic density in the olfactory tubercle, in conjunction with the occurence of short- or long-lasting degeneration, may account for known differences in neuropil reorganization after bulb lesions at different ages.

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582 THE HALTERE-LIKE PROJECTION OF SEGMENTALLY TRANSLOCATED WING RECEPTORS IN DROSOPHILA HOMEOTIC MUTANTS. Margrit Schubiger* and John Palka, Dept. Zoology, Univ. Washington, Seattle, WA. 98195.

When sets of sensory cells are experimentally moved from one location to another, their axons often assume a distribution within the c.n.s. that characterizes their place of origin, as when the ganglion cells of an inverted amphibian eye project to the tectum according to their original orientation relative to the body. Certain receptors on the wing of Drosophila behave in this way when their axons are caused to enter the c.n.s. in the metathoracic segment by the mutation bithorax which transforms the halteres into a second set of wings. However, each investigated class of wing receptors responds differently to such a segmental translocation [Palka, J., Lawrence, P.A., and Hart, H.S., Develop. Biol. 69, 549-575 (1979)]. We present here an analysis of the small campaniform sensilla whose axons, instead of going "home" to the mesothorax, follow the paths of the haltere receptors they have replaced.

The wings and halteres of wild type flies both carry small campaniform sensilla. Their morphologies are similar but not identical and their central pathways are likewise very close to each other in many places but are not overlapping; in certain locations they are entirely distinct. The small campaniform sensilla of halteres homeotically transformed into wings follow the path of wild type haltere fibers in every respect we have examined, even though they come from wing tissue and their external morphology is clearly of wing type.

A more wing-like projection might be obtained from these fibers if the normal mesothoracic wings were removed prior to the time when sensory fibers reach the c.n.s., thus leaving a substrate vacant and ready for occupancy by the homeotic wing fibers. We have produced wingless flies both genetically by using a wingless; bithorax stock, and surgically by extirpating one wing disc in bithorax prepupae. In silver-intensified cobalt fills we have not been able to detect any differences in the course of the homeotic fibers in flies with and without normal wings. Thus, an occupied wing substrate does not appear to be responsible for the haltere-like course taken by the axons of the small campaniform sensilla of the homeotic wing.

583 DEVELOPMENTAL SPECIFICATION OF COLUMNAR AND RETINOTOPIC STRUCTURE OF RETINO-GENICULO-STRIATE SYSTEM VIA A MOVING BOUNDARY VALUE DIFFUSION DECAY SYSTEM. H.Schwartz* & E.L.Schwartz (SPON: E. R. John) Brain Research, Dept. of Psychiatry N.Y.U.

In previous work, it has been shown that the pattern of afferent input to striate cortex may be described in terms of a concatenated complex logarithmic mapping (Schwartz,1977a), and that receptotopic structure in general may be characterized in terms of solutions to a variational problem in which the magnitude of the magnification factor is minimized,subject to prescribed boundary conditions imposed by retinal cell density and cortical anatomy (Schwartz,1977b). In the present work, it is shown that the moving boundary value diffusion-decay system that has recently been proposed as a phyllotactic mechanism by Lindenmeyer and Veen (1977) may be adapted by a conformal transformation to specify the columnar and retinotopic structure of the retino-geniculo-striate system. The solution to the modified Lindenmeyer-Veen equation possesses the correct spatial structure to describe the receptive field density of LGN direction columns on a local scale, as well as retino-cortical inhomogeneity on a global scale. Remapping this spatial pattern to the cortex via the variational principle proposed in Schwartz(1977b) results in an accurate model of local and global retino-geniculo-striate functional architecture. Ocular dominance and orientation column structure are provided with the correct relative column scale factors, and it is shown that the Ising model method allows an analytic study of the (phase) transition from overlapped to columnar ocular dominance pattern. Finally, a general variational principle for fiber sorting is proposed, which includes most other models as special cases, and which is implimented by a convenient computer algorithm which may be used to solve the general receptotopic encoding problem. This work indicates that the details of retino-geniculo-striate map development may be simulated quantitatively and analytically, and that the variational principle-field theoretic approach to developmental specification(Schwartz,1977b) may provide a comprehensive description of receptotopic formation.

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584 PSYCHOPHARMACOLOGICAL EFFECTS OF APOMORPHINE DURING ONTOGENY. Ismail A. Shalaby* and Linda Patia Spear. Dept. Psychology, SUNY-Binghamton, Binghamton, N.Y. 13901.

Behavioral time-sampling methods were used to assess the ontogenetic patterns of psychopharmacological responsiveness to the dopaminergic agonist, apomorphine. Male and female Sprague-Dawley rats were given 0, 0.05, 0.1, 1.0 or 3.0 mg/kg apomorphine hydrochloride and tested on either postnatal day (P)7, 14, 21, 28 or 35. During the first two weeks of life, all doses of apomorphine markedly increased wall climbing behavior (forepaw treading against walls) and the number of matrix crossings. At these ages, only slight amounts of sniffing behavior were seen even after the highest doses of apomorphine. By P28, the adult pattern of apomorphine responsiveness was seen. At this age, 0.05 and 0.1 mg/kg apomorphine decreased the number of matrix crossings, presumably a result of preferential activation of autoreceptors at these low doses of apomorphine (e.g., Martres et al., Br. Res. 136 (1977, 319). Also at P28, administration of either 1 or 3 mg/kg apomorphine increased matrix crossing behavior and induced marked stereotyped sniffing, gnawing, or licking behavior.

Apomorphine-induced wall climbing behavior was seen only during the first two postnatal weeks. We have also observed that the α-noradrenergic agonist clonidine similarly induced wall climbing behavior only during this postnatal interval, although the noradrenergic reuptake inhibitor cocaine did not induce wall climbing at any age (Spear & Brick, Behav. Neural Biol., in press). Moreover, wall climbing can also be induced by footshock (Fish, personal communication) or wall shock (Stehouwer & Campbell, Develop. Psychobiol., 1979, in press) at early postnatal ages. Possible explanations of these similarities in insult-induced behavior at early postnatal ages, and their subsequent decline during ontogeny, will be discussed.

585 DEVELOPMENT OF PIONEER AND SENSORY AFFERENT PROJECTIONS IN THE GRASSHOPPER EMBRYO. Marty Shankland* (SPON: D. Bentley) Neurobiology Group, Univ. Calif., Berkeley, Ca. 94720

The embryonic formation of a sensory projection will be described in the grasshopper, Schistocerca nitens. Cobalt diffusion staining (silver intensified) of the cercal nerve depicts sequential ingrowth and central branching of both pioneer and sensory axons.

Pioneer neurons (Bate, '76, Nature 260: 54-56; Edwards and Chen, Roux's Arch. Dev. Biol., in press) in the cercal lumen establish the nerve prior to sensory axon differentiation. I have stained the central processes of pioneer axons as early as 50% of embryogenesis. At this stage many central neurons have not yet differentiated, and the neuropil is restricted to pairs of segmental commissures linked by two bilateral longitudinal fiber tracts (LFT). At least five pioneer axons are seen in the cercal nerve. They enter the LFT at its junction with the most posterior commissure and form numerous fine processes. Two or three thicker axons that end in growth cones travel several segments anterior as part of the LFT. These axons do not branch, but bear short, fine processes which later disappear. The four posterior ganglion rudiments fuse at 60% of embryogenesis. Sensory axons enter this fused ganglion at 65% and obscure the projection, but the pioneer neurons still persist. Unbranched, multisegmental longitudinal axons are present even in the adult. Furthermore, a cobalt fill into the hatchling cercus stains not only epidermal sensory cells but also a pair of neurons within the lumen (pioneers) whose dendrites do not contact sensilla. Hence, at least some pioneer neurons survive after embryogenesis.

The pioneers have been described as pathfinders, leading sensory axons into the ganglion. Cobalt stains show that some sensory axons continue to follow the pioneer tract within the ganglion, but the majority diverge upon entry. At 65% they form two ipsilateral patches of dense arborization which demarcate the eventual cercal glomerulus. The Medial Giant Interneuron is first visible in silver stained sections at this same age. The giant is a major synaptic target for the sensory afferents, and the glomerulus develops as a paraboloid sheet of afferent arborization around its main dendrite. By 80% the embryonic projection resembles the adult projection in all main features. The neuropil continues to expand, but no new tracts are added by the postembryonic ingrowth of new axons.

The regional characteristics of this sensory projection are thus established during embryonic formation of the neuropil. Pioneer and sensory axons grow along specific paths within the ganglia and contribute to the structural differentiation of the neuropil.

586 THE DEVELOPMENT OF ORDERING IN THE OPTIC AXONS OF Rana pipiens. Sansar C. Sharma. Dept. Ophthalmology, New York Medical College, Valhalla, N.Y. 10595.

The time of origin of ganglion cell fibers in the developing Rana pipiens retina has been studied using electronmicroscopy, silver stained sections and whole mount preparations. The first ganglion cell axons appear at Shumway stage 17-18. The developing fibers group together and form a fascicle. The individual fibers within the fascicle are directed towards the optic disc and travel into the optic stalk, which is still intact at stage 18. Subsequent to the formation of the initial optic fascicle, more fascicles appear in later stages, and they are also oriented towards the optic papilla. In later stages a fan-like network of fascicles appears which joins at the optic papilla. The initial nerve outgrowth is not dependent upon the choroidal fissure, which develops later. Instead, first-formed axons and their fascicle formation act as a guiding source for the new fascicles. These fascicles maintain parallel alignment in the optic nerve and are apparently responsible for the retinotopic order in the optic nerve and subsequent termination in the optic tectum.

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587 Birthdates Of Ocular Motoneurons In Rabbits. Marjorie D. Shaw*, and Keith E. Alley. Dept. Anat., Case Western Reserve University, Cleveland, Ohio 44106.

Temporal gradients constitute a critical variable in many developmental processes. A knowledge of neuron birthdates (time of final mitosis) may reveal new principles in the developmental construction of brain pathways and nuclei.

Injections of 12 uCi of tritiated thymidine into the large rabbit blastocyst demarcate all newly emerging neurons with heavy radioactivity. On the ninth day of gestation, such an injection reveals the earliest beginnings of ocular motoneuron formation. By day 13, production of ocular motoneurons ceases.

The generation of the oculomotor, trochlear and abducens nuclei proceed on similar timetables. Heavily radioactive cells form approximately 1% of the total neurons of each nucleus in specimens injected on day 9. The percentage rises to about 5 on day 10, peaks at 20 on day 11, then falls to 5 again on day 12. The neurons of the accessory abducens nucleus (recently recognized as the motoneurons for the retractor bulbi muscle of the eye) emerge at a faster rate. On day 10, 30% of the cells show heavy labelling, but by day 12 all label disappears.

In the oculomotor nucleus, the earliest neurons tend to settle most rostrally, while later forming cells migrate preferentially to the caudal end. A rostro-caudal gradient emerged clearly within the oculomotor nucleus, but appears less marked between the nuclei.

588 GUIDANCE AND TOPOGRAPHIC PATTERNING OF RETINAL GANGLION CELL AXONS. Jerry Silver* (SPON: G.A. Kevetter). Dept. Anat., Sch. Med., Case Western Reserve Univ., Cleveland, Ohio 44106

In order to examine the factors that may guide the earliest developing optic axons in mice, the stretch of primitive neuroepithelium located along the potential route of the pioneer optic nerve fibers has been examined in three dimensions with the use of computer graphic reconstruction of serial, 1μm sections. The study has revealed a system of wide-bored intercellular channels (averaging about 25 μm^2 in cross sectional area) within the marginal zone of the primitive retina and continuing without interruption into the underside of the optic stalk (adjacent to the optic fissure) and toward the presumptive optic chiasm. The spaces appear well in advance of the morphological differentiation of the retinal ganglion cell neurons.

The openings are arranged with a definite directionality. Thus, the spaces at the region of the prospective optic disc appear to coalesce and form segregated sets of long, interconnecting tunnels oriented in the direction of the stalk. More peripherally, toward the back and rim of the cup, the spaces form blind, radially arranged pockets. Previous studies have established that the first developing retinal ganglion cells with axons are generated immediately dorsal to the optic stalk (Mann,'69; Kahn, '73), that is, at the junction between the open tunnels and closed pockets. Given the constraints of their extracellular surroundings it is conceivable that the earliest optic axons may be confronted with alternative environments depending on their direction of movement and may literally be compelled to exit the eye in the proper direction. Indeed, the path taken by the first outgrowing optic fibers is identical to the one previously established by the intercellular tunnels and always in the direction of the stalk.

Over their entire course, the tunnels in the region of the disc strictly maintain their positions in relation to the optic fissure and, thus, particular segments of the retina become connected by continuous openings with equivalent regions in the stalk. As the later forming optic axons (those near the back and periphery of the cup) add on sequentially, perhaps by fasciculation, they are never given the option of growing towards the pupil since the pockets interposed between them and the rim of the cup always remain closed. Providing that the first optic axons approach the disc in register, the layout of these specialized extracellular spaces may not only provide directional information to the first forming optic axons but topographic cues as well.

589 THE BLUEPRINT HYPOTHESIS OF CENTRAL NEURITE PATTERNING DURING REGENERATION AND EMBRYOGENESIS. Marcus Singer, Ruth Nordlander* and Margaret Egar*. Dept. Anat., Sch. Med., and Dev. Biology Ctr., CWRU, Cleveland, OH 44106.

The tail of the lizard and of the newt including the spinal cord regenerates after amputation. The spinal cord is formed at first by outgrowth of the ependyma from the amputated end of the cord, forming a tube which ends blindly. Fibers descending from the cord stump follow the primitive ependyma; and in the case of the newt the ependyma also gives rise to new central neurons and glia. The ependymal cells, oriented around the central canal, have radiating processes which contact the basal lamina beneath the developing pia mater. Between these processes there are "spaces" which are in register to form longitudinal channels and descending axons are located in these channels. A salient fact is that the channels form in advance of the first neurites; and it is, therefore, these channels or their internal surfaces which guide the neurites toward the as yet undeveloped target. A similar mechanism of guiding neurites was observed during embryonic and larval development in the newt and in other animals. These observations led us to propose the so-called blueprint hypothesis of neurite patterning which states that the primitive ependyma and its derivative primitive glia provide the itinerary directing the pioneer neurites to their destination. The hypothesis includes the concepts of trace pathways on the epithelial surfaces, the ability of neurites to follow specific traces and the possibility that growing axons may act as evocators of the blueprint.

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590 MORPHOLOGY OF RETINOTECTAL CONNECTIONS IN FETAL MOUSE EXPLANT CO-CULTURES. Neil R. Smalheiser, Dept. of Neuroscience, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

Electrophysiologic studies of retinotectal co-cultures (Smalheiser and Crain, Br. Res. 148:484,'78) have now been extended by horseradish peroxidase (HRP) tracing of retinal ganglion cell axons within tectal (superior collicular) explants. Extracellular iontophoretic HRP injections (Smalheiser, Crain and Peterson, Soc. Neurosci. Abstr. 4:479,'78) resulted in a solid Golgi-like filling of cell bodies and neurites. Using 13-15 day fetal mice, retinal halves were placed adjacent to, or ~ 0.5 mm away from, whole tecta. In the latter case a collagen gel overlay (Elsdale and Bard, J. Cell Biol. 54:626,'72) enhanced organized "optic nerve" outgrowth. HRP injections into the retinal or tectal explants filled retinal ganglion cells whose axons entered the optic fiber layer and converged to the site of the optic nerve head, as occurs in situ. In co-cultures, some of these axons often became myelinated after 2-3 weeks in vitro. The axons formed long fascicles that emerged from the retina during the first few days in vitro. The majority of ganglion cell axons that did not reach the tectal explant degenerated after the first week. If they entered the tectum, many of them survived for at least five weeks. Within the tectum, the retinal fibers tended to be smooth, with few swellings; pursuing a wavy course, usually with few bifurcations. Thin stems came off at frequent intervals which varied from simple short thorns to long, thin preterminal ramifications bearing elaborate nests of boutons. The existence of these terminal arborizations had, in fact, been predicted from our earlier analyses of retinally-evoked action potentials within the tectum. Ganglion cell axons surviving upon collagen or nonneural cell layers showed no arborizations -- at most, bouton-like swellings along the fibers. Oriented co-cultures are being analyzed after HRP injections to evaluate whether retinal fiber growth or arborizations from retinal halves are preferentially located in correct tectal regions as occurs in situ.

Neurites also grew out from retinal interneurons, emerging radially from the retinal explant after the first week in vitro. These had 'en passant' varicosities and bouton-like structures, and were tipped with growth cones (even after several weeks in vitro). Their appearance was similar whether growing within the tectum or upon inert collagen substrates. Some of these neurites showed catecholamine fluorescence.

These morphological observations further demonstrate the potential of retinotectal co-cultures for exploring mechanisms of synaptic specificity.

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591 LATE DEVELOPMENT OF DENDRITIC STRUCTURE IN N. LAMINARIS D.J. Smith and E. W Rubel. Neuroscience Program and Dept.s of Otolaryngology and Physiology, Univ. Virginia Med. Ctr., Charlottesville, VA. 22908.

N. laminaris (NL) is a third-order auditory nucleus in the avian brain stem. It is composed of a monolayer of cell bodies, and two neuropil regions, one dorsal to the cell bodies, and one ventral. The dorsal neuropil receives tonotopic input from the ipsilateral cochlea via the ipsilateral n. magnocellularis (NM), and the ventral neuropil receives matching input from the contralateral NM. The dendritic structure of NL cells is such that they have a dorsal and a ventral group of dendrites, with few dendrites issued laterally. The dendritic organization of NL in 5 day-old chicks has been previously described (Smith and Rubel, J. Comp. Neur. in press). At this age, NL has been functional for two weeks. At 5 days the total dendritic length (the sum of the dorsal and ventral dendritic lengths) of NL cells increased 3.5-fold from the rostromedial to the caudolateral poles of the nucleus. The gradient of this increase is identical to the gradient of tonotopic organization in NL, and accounted for 50% of the variance in total dendritic length. Separate analyses of the dorsal and the ventral dendritic lengths revealed divergent gradients for the dorsal and ventral dendrites; each was symmetrically skewed to either side of the frequency gradient. The cell-by-cell correlation of dorsal with ventral dendritic lengths was 0.47.

In this study the late development of dendritic structure in NL was examined in 25 day-old chickens. Camera lucida drawings of Golgi-Kopsch impregnated cells were analysed for dendritic length and number of primary dendrites. The gradient of total dendritic length across NL remains in correspondence with the tonotopic gradient, and accounts for 72% of the variance: there is a 10-fold increase in total length from rostromedial to caudolateral NL. The dorsal and ventral length gradients become identical by 25 days, and both gradients correspond to the tonotopic organization. The cell-by-cell correlation of dorsal with ventral dendritic lengths is increased to 0.84.

Thus, during the postnatal period the degree of change in dendritic length from rostromedial (high Hz) to caudolateral (low Hz) NL becomes greater. Also, the lengths of the dorsal and ventral dendritic trees become more closely correlated: the orientations of the dorsal and ventral length gradients realign to coincide with the gradient of tonotopic organization. These data indicate that the organization of dendritic size is progressively tuned to a greater correspondence with the tonotopic organization, emphasizing the importance of afferent organization in determining the organization of dendritic structure. (Support: NSF #BNS 78-04070; Deafness Research Found.; NIH #MH-05949)

592 KINETICS OF ^{22}Na AND ^{36}Cl DISTRIBUTION IN THE POSTNATAL RAT CHOROID PLEXUS. Quentin R. Smith, Conrad E. Johanson, and Dixon M. Woodbury. Dept. Pharmacology, Univ. of Utah College of Medicine, Salt Lake City, Utah 84132.

Maturation of the blood-CSF barrier involves changes in the transport properties of the choroid plexus (CP). Notably, CP vascularity and fluid production increase while permeability decreases. To evaluate the effect of these alterations on two ions (Na and Cl) closely linked to fluid elaboration, the kinetics of ^{22}Na and ^{36}Cl distribution in the postnatal choroid plexus were analyzed. The data for ^{36}Cl are reported herein.

Four-hr nephrectomized rats (1 wk, 2 wk, and adult) were sacrificed 1/12, 1/6, 1/4, 1/2, 1, 2, 4, and 8 hr after i.p. injection of 100 uc/kg ^{22}Na or ^{36}Cl. Samples from the central nervous system (CP, CSF, cerebral cortex, and cerebellum) and the periphery (skeletal muscle, plasma, submaxillary salivary gland, and thyroid) were assayed for radioactivity. Values were reported as a space (%) = 100 x (dpm/g tissue)/(dpm/g extracellular fluid H_2O). The uptake of ^{36}Cl by the 1 wk rat CP was markedly slower than that of the adult, though similar steady-state values were obtained (~54%). For example, the 5, 10, and 15 min post-injection spaces (which reflect the filling of the CP extracellular space) were ~35% those of adult values. In contrast to the CP, ^{36}Cl spaces of the 1 wk rat cerebral cortex were equal to or greater than those of the adult at all time periods. Because of the greater extracellular volume, the cerebral cortex steady-state space of the neonate was larger than that of the adult. Comparable results were obtained with the cerebellum.

The slower rate of uptake at early time periods by the 1 wk rat CP compared to the adult may reflect the less developed vascularity of the neonatal CP. Similarly, the steady-state Cl content of the 1 wk CP may indicate that mechanisms for accumulating Cl are present in the CP at that age. In the cerebral cortex and cerebellum, differences in ^{36}Cl uptake with age may be explained by a larger extracellular space and a greater membrane permeability in the neonate. (Supported by NIH Grant GM07579, NS13988, and AM20935.)

593 DEVELOPMENT OF HYPOTHALAMIC LHRH AND SERUM LH, FSH AND PROLACTIN IN INTACT AND NEONATALLY GONADECTOMIZED MALE AND FEMALE RATS AND NEONATALLY ANDROGENIZED FEMALE RATS. W.E. Sonntag*, A.A. Gerall, J.C. King and A. Arimura*. Department of Psychology, Tulane University, and Veterans Admin. Hospital, New Orleans, LA 70118.

Hypothalamic LHRH, serum LH, FSH and prolactin were measured by RIA on days 10, 20, 29, 36 and 52 in intact and castrated male and female rats and in intact females injected with 1.25 mg testosterone propionate on dav 3. LHRH was assayed in rostral (MPOA-OVLT) and caudal (arcuate-median eminence) areas. In intact animals, LHRH content was higher on day 20 and 52 than other davs. No sex differences in LHRH content were observed in either hypothalamic area until dav 52 when estrous females had lower LHRH than either males or diestrous females (P<.01). On day 10, LHRH content was not different among any groups. About day 20, gonadectomized females had a lower hypothalamic LHRH content in caudal areas compared to intact controls (P<.05). A similar difference was not observed in gonadectomized males until day 25. In androgenized females, LHRH content was similar to that in intact animals until days 36 and 52 when it exceeded that in intact animals (P<.01). Although LHRH was substantially lower in rostral than in caudal areas, their developmental pattern was similar. Serum LH was uniformly low in intact and androgenized animals throughout development. Gonadectomized animals had similar serum LH as intact animals on day 10, but these LH values increased around dav 20, and stabilized around day 29 to a level above that in intact animals. Serum FSH was elevated in intact females compared to intact males between days 10 and 20 (P<.01, each), but serum FSH in intact males was significantly higher than in intact females on days 29, 36 and 52 (P<.01. each). Androgenized females had FSH values intermediate to intact males and females at all ages. Although gonadectomized animals had higher serum FSH concentrations from days 10 to 52 than intact animals (P<.01), high est values were found around day 20. Serum prolactin concentrations increase from day 20 to dav 29 and then decrease in all animals except androgenized and estrous females.

Despite differences in serum FSH concentration between males and females and between intact and gonadectomized animals as early as day 10, differential hypothalamic LHRH content between intact and gonadectomized animals was not evident until after day 10 in female rats and after day 25 in male rats. Androgenized females do not demonstrate an increase in LHRH content until the peripuberal period. This increased hypothalamic LHRH might be related to the tonic secretion of estrogen or possibly, to the high concentrations of prolactin occurring after puberty in the androgenized animals.

594 DEVELOPMENTAL CHANGE IN AUDIOGENIC SEIZURE SUSCEPTIBILITY IN DBA/2J MICE, POSSIBLY MEDIATED BY SEROTONIN RECEPTOR MODIFICATION. David L. Sparks* and N.S. Buckholtz, Depts. of Biochemistry and Psychiatry, Medical Univ. of S.C., Charleston, S.C. 29403

DBA/2J mice are maximally susceptible to AGS at 21 days of age and demonstrate almost no AGS Susceptibility (AGS-S) at 28 days. AGS-S in the DBA/2J mouse is possibly a result of reduced neurotransmitter levels leading to reduced receptor interaction. 5-HT system may mediate the seizure attenuating action of the drugs chlorimipramine (CIMI), pargyline, and certain ß-carbolines by increasing 5-HT interacting at its receptor or by the drugs themselves acting directly at the 5-HT receptor.[1] It would thus seem that the 5-HT receptor agonists Quipazine or 5-Methoxy-DMT would block AGS, but they do not.[1] This report presents evidence that the effect of these drugs on AGS changes developmentally and this may be due to a modification in the 5-HT receptor.

AGS testing was done by placing drug or saline injected DBA/2J mice, one hour post injection, into a bell jar and engaging a bell (119 ± 2 db). AGS was scored for the following components: wild run, clonic seizure, tonic seizure and death. Saline injected control animals demonstrated the expected developmental reduction in AGS-S starting after 24 days of age. The receptor agonist 5-MeO-DMT (10 mg/kg) was shown to block the death, tonic, and clonic components of AGS in DBA/2J mice from 22 to 26 days of age after which time it increased AGS-S as compared to control animals. The receptor agonist Quipazine (10 mg/kg) blocked death and the tonic seizure component until age 26 after which time it increased clonic and tonic components as compared to control animals. The selective 5-HT re-uptake inhibitor CIMI (25 mg/kg; which would increase 5-HT levels at its receptor) demonstrated the same phenomenon, whereas the monoamine oxidase inhibitor pargyline (100 mg/kg; which would increase 5-HT intraneuronally) did not.

These results suggest that a developmental change in the 5-HT receptor occurs at day 26 or 27, which can be observed by increased AGS-S in response to increased 5-HT or 5-HT like activity at post synaptic receptors. One might speculate that the post-synaptic 5-HT receptor or a specific pool of these receptors undergoes a shift in functionality or maturation which may be related to the isozymal shift in activity of Ca^{2+}-Mg^{2+} dependent ATPase activity at 25 days in DBA/2J mice.[2] This would lead to an hypothesis that decreases in neurotransmitter levels contribute to the severity of AGS but it is the presence of unmatured receptors that imparts susceptibility to AGS in DBA/2J mice.

Supported by PHS Grant MH26712 & S.C. Gen. Med. Fac. Res. Appr.

1) Sparks, D.L. and Buckholtz, N.S., Soc. Neurosci. Abst. 3:449, 1977.

2) Rosenblatt, D.E. et al., J. Neurochem. 27:1299-1304, 1976.

595 DISTRIBUTION OF ENZYME ACTIVITIES AND AXONALLY TRANSPORTED GLYCOPROTEIN IN HAMSTER OPTIC NERVE SYNAPTOSOMES DURING POSTNATAL MATURATION. Susan Corey Specht and Teresa Candelas*. Dept. Pharmacol., Sch. Med., Univ. Puerto Rico, San Juan, PR 00936.

Optic nerve endings of golden hamsters were labeled by axonal transport following intraocular injection of ^{3}H-fucose at postnatal ages 12 days (P12), P16, 3 wks and adults. Synaptosomal subfractions were separated on a 4-step sucrose gradient by the method of Cohen et al. (J. Cell Biol. 74:181, 1977) and analyzed for acid-precipitable label or enzymatic activity. Incorporated radioactivity was measured 1, 7 and 15 days following injection. At both P12 and P16 over 75% of K^+-dependent, ouabain-sensitive p-nitrophenyl phosphatase and 5'-mononucleotidase activities were found in subsynaptosomal fractions layering at 0-0.85 M (A) and 0.85-1M (B); cytochrome oxidase activity was divided between the 1-1.2M (C) interface and the pellet (D). In 3 wk and adult hamsters the membrane markers were concentrated at the denser B and C interfaces; cytochrome oxidase activity was concentrated in D. The proportion of total synaptosomal protein in these fractions increased only slightly during development. Incorporated ^{3}H-fucose was recovered predominately from the C and D fractions in adults and from the less dense A and B fractions in P12 hamsters. In P16 hamsters synaptosomal label was initially distributed according to the P12 pattern but shifted to resemble the adult pattern 7 and 15 days after intraocular injection. Label disappeared from adult synaptosomes at the same rate as from other optic nerve fractions but was accelerated in synaptosomes isolated from hamsters injected at P12 and P16. The results demonstrate a maturational shift of membrane marker enzymes into denser fractions. ^{3}H-fucose incorporated at P16 showed a similar shift, suggesting that incorporation by retinal ganglion cells at P16 may be directed principally to plasma membrane precursors which appear first in light fractions and are later inserted into the heavier membranes. At P12 incorporation may be directed to less dense synaptosomal components. Active synaptic remodeling in neonatal optic nerve endings is suggested by the relatively more rapid turnover of synaptosomal ^{3}H-fucose. (Supported in part by NIHPHS grant EY02334.)

596 PHYSIOLOGICAL DEVELOPMENT AND SEGMENTAL DIFFERENCES OF NEURONS FROM AN IDENTIFIED PRECURSOR DURING GRASSHOPPER EMBRYOGENESIS. N.C. Spitzer, M. Bate*, and C.S. Goodman. Dept. of Biology, UCSD, La Jolla, CA, 92093, and Max-Planck-Institut für Virusforschung, Tübingen, F.D.R.

The "H" neuron, one of the two progeny of the midline precursor cell 3 (MP3) in the grasshopper, undergoes a morphological transformation early in embryogenesis. During this transformation in the metathoracic ganglion it acquires many of the same physiological properties as the first progeny of the DUM neuroblast in the same ganglion, at the same time that the latter cells are differentiating. The transformation of the "H" cell takes place while still electrically coupled to its mitotic sibling. The original process appears inexcitable throughout the course of its transient existence. This cell becomes uncoupled from its sibling and loses its original axon. The onset of electrical excitability is first seen in the new processes and shortly thereafter in the soma. It stains with neutral red, is depolarized by ACh, and receives spontaneous synaptic input. In those cases in which the sibling persists, its axon but not its cell body becomes electrically excitable.

Although all of the segmental ganglia come from relatively similar sets of embryonic precursor cells, there are striking differences in the number and properties of the neurons in the mature ganglia. In the meso- and metathoracic ganglia (T2 & T3), one of the two progeny of MP3 is transformed into the "H" cell. In many of the abdominal ganglia, beginning with the fourth (A4), both MP3 progeny disappear, at the time of the transformation of the "H" cell in T2 and T3. A gradient of transformation and cell death is seen in the first three abdominal ganglia (A1-3), at the boundary between thorax and abdomen. From A1 to A3 the MP3 progeny acquire fewer of the morphological phenotypes of the "H" cell: they lose the original process less often, and extend fewer of the new axons. There is also variability in the apparent gradient of the number of progeny persisting from A1 to A3. Finally, the acquisition of only a fraction of the morphological phenotypes is paralleled by the partial acquisition of the physiological phenotypes: the progeny of MP3 in A1 to A3 develop excitable axons but do not develop overshooting soma spikes.

Thus cells that are the progeny of the same precursor cell in different segments can develop different morphological and physiological properties. In contrast, cells which are the progeny of different precursor cells in the same segment can develop similar phenotypes.

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597 SELECTIVE OPTIC FIBRE PROJECTION FROM COMPOUND EYES TO THE TECTUM DURING DEVELOPMENT IN XENOPUS. Charles Straznicky[1,3], R.M. GAZE[2]* and M.J. KEATING[2]*. [1]Sch. Med., Flinders Univ of South Australia, Bedford Pk. 5042, Australia. [2]Natl. Inst. Med. Res., London NW7 1AA, UK.

Compound eyes were formed in Xenopus embryos at stage 32 by the fusion of two nasal (NN), two temporal (TT) and two ventral (VV) halves of the eye blastema. ^{3}H-proline was administered both into the operated right and the left intact eyes at stages 50, 52, 56, 60, 63, 66 and 2 and 6 weeks after metamorphosis and animals were killed 24 hours after injection in order to map the retinotectal projections autoradiographically. The visuotectal projections in animals from stage 56 onwards were also mapped electrophysiologically to demonstrate the success of the compound eye operation.

The projection from TT eyes was deficient in comparison with the normal side in that only the rostrolateral part of the tectum was innervated. In the autoradiographic time series the TT eye projection gradually expanded caudiomedially and finally reached the caudomedial tectal margin about 6 weeks after metamorphosis. Optic fibres from VV eyes initially projected, via the medial branch of the optic tract, to the medial portion of the developing tectum. At later stages this projection gradually expanded laterally reaching the lateral tectal margin only after metamorphosis. In contrast to TT and VV eye projections, optic fibres from NN eyes projected across the entire normally innervated extent of the tectum at all developmental stages.

These observations suggest that the initial tectal distribution of fibres from a compound eye is selective at least as far as concerns the polarity of the projection. Secondary spreading of the projection over the whole extent of the tectum then occurs. The extension of the NN projection over the whole tectum at all stages of development reflects the fact that the first part of the tectum to be innervated is the rostral part, even for nasal retina. In normal animals this rostral innervation by nasal fibres is pushed caudally during development by incoming temporal fibres; but with an NN retina there are no such fibres and the nasal innervation persists.

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598 NEUROTRANSMITTER-SPECIFIC PROTEINS OF SYMPATHETIC NEURONS. Kathleen J. Sweadner and Sarah J. Braun*. Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115.

Sympathetic neurons from superior cervical ganglia of neonatal rats can be maintained in dissociated cell culture in the absence of non-neuronal cells, where they develop into mature, differentiated neurons. The choice of neurotransmitter they synthesize, store, and release at synapses can be controlled: they develop as adrenergic cells when grown in depolarizing media, and as cholinergic cells when grown in media conditioned by certain non-neuronal cells (Walicke, Campenot, & Patterson, PNAS 74: 5767, 1977). We have examined the protein composition of adrenergic and cholinergic cells to identify transmitter-specific proteins involved in the formation and function of these two types of synapses.

Transmitter specific proteins might be found in the plasma membrane, in the extracellular matrix, in synaptic vesicles, and as soluble proteins free to diffuse between cells. To separate and characterize the transmitter-specific proteins, we have analyzed each of these subclasses of neuronal proteins separately: (1) Membrane glycoproteins were labeled either metabolically, by incorporation of [^{3}H] sugar precursors, or at the cell surface, by oxidation with periodate or galactose oxidase, followed by reduction with [^{3}H]BH_4^-. Both methods labeled a similar group of proteins. (2) Extracellular glycoproteins are left bound to the collagen substratum after the neurons are removed. These were then labeled by periodate or galactose oxidase and [^{3}H]BH_4^-. (3) Proteins are released into the culture medium when transmitter release is evoked by depolarization, Ba^{+2}, or black widow spider venom. These were labeled metabolically by incorporation of [^{3}H] leucine. (4) Soluble proteins are secreted and/or shed into the culture medium under normal growth conditions. These were also labeled metabolically by incorporation of [^{3}H]leucine.

Proteins were analyzed by high resolution one dimensional (SDS) and two dimensional (isoelectric focusing/SDS) gel electrophoresis and autoradiography. Data will be presented that shows that each of these subclasses of cellular proteins is distinct. Each contains from five to more than twenty different polypeptides. It has been possible to identify adrenergic- and cholinergic-specific proteins in each subclass, and a preliminary characterization of these will be described. Determination of neuronal transmitter choice thus involves not only the enzymes required for transmitter synthesis, but also a number of other proteins which are extracellular or exposed on the surface of the cell.

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599 DIFFERENTIATION OF PNMT IN CULTURED ADRENAL GLANDS FROM RAT EMBRYO. Gladys Teitelman, Dong H. Park, Tong H. Joh and Donald J. Reis (SPON: R.A. Ross). Lab. of Neurobiol., Dept. of Neurol., Cornell Univ. Med. College, New York, NY 10021.

In the rat embryo, the prospective adrenal medullary cells (phaeochromoblasts), of neural crest origin, reach the adrenal cortical anlage between prenatal days 15 and 16. At this stage, the CA synthesizing enzymes tyrosine hydroxylase (TH) and dopamine-β-hydroxylase (DBH) are detected in these cells by immunohistochemical techniques. However, it is not until day 18 (d18) of development that the enzyme required for adrenaline synthesis, phenylethanolamine-N-methyl transferase (PNMT), is first expressed (Teitelman et al., PNAS 76:509, 1979). To study some of the possible factors responsible for the initiation of PNMT synthesis within the phaeochromoblasts, we have sought to determine: a) whether PNMT would be expressed in adrenal glands removed from the embryo at day 16 and cultured in vitro; b) if the time course of appearance of PNMT in vitro is similar to that in vivo; and 3) whether ACTH and/or glucocorticoids are required for the initiation of PNMT synthesis in vitro. Adrenal glands were removed from 16d rat embryos, bisected, transferred into culture flasks and incubated. At different times in culture, the tissues were fixed in 4% paraformaldehyde, embedded in sucrose and cut in a cryostat microtome. Using the PAP technique, the sections were stained with specific antibodies to the enzymes TH, DBH, or PNMT. After 2 days in vitro, the adrenals contained many cells stained for TH and DBH. On the other hand, PNMT was detected in only a few cells, as in 18d in vivo. After 5 days in culture, the number of cells containing PNMT had increased greatly. The distribution of cells stained with either TH, DBH or PNMT was similar: after 5 days in vitro, they tended to aggregate into several large groups and, in many cases, surrounded the unstained adrenal cortex. To determine whether the expression of PNMT was due to ACTH or glucocorticoids in the culture media, adrenals removed at d16 were cultured in medium containing dialyzed serum, which presumably lacks these molecules. Incubation in dialyzed serum did not modify the time of the appearance of PNMT nor the subsequent increase in the number of cells containing the enzyme. We conclude: a) PNMT can be expressed in embryonic adrenal medullary cells in vitro; b) such differentiation is not a consequence of small molecules present in the culture medium; and c) the time course of appearance of PNMT in vitro is similar to that in vivo. The cultured embryonic adrenals may serve as a useful model for the analysis of the factors involved in the initiation of PNMT synthesis.

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600 SYNAPTOGENESIS IN THE DEVELOPING ANTENNAL LOBES OF THE BRAIN OF THE MOTH *MANDUCA SEXTA*. Leslie P. Tolbert. Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115.

The antennae and antennal lobes of the brain of the moth *Manduca sexta* arise during the 18 days of metamorphic adult development, and therefore provide a convenient preparation in which to study the developmental interactions between neurons that will come to be synaptically related. As a basis for a detailed understanding of the correlation between the neurochemical and cytological maturation of the antennal lobes, we have undertaken a study of the synaptic connections in the adult antennal lobe and of the formation of synapses in the developing antennal lobe.

In the antennal lobe, the antennal sensory axons synapse with second order cells in light-microscopically identifiable regions of condensed neuropil, the glomeruli. Electron-microscopic examination of these glomeruli in adult moths reveals that they comprise tightly packed neuronal processes, most of which contain vesicles. Several types of synaptic processes can be distinguished on the basis of the sizes and shapes of their vesicles and whether they are presynaptic or postsynaptic or both. Following removal of the antenna, profiles containing round, electron-lucent vesicles exhibit degenerative changes, suggesting that these profiles represent the primary afferent input to the lobe.

Although growing antennal axons begin to reach the antennal lobe on about day 3 of adult development, synapses do not appear until much later. On day 10, many neurite profiles contain vesicles; only a few have begun to elaborate small, weakly staining membrane specializations. During the next two days of development, membrane-associated densities expand and acquire their adult staining characteristics; synaptic vesicles in the presynaptic elements cluster around the presynaptic densities. By day 12, mature synaptic complexes are distributed throughout the glomeruli. Previous studies in our laboratory (Dev. Biol. *52*: 105, 1976; Brain Res. *119*: 389, 1977) have shown that the antennal axons appear to use acetylcholine as neurotransmitter. On day 12, when morphologically mature synapses are first seen, levels of ACh and its synthetic enzyme, choline acetyltransferase, in the antennal lobe are just reaching their mature levels, suggesting that the newly formed synapses may be functional. Intracellular recordings by S.G. Matsumoto (this volume) corroborate this idea. They show that synaptic transmission in the antennal lobe develops between days 9 and 13 of development.

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601 PATTERNS OF NEUROGENESIS IN THE VENTRAL LATERAL GENICULATE NUCLEUS OF THE CHICK. C. J. Uchwat* and W. J. Crossland, Department of Anatomy, Wayne State University School of Medicine, Detroit, Mich. 48201.

This study was conducted to determine the temporal and spatial patterns of neurogenesis in the chick ventral lateral geniculate nucleus (GLv). A single injection of 20 µCi of ^{3}H-thymidine was applied to the yolk sac of 49 chick embryos from stage 17 through stage 36. The embryos were sacrificed at stage 42 or 43 and the brains embedded in paraffin. Since the ^{3}H-thymidine is available to the embryo from the yolk for some time, a cumulative labeling analysis was employed.

The results of our autoradiographic studies indicated that the lateral diencephalic nuclei are produced before the medial nuclei. Similarly, ventral nuclei precede dorsal; caudal nuclei precede rostral. These patterns are similar to those already described for mammals.

Neuronal proliferation is somewhat late in the GLv in spite of the ventrolateral position of the nucleus in the diencephalon. A detailed analysis of cell production in the GLv was made by determining the percentage of unlabeled cells in both GLv lamina (internal lamina and neuropil lamina) from thionin stained autoradiographs. Two gradients of cell production were apparent. 1) Neurons in the caudal GLv were produced before the rostral GLv neurons. 2) Although unlabeled cells appeared in both GLv laminae at stage 21 and both laminae were unlabeled by stage 34, the internal lamina became unlabeled more rapidly than the neuropil lamina. Thus the GLv has an "inside-out" (dorsomedial to ventrolateral) gradient of cell production in contrast to the over-all "outside-in" (ventrolateral to dorsomedial) pattern in the diencephalon.

Planimetric measurements of camera lucida-traced neurons in a stage 25 embryo showed that unlabeled cells in both GLv laminae have a larger mean area than labeled cells. Furthermore, the mean neuronal area in the internal lamina is significantly greater than the mean neuronal area in the neuropil. Thus the pattern of neurogenesis in the GLv appears related to cell size rather than to the general gradients of cell production in the diencephalon.

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602 ALTERED ISTHMO-TECTAL TOPOGRAPHY AFTER EYE ROTATION IN XENOPUS TADPOLES. Susan B. Udin and M.J. Keating*, National Institute for Medical Research, Mill Hill, London NW7 1AA, England.

In normal Xenopus, the tectum receives a direct visuotopic input from the contralateral eye and an indirect input from the ipsilateral eye. Each eye projects directly to the contralateral tectum; in turn, each tectum projects to its ipsilateral nucleus isthmi (NI) and the NI then projects across the midline to the tectum ipsilateral to the eye of origin. The tectal projections from the two eyes are in register with each other; each point in the binocular visual field is represented at a single point in each tectum via both eyes.

If one eye is rotated during mid-larval stages, each point on the retina still projects to its appropriate site in the contralateral tectum; thus, the visuotectal map on the contralateral tectum is rotated. The indirect ipsilateral visuotectal map from the normal eye develops in such a way that the two visuotectal projections are in register, i.e., the indirect ipsilateral projection from the normal eye becomes rotated. On the other tectum, the normal eye still produces a normally-oriented direct visuotectal map; and again, the indirect ipsilateral projection is in register, i.e., the rotated eye produces a normally oriented ipsilateral projection. We have studied the tecto-isthmo-tectal pathway in an attempt to reveal whether this plasticity comes about by means of rearrangements of the terminals of the crossed isthmo-tectal projection.

To test this hypothesis, we have made localized horseradish peroxidase (HRP) injections in the tectum to determine the topographic relationship between the tectum and the NI in normal and eye-rotated frogs. Our results show that in normal Xenopus, each tectal HRP injection labels a discrete group of tecto-isthmic fibers in the ipsilateral NI. This tecto-isthmic topography appears to be identical in eye-rotated frogs. In normal frogs, each injection also labels a discrete group of cells in the contralateral NI. In eye-rotated animals, however, a single HRP injection usually labels two groups of cells in the contralateral NI. One group corresponds to the normal population; the other consists of cells which normally would be labelled by an HRP injection in a different tectal site. We interpret our results to mean that a new population of crossed isthmo-tectal cells is arborizing at the injection site and that the original population is labelled because they are either passing through their original positions en route to new sites or are forming physiologically inactive arbors at their original sites. Thus, it appears that the anomalous topography of the indirect ipsilateral projection results from alterations in the termination sites of the crossed isthmo-tectal fibers.

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603 FEMALE SEXUALITY: PREDICTION BY FETAL LEVELS OF TESTOSTERONE IN SERUM AND AMNIOTIC FLUID OF MICE. Frederick vom Saal* and Claude Desjardins* (SPON: L.W. Hamilton). University of Texas, Austin, Tx

Androgen exposure during fetal life produces changes characteristic of the masculine phenotype in mice as evidenced from developmental anomalies and the administration of exogenous androgen. In sharp contrast, a novel approach to the study of sexual differentiation has involved the examination of a naturally occurring phenomenon in multiple-birth species; namely, the effect of intrauterine proximity of females to male fetuses (vom Saal and Bronson, 1978). Specifically, female mice that develop in utero between two male fetuses (2M females) and females that are not contiguous to a male fetus (0M females) differ in a variety of characteristics: for example, 0M females are more sexually attractive to males, have shorter estrous cycles as adults, and enter puberty later when grouped; 2M females, on the other hand, are more aggressive in a variety of situations and have larger anogenital spaces at birth. It was hypothesized that androgen produced by a male fetus might be transfered to an adjacent female fetus via the amniotic fluid and thus be present in higher concentrations in both the amniotic fluid and blood of a 2M female relative to a 0M female. To test this hypothesis, mouse fetuses were delivered on Day 17. Amniotic fluid was collected on filter paper, and blood serum was taken from 75 2M and 75 0M females. Testosterone, progesterone and 17β-estradiol were measured by radioimmunoassay after chromatographic separation.

Significantly higher levels of testosterone were detected in the serum and amniotic fluid of 2M females (serum: 2M females = 1,090±66 pg/ml; 0M females = 878±43 pg/ml; $t = 2.7$, $p < .05$, df=8; amniotic fluid: 2M females = 128±2 pg/fetus; 0M females = 102±6 pg/fetus; $t = 4.0$, $p < .01$). Estradiol and progesterone levels in amniotic fluid and serum did not differ ($p > 0.1$). The results support the hypothesis that 2M and 0M females differ as a result of 2M females being exposed to androgen produced by contiguous male fetuses. The findings from previous studies involving the intrauterine-position phenomenon demonstrate that many androgen-mediated characteristics, traditionally considered sexually dimorphic (e.g. aggressiveness), exhibit considerable variability within a population of females. Rather than consider these characteristics as dimorphic, in terms of male vs female, it now appears to be more appropriate to consider that a continuum exists for females with regard to characterististics such as aggression and sexual attractiveness. The implications of the present findings are that the point on a continuum of these characteristics that a female occupies is correlated with serum levels of testosterone during prenatal development.

vom Saal, F. & Bronson, F. Biol. Reprod., 19: 842-853, 1978.

604 CONSIDERATION OF Ca^{2+} AND CYCLIC AMP AS SECOND MESSENGERS IN THE EFFECTS OF ELECTRICAL ACTIVITY ON NEUROTRANSMITTER CHOICE BY SYMPATHETIC NEURONS. Pat Walicke* and Paul Patterson (SPON: E. J. Furshpan). Dept. Neurobiology, Harvard Med. Sch., Boston, MA 02115

Sympathetic neurons taken from superior cervical ganglia of neonatal rats and maintained in cell culture can develop either adrenergically or cholinergically depending on culture conditions. Both chronic depolarization and electrical activity can stabilize the choice of adrenergic development, even in the presence of the cholinergic inducing factor secreted by certain nonneuronal cells. Blockers of Ca^{2+} influx (Mg^{2+}, D600, EGTA) inhibit this effect of depolarization, indicating that Ca^{2+} may be the coupling factor. Depolarization elevates neuronal cAMP content, and exposure to dibutyryl cAMP or agents elevating cAMP (adenosine, PGE_1, dibutyryl cGMP) also stabilize the adrenergic choice, suggesting that cAMP may be the second messenger.

To clarify the roles of Ca^{2+} and cAMP, some of their interrelationships were studied. When neurons were depolarized in the presence of Mg^{2+} or D600, no increase in cAMP was seen. On the other hand, EGTA, diphenylhydantoin, and theophylline did not alter the elevation in cAMP but did reverse the developmental effect of depolarization. Thus Ca^{2+} appears to be more directly involved in the depolarization effect, since manipulating its cellular distribution can uncouple the cAMP increase from stabilization of the adrenergic choice. Mg^{2+} and D600 are probably more efficient than the other agents at blocking Ca^{2+} influx through voltage-dependent channels, so their additional effect on cAMP could indicate that the increase is in response to this type of Ca^{2+} entry. α and β adrenergic blockers and adenosine blockers had no influence on cAMP, so release of these substances is not an intermediate step.

Restricting Ca^{2+} availability with EGTA also reversed the effect of dibutyryl cAMP on transmitter choice. Furthermore, neurons exposed to adenosine, PGE_1, or dibutyryl cGMP in the presence of EGTA responded with a normal increase of cAMP but failed to remain adrenergic. These results are consistent with the interpretation that these agents are acting by increasing neuronal Ca^{2+}, though the possibility remains that Ca^{2+} and cAMP act additively at a later step controlling transmitter choice. In summary, the Ca^{2+} and cAMP systems of these neurons appear to be multiply interconnected, but Ca^{2+} appears to be more directly linked with the control of transmitter choice. (Supported by a NINCDS grant to Paul Patterson.)

605 FURTHER OBSERVATIONS ON POSTNATAL NEUROGENESIS IN THE SNAKE'S VOMERONASAL EPITHELIUM: USE OF ^{3}H-THYMIDINE AUTORADIOGRAPHY TO TRACE THE DIFFERENTIATION, MATURATION AND MOVEMENT OF BIPOLAR NEURONS. R.T. Wang, A. Vagvolgyi*, B. Mendelsohn* and M. Halpern. Dept. of Anatomy and Cell Biology, SUNY Downstate Medical Center, Brooklyn, N.Y. 11203.

The vomeronasal epithelium of adult garter snakes is capable of postnatal neurogenesis under normal conditions and following vomeronasal axotomy (Wang et al., Society for Neuroscience Abstracts 3:85, 1977; 4:93, 1978). The undifferentiated cells (UD)--the precursors of bipolar neurons (BP)--can be labeled with ^{3}H-thymidine and identified using autoradiographic techniques (Wang et al., 4:93, 1978). Application of this technique to animals with long postinjection and post lesion survival times has permitted us to trace the genesis of the BP in the epithelium.

Four groups of adult garter snakes (3 snakes in each group) were injected intracardially with ^{3}H-thymidine (1 μCi/gm body wt). On the day following injection, unilateral vomeronasal nerve lesions were made in snakes in three of the experimental groups. The fourth group was sacrificed 24 hours following injection without being subjected to the lesion-making procedure. The remaining snakes survived 2 weeks, 4 weeks or 8 weeks following surgery. The heads of all animals were processed for light microscopic autoradiography.

Graded movement of ^{3}H-thymidine-labeled cells from the basal, UD layer toward the apical region of the BP layer was observed in both the normal VN organ and the deafferented VN organ at different survival times. Cell migration distances were positively correlated with post operative survival time in both normal and deafferented organs. However, the apical labeled cells in the deafferented VN organ always progressed further than their counterparts in the normal VN organ at each respective survival time. Labeling in cells at the migrating front was very heavy at all survival times, but the labeling in the basal UD cells was gradually diluted at long survival times.

Despite the difference in distance of cell migration which occurred between the normal VN organ and the deafferented VN organ at each survival time, labeled cells which had been tagged for 2 weeks in both organs were still in the undifferentiated stage. The apical labeled cells underwent further neuronal differentiation in 4 weeks and subsequently became mature neurons in 8 weeks. (Supported by NIH contract NO1RR82139).

606 DO CATECHOLAMINES MONITOR BRAIN DNA SYNTHESIS ? Claude G. Wasterlain†, Matilde Fando* (SPON: R. Lolley). Epilepsy Research Laboratory, VA Hospital, Sepulveda, CA 91343, Dept. Neurol. and Brain Research Institute, UCLA Sch.Med.

Catecholamine agonists and antagonists can have lasting effects on the developing brain. Since L-DOPA is used in treating cerebral palsy, and since cAMP and/or catecholamines monitor the rate of cell division in many systems, we investigated the relationship between L-DOPA treatment and brain DNA synthesis in immature rats. L-DOPA (30 to 500 mg/Kg) inhibited DNA synthesis in forebrain in a dose-dependent fashion. A DOPA-decarboxylase inhibitor (RO4-4602, 800 mg/Kg), which raises brain L-DOPA but not catecholamines, blocked the inhibition of DNA synthesis by L-DOPA, while monoamine oxidase inhibitors potentiated this effect. This inhibition was maximal in the first 10 days of life in forebrain, but was not manifest until 10 days in cerebellum, suggesting that it depended on the stage of maturation of the tissue. In vitro, norepinephrine and dopamine in concentrations sufficient to elevate cAMP (0.1 to 1 mM) and dibutyryl cyclic AMP inhibited DNA synthesis in brain slices.

These results suggest that therapeutic amounts of L-DOPA can alter brain DNA synthesis and presumably brain development; whether this effect is mediated by catecholamines and cAMP requires further investigation. Until that hypothesis is ruled out, the possibility of adverse effects on development should be kept in mind when using neurotransmitter precursors, agonists or antagonists in the neonate.

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607 THE DEVELOPMENT OF β-ADRENERGIC RESPONSIVENESS IN CNS MOUSE REAGGREGATE CULTURES

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Mouse CNS reaggregate cultures were prepared using fetuses after 17 days of gestation. Whole fetal brains were removed and mechanically dissociated into single cells as described by Honneggar and Richelson (Brain Res. 109:338, 1976), and maintained in DMEM + 15% horse serum. The development of β-adrenergic hormone responsiveness was examined as a function of time in culture. The time course of cAMP synthesis (using intact reaggregates) in response to 10^{-7}M Isop. revealed that a major peak of cAMP production (after 10 min of hormone exposure) appeared at 11-13 DIV (equivalent to 8-10 days after birth). This corresponds to the development of catecholamine response in cerebral cortex described by Harden et al. (Brain Res. 125:99, 1977). The stimulation of cAMP production was dependent upon the dose of Isop. (10^{-9} - 10^{-5}M), and was blocked by the addition of β-propranolol. Adenylate cyclase activity was also measured using homogenates prepared from reaggregates at 4, 14, 21 DIV. Basal and fluoride-stimulated activity increased as a function of time in culture. Isop. (10μM) or GTP (50μM) did not stimulate adenylate cyclase activity in homogenates of 4DIV reaggregates. However, adenylate cyclase activity was stimulated 2-3 fold by Isop. or GTP and 7-8 fold by Isop. + GTP at 14 & 21 DIV. These experiments indicate that β-adrenergic responsiveness appears in reaggregate CNS cultures at approximately the same time that such a response appears in vivo. (Supported by NIH Grant NS-14436.)

608 DEVELOPMENT OF THE PARASYMPATHETIC INNERVATION OF THE CHICK HEART. Thomas A. Weidman*, Margaret L. Kirby, John W. McKenzie*. Dept. Anat., Sch. Med., Medical College of Georgia, Augusta, GA 30901.

There have been several studies of the development of parasympathetic innervation of the heart using silver impregnation techniques. The results of these experiments are conflicting and indicate that the heart is innervated either on the 3rd or 5th day of incubation. Using electron microscopy and the Holmes silver impregnation for light microscopy, we have determined the sequence of innervation of the chick heart. At 72 hours of incubation cardiac ganglion cell precursors were aggregating adjacent to the bulbus arteriosus and were partially encapsulated. The cells appeared to be streaming lateral to the pharynx into the bulbar region. No neuronal processes were seen ultrastructurally and no preganglionic fibers could be found either in silver preparations or in electron micrographs. Preganglionic axons were traced from the neural tube to the cardiac ganglia on the 6th day and in electron micrographs preganglionic terminals could be seen in the region of the cardiac ganglia. The earliest synapses of preganglionic axons were observed at 8 days. The ganglion cells sprouted on the same day, and postganglionic nerves were found in the heart. The terminals contained clear vesicles of the type associated with cholinergic transmission. On the 9th and 10th days the number of postganglionic nerves increased and en passant synapses were present on cardiac muscle fibers. These results correlate very well with physiological studies which have shown that cardioinhibition between the postganglionic neurons and sinoatrial pacemaker cells can be detected first on the 12th day of incubation. In the presence of physostigmine, a cholinesterase inhibitor, cardioinhibition can be elicited on day 10 of incubation. The effect of physostigmine has been attributed to inhibition of cholinesterase which allows the small amounts of released acetylcholine to reach postsynaptic receptors in concentrations sufficient to inhibit pacemaker activity (Pappano AJ, Pharmacol Rev 29:3-33, 1977).

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609 CELL LINEAGE AND REGULATION DURING LEECH NEUROGENESIS AS REVEALED BY ENZYME INJECTION OF IDENTIFIED EMBRYONIC CELLS. David A. Weisblat*, Seth Blair*, and Gunther S. Stent. Dept. Mol. Biol. UCB, Berkeley, CA 94720.

Glossiphoniid leeches are highly suitable for neurodevelopmental studies because the early embryo and the segmental ganglia of the adult nerve cord both consist of large, identifiable cells, readily accessible to observation and experimental manipulation. The egg of these leeches undergoes stereotyped cleavages that produce an early embryo containing three macromeres (A, B, and C), one bilateral pair of mesoteloblasts (M) and four bilateral pairs of ectoteloblasts (N, O, P, and Q). The teloblasts give rise to columns of stem cells, that merge to form left and right germinal bands; the germinal bands migrate ventrolaterally across the surface of the embryo, eventually coalescing in a rostrocaudal progression on the ventral midline to form the germinal plate, from which the segmental tissues, including the nerve cord ganglia, arise.

Lines of descent of cells comprising the ganglia have been ascertained by injection of horseradish peroxidase (HRP) into identified cells of early embryos of the glossiphoniid leech Helobdella triserialis. Such experiments show that the central nervous system has a complex embryological origin. Most of the neurons of the hemilateral segmental ganglia derive from the ipsilateral N ectoteloblast, but a spatially coherent fraction derives from the ipsilateral OPQ ectoteloblast precursor. Some, possibly all, of the segmental neuroglia derive from the mesoteloblast M, whereas the cells of the frontmost, supraesophageal ganglion derive from the A, B, or C macromeres. The position of the boundary between unstained (rostral) and stained (caudal) tissues in preparations in which N teloblasts were injected after initiation of stem cell production showed that each hemiganglion contains the descendants of more than one, probably two, primary N-derived stem cells.

In normal embryos, HRP-stained ganglionic cell bodies are found only ipsilateral to the injected teloblast progenitor, indicating that innervation of the contralateral musculature by segmental motor neurons is attributable to a developmental decussation of their axons, rather than to an initially uncrossed projection followed by reciprocal migration of homologous cell bodies across the midline. However, if, following HRP injection of one N teloblast, the contralateral N teloblast is killed by intracellular injection of Pronase (Parnas and Bowling, Nature 270: 626, 1977), stained cell bodies are present on both sides of the segmental ganglia, indicating a regulative contribution by the surviving N teloblast to the contralateral nerve cord.

610 A GOLGI ANALYSIS OF EARLY DIFFERENTIATION OF MOTOR NUCLEI IN THE HINDBRAIN OF THE MOUSE EMBRYO. Lee E. Wentworth. Dept. Anat., Sch. Med., Univ. of California, San Francisco, CA 94143

To this date no one has described the very early differentiation of mammalian cranial nerves using the Golgi technique. Last year, using a modified Golgi technique, we described (Anat. Rec., 190: 580-581) neuronal differentiation throughout the CNS in a mouse embryo of ten days gestation (E10; E0=vaginal plug). Cells from younger and older embryos have been successfully impregnated since then. At E9-9 1/2 axons of hypoglossal neurons exit in a direct line with the ventral roots of cervical nerves. By contrast, axons of cells of the nucleus ambiguous complex, facial motor nucleus, and trigeminal motor nucleus exit at the level of the sulcus limitans. Their cell bodies are located in a position similar to that of spinal accessory neurons at this age, i.e., ventromedial to somatic efferent neurons, e.g., the hypoglossal nucleus. The bulbar portion of C.N. XI and the vagus nerve appear to be part of a continuous complex at this age. A few of the cells of the trigeminal motor nucleus have started to migrate towards their existing root. By E10 the hypoglossal nerve is well developed and some cells are becoming multipolar. The majority of the cells of the nucleus ambiguous complex still reside ventral to hypoglossal neurons. Occasionally a cell is seen located ventral to and projecting a process into the exiting root of the vagus. These cells may be early cells of the dorsal motor nucleus of the vagus. A row of motor neurons of the trigeminal nerve extends along the lateral border of the neural tube from a most ventral position to almost the level of exit of the motor root. Although many of the most ventral cells are still oriented radially, some of the cells which have migrated closer to the exiting root have reached the bipolar stage. By E11 some cells of the X-XI nucleus ambiguous complex have migrated dorsolaterally to the level of exit of the hypoglossal nerve. A well-developed facial nerve passes between the facial ganglion laterally and the acoustic ganglion medially. Occasionally a cell that may belong to the developing superior salivatory nucleus is seen ventromedial to the exiting root of the facial nerve. A few exiting fibers of the abducens nerve make their first appearance during E11.
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611 SYNAPSE DEVELOPMENT IN OLFACTORY CORTEX. Lesnick E. Westrum and Elizabeth Miller.* Departments of Neurological Surgery and Biological Structure, Univ. of Washington, Seattle, Wa. 98195

Layer I of rat prepiriform cortex (area 51A) is being studied electron microscopically at several consecutive postnatal ages from birth to adulthood, emphasizing the maturation of synaptic patterns. Previous qualitative studies have suggested that dramatic increases in numbers and changes in proportions of synaptic types occur during the first two weeks of life (J. Neurocytol. 4:713). Attempts have now been made to quantify these estimates. Consecutive non-overlapping micrographs were taken throughout layer I starting at the olfactory tract and finishing at the cell body layer II. Three animals were used at each of the ages of 1, 3.5, 4.5, 7, 14, and 30 days after birth. Mature synapses were counted in all of the micrographs and averages were determined for density of contacts and proportions of the major types of synapse. With rare exceptions, the area contains two major types of synaptic contact: presynaptic terminals with primarily round synaptic vesicles (R) and presynaptic terminals with mainly flattened or elliptical synaptic vesicles (F). The R terminals usually form asymmetric contacts and the F form symmetric appositions. Thus far over 10,000 total synaptic contacts (R + F) have been counted in approximately 900 electron micrographic fields (of 140 sq. μm each). At 1 day of age, there are approximately 5½ total synaptic contacts per field studied (about 0.038 synapse/sq. μm or 3.8/100 sq. μm) and 10% of these are F synapses. The numbers of total synaptic contact increase gradually over the first week to about 9 per field at 7 days of age (0.063/sq. μm or 6/100 sq. μm) with 11-12% of these being F contacts. A striking finding, however, was the relative proportion of F contacts seen at 3-4 days of age forming approximately 20% of the total contacts. By 14 and 30 days of age, the density of all synapses increases to 16-20 per field (0.12-0.15 synapse/sq. μm) with the proportion of F terminals decreasing to about 10% of the total. The thickness of layer I increases from about 60 μm at birth to about 150 μm at 14-30 days of age. The observations document the increase in total number and proportions of synaptic contacts during the first month after birth, and suggest a critical age range (3-5 days) during which there is an accelerated maturation specifically of the F type of synaptic contact. This is followed at 5-14 days by a proportionally greater maturation of the R synaptic contacts. These patterns may be related to developmental sequences in specific pathways of this region. (LEW is an affiliate of the CDMRC. Aided by NIH Grants NS09678, NS04053, and DE04942).

612 CENTRAL CONTROL OF ULTRASONIC VOCALIZATIONS OF NEONATAL RATS: BRAIN STEM MOTOR NUCLEI. Daniel M. Wetzel, Darcy B. Kelley, and Byron A. Campbell. Department of Psychology, Princeton University, Princeton, N.J. 08544.

Ultrasonic vocalizations of rodents show dramatic developmental changes. This system may prove a useful model to study maturational changes in the mammalian brain and subsequent effects on behavior. Neonatal rats emit pulses (≅ 100 ms duration) of intense (59-85 dB re 2 x 10^{-5} NM^{-2}) ultrasound (≅40 kHz) in response to cold stress. The rate of ultrasounding declines as the pup matures, from a high of >100 pulses/min on postnatal day 10 to <10 pulses/hr by day 20. To elaborate the possible neural bases of this change, the laryngeal nerves controlling the production of ultrasounds and the motor nuclei of origin of these nerves were investigated.

Transections of the laryngeal nerves were used to study the laryngeal production of ultrasounds. Bilateral and unilateral transections of the inferior laryngeal (IL) and superior laryngeal (SL) nerves were made on postnatal day 8, as were sham operations. Ultrasounds were elicited on day 10 by placing the pups in a sound isolation chamber with an air temperature of 0°± .1°C. Both unilateral and bilateral transections of the IL reduced ultrasounds to undectable levels, while sham animals were no different from controls. The harmonic structure of audible squeaks,elicited by tail pinch, was only slightly affected by bilateral transections of the IL. Transections of the SL reduced the intensity (86 dB to 77 dB) and increased the fundamental frequency (from 40 kHz to 49.5 kHz) of the ultrasounds; bilateral transections more so than unilateral. Squeaks remained unchanged after SL transections. It appears that the ultrasound is produced by the actions of the muscles innervated by the IL on the airflow, while the muscles innervated by the SL act to further modify the ultrasounds' physical characteristics.

The motor nuclei of origin of the IL and SL were explored with retrograde transport of horseradish peroxidase (HRP). On day 8 bilateral injections of HRP were made into the intrinsic muscles of the larynx with only one IL or one SL intact, and all other laryngeal nerves cut. After 48 hrs survival, brain sections were reacted with tetramethylbezidine. Cells heavily labeled with HRP reaction product were consistently found in the dorsal formation of the nucleus ambiguus ipsilateral to the intact IL. A few lightly labeled cells were also found in the dorsal motor nucleus of the vagus; possibly gamma motor or secretomotor neurons. The ventral formation of the nucleus ambiguus contained heavily labeled cells ipsilateral to the intact SL. The results suggest that the dorsal and ventral formations of the nucleus ambiguus are the cranial motor nuclei controlling the laryngeal production of the ultrasonic vocalizations of rat pups.

613 DEVELOPMENTAL CHANGES OF MEMBRANE-BOUND NEURAMINIDASE ACTIVITY IN PRE- AND POSTNATAL STAGES OF WILDTYPE MICE AND ALTERATIONS IN NEUROLOGICAL MUTANTS. Wolfgang Wille* and Ekkhart Trenkner Dept. of Neuroscience, Children's Hospital Med. Ctr., Harvard Medical School, Boston, MA 02115

Mouse cerebellum cells change their cell surface carbohydrate pattern during their development as shown with carbohydrate specific antibodies (Trenkner & Sarkar, J. Supr. Struct., 6:465, 1977). In the cerebellar mutant 'staggerer' (sg/sg) neuraminic acid containing sugar polymers pertinent to prenatal cell surfaces are maintained throughout early postnatal development (Trenkner, Nature, 277:566, 1979). In this study we focus on the enzyme neuraminidase which is presumably involved in alterations of cell surface carbohydrates.

Neuraminidase (N-acetylneuraminate glycohydrolase, =sialidase, EC 3.2.1.18) is, at least in one form, a plasmamembrane-bound glycosidase which cleaves N-acetylneuraminosyl linkages. In our test system we used the substrate α-D-N-acetylneuraminosyl-(2'→3')lactit[^{3}H]ol. After cleavage the free lactitol was separated from uncleaved neuraminlactitol on ion-exchange column (Bio-Rad AG1-X2, formate). The eluted radioactivity was related to the protein concentration of the enzyme containing membrane fraction of the homogenized tissue.

Neuraminidase activity was determined in cerebellum, cerebrum, and liver. The age-dependent changes of neuraminidase activity in all the tissues show only one significant maximum. While three days before birth (E16) the specific activities were low (liver: 5.0, cerebellum: 2.5, and cerebrum 1.5 nmoles cleaved neuraminlactitol/mg protein/3 h at 37°C and pH 4.0) the maximum activity was reached within 5-6 days (4 fold). The activities then decreased rapidly to values equivalent to E16 at approx. 20 days after birth (P20). The order of tissue specific activities as seen at E16 remained unchanged throughout development.

In contrast neuraminidase activity of the 'staggerer' cerebellum was considerably higher (2 fold at P7, 5 fold at P17) than wildtype whereas activities in cerebrum and liver remained unchanged. The cerebellar mutant 'weaver' (wv/wv), on the other hand, did not exhibit significantly different neuraminidase activities compared to normal littermates.

614 ^{3}H-THYMIDINE LONG-SURVIVAL AUTORADIOGRAPHY AS A METHOD FOR DATING THE TIME OF ORIGIN IN THE CHICK EMBRYO: DEVELOPMENT OF THE LOCUS COERULEUS-CEREBELLAR SYSTEM. L. Yurkewicz*, J. M. Lauder, E. Giacobini and M. Marchi*. Dept. of Biobehavioral Sci., Univ. of Connecticut, Storrs, CT. 06268. (SPON: E. Shaskan).

Since the chick embryo offers numerous advantages for developmental studies, we are attempting to establish a model system to explore the interactions between noradrenergic (NA) neurons and their target cells during brain development in this experimental animal. As investigated by histofluorescence, the locus coeruleus (LC) has been reported to project to the cerebellar cortex of the adult chicken (Mugnaini and Dahl, J. Comp. Neurol. 162 (4):417, 1975). In the present study we have confirmed this report using the horseradish peroxidase tracing technique. In the rat LC-cerebellar system, the time of origin (last cell division) of LC cells precedes that of cerebellar Purkinje cells by several days (Lauder and Bloom, J. Comp. Neurol. 155 (4): 469, 1974), a pattern we have now examined in the chick. The amount of ^{3}H-thymidine (^{3}H-T) (s. a. 57 Ci/mMol) available to the embryo at various survival times following a single injection (25 uCi) into the yolk sac at 1-2 days of incubation (d. i.) was determined by thin layer chromatography of acetic acid extracted yolk, white or embryonic brain tissue. Contrary to previous assumptions, results of this study indicate that ^{3}H-T is not continuously available to the chick embryo after a single injection, but rather constitutes a 1-2 day pulse. This finding permits the use of long-survival ^{3}H-T autoradiography in chicks to date the time of last cell division within a 2 day range using the adult brain rather than that of the embryo, greatly facilitating accurate identification of cell populations in discrete brain regions. Therefore, we have used this method to determine the time of last cell division of LC cells and cerebellar Purkinje cells in the chick. A single dose (25 uCi) of ^{3}H-T was injected into the yolk sac of fertilized eggs on day 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7 or 8 d. i. Chicks were sacrificed between 29 and 44 days posthatching and the brains prepared for autoradiography. Cell proliferation ceased in the chick LC from 2-6 d. i., with a peak of heavy labeling between 3-5 d. i., whereas this event occurred in the Purkinje cell precursor population from 3-7 d. i. with a peak of heavy labeling between 4-6 d. i., indicating that the chick LC begins to develop prior to the cerebellar Purkinje cells in a manner similar to that observed in the rat. This finding, together with our data confirming a specific LC-cerebellar projection in the chick, provides the basis for the use of this system as a model for examining NA neuron target cell interactions during avian embryonic development. Supported by U of Connecticut Research Foundation.

615 POSTNATAL BRAIN DEVELOPMENT FOLLOWING PRE- AND POSTNATAL EXPOSURE TO TRITIATED WATER FOR 5 GENERATIONS. Stephen Zamenhof and Edith van Marthens*. Mental Retardation Res. Cntr., and Brain Res. Inst., Sch. Med., UCLA, Los Angeles, Ca. 90024.

In view of the anticipated increased use of atomic energy in industry, in continuation of our previous work (Radiation Res. 77, 117, 1979) we studied the possible long term effect of chronic radiation exposure. Rats were given tritiated drinking water (3 μCi/ml) before pregnancy, during pregnancy and thereafter, continuously through 5 generations (F_1 to F_5); females were mated with control males. The average total daily intake was 57 μCi. At this dose, no signs of radiation illness were observed. In each generation, 30 day old male and female pups were sacrificed and their brains assessed for accumulation of radioactivity and for development.

Radioactivity significantly increased through generations: as compared with F_1, the radioactivity in F_3 and subsequent generations was up to 136% higher in the blood and up to 88% higher in the cerebral cortex; the highest increase was in the cerebellum (209%). The highest specific activity was in the cortical DNA fraction (up to 85000cpm/mg); in the protein fractions the highest specific activity was in the diencephalon (up to 2000 cpm/mg) and brain stem (up to 1780 cpm/mg).

The damages to the developing brain were also significantly intensified through generations: as compared to the control, the decrease in DNA (cell number) and protein in F_5 was 3 to 4 times more severe than in F_1. The most severe decreases were: DNA (cell number) of brain stem (-31%), diencephalon (-25%), cerebral cortex (-23%) and cerebellum (-18%); protein of diencephalon (-32%), brain stem (-30%), cerebral cortex (-28%) and cerebellum (-27%); all these differences were statistically highly significant ($p < 0.001$); on the average for both DNA and protein they were 1½ times more severe in diencephalon than in the cortex. These damages at 30 days were also 2 to 3 times higher than in the newborns.

Several phenomena may account for all these results. Postnatally proliferating cells (glia in cerebral cortex, most neurons and glia in cerebellum) may be more sensitive to radiation damage than the prenatally proliferating neurons. RNA involved in protein synthesis (mostly postnatal) is repaired less efficiently (if at all) than the prenatally proliferating neuronal DNA. (Supported by DOE grant EY-76-S-03-0034 and NIH grants HD-05615 and AG-00162).

EPILEPSY

616 **DIFFERENTIAL EFFECTS OF ANTICONVULSANT DRUGS ON CORTICAL- AND AMYGDALA-KINDLED SEIZURES IN THE RAT.** Penny Albright* and W. M. Burnham. Dept. of Pharmacology, Univ. of Toronto, Toronto, Ontario, Canada.

Previous studies involving the kindling model have indicated that seizures kindled from the cortex and from the amygdala respond differentially to therapeutic drugs. The present study was designed to explore this difference in more detail and to determine whether the amygdala-kindled seizures might resemble human temporal lobe epilepsy in terms of their drug response. No good pharmacological model for temporal lobe seizures exists at present.

Rats with well-established seizures of the amygdala- or cortical-generalized type were therefore injected with multiple doses of the following anticonvulsant drugs: carbamazepine, clonazepam, diazepam, ethosuximide, methsuximide, phenobarbital, phenytoin, and sodium valproate. The effects of these drugs were analyzed on two components of the seizure: 1) the long generalized convulsion 2)and the partially generalized and focal seizure. With respect to the generalized component all the anticonvulsant drugs with the exception of ethosuximide suppressed this aspect of the seizure at non-toxic doses. Thus the kindling model responds appropriately to antiepileptic drugs and appears to be a viable model of grand mal epilepsy. Furthermore there were no differences between amygdala and cortical groups in the suppression of the generalized convulsion by drugs. This finding coincides with the hypothesis that the generalized seizure is similar in mechanism regardless of the location of the stimulated focus.

Analysis of the focal and partially generalized component revealed dramatic differences between amygdala and cortex. Epileptic activity in the cortical focus was easily inhibited by drugs at doses only slightly higher than those required to block the generalized convulsion. The amygdala focus was extremely resistant to anticonvulsant action however and was never fully suppressed by drugs. In addition the drugs most successful in treating human temporal lobe epilepsy were also most effective at suppressing amygdala-kindled seizures in the rat. These results suggest that the amygdala-kindled seizure may provide a useful pharmacological model for temporal lobe epilepsy.

617 PROCAINE-INDUCED SEIZURES IN MONKEYS WITH BILATERAL HIPPOCAMPAL ALUMINA FOCI. Thomas L. Babb, Kent M. Perryman*, Jeffrey P. Lieb, David M. Finch and Paul H. Crandall. Depts. Neurology, Neurosurgery and Brain Research Institute, UCLA, Los Angeles, CA. 90024.

Intravenous injections of local anesthetics at low doses have been shown to depress central nervous system functions (Livingston and Perrin, 1972; Julien, 1973; Demetrescu and Julien, 1974) and has been recommended for treatment of status epilepticus (Bernhard and Bohm, 1955; French et al., 1957). However, at higher doses a variety of cocaine derivatives precipitate seizures in animals (Wagman et al., 1967; Julien, 1973; Munson et al., 1969) and man (de Jong and Walts, 1966). This paradoxical activation of central synapses has been attributed to a selective conduction block of inhibitory fibers (Warnick et al., 1971). Furthermore, it has been suggested that anesthetics such as lidocaine and procaine preferentially activate the limbic system before affecting the neocortex (de Jong and Walts, 1966; Wagman et al., 1967; Racine et al., 1975). For these reasons, de Jong (1970) and Livingston (1978) have proposed that lidocaine and procaine may induce typical auras and electrographic seizures in patients with subcortical limbic epilepsy while preventing secondarily-generalized seizures, which are a common threat when using seizure-provoking drugs such as pentylenetetrazol.

Intravenous procaine HCl given at low doses (0.5-2.5 mg/kg) to two monkeys with bilateral alumina hippocampal foci depressed interictal spiking or had little effect. At 5.0 mg/kg unilateral limbic activation occurred. At 10.0 mg/kg unilateral or bilateral limbic activation and generalized seizures could be evoked within 3-10 minutes. At higher doses (15 and 20 mg/kg) bilateral limbic activation or brief (one minute) generalized seizures occurred. The unilateral-onset psychomotor seizures were not identical to spontaneous psychomotor seizures, and the generalized seizures never occurred spontaneously in these monkeys. However, these results do indicate that procaine challenges may selectively activate limbic epileptogenic areas without activation of debilitating generalized tonic-clonic seizures.

618 ANTAGONISM OF GAMMA-HYDROXYBUTYRIC ACID INDUCED FREQUENCY SHIFTS IN THE CORTICAL EEG OF RATS BY DIPROPYLACETATE. Larry J. Bearden and O.C. Snead, III. Neurosciences Program and Dept. Pediatrics, Univ. Ala. in Birmingham, Birmingham, Ala. 35294.

Gamma-hydroxybutyric acid (GHB) is an endogenous neural constituent, of undefined significance. This compound has been shown to produce EEG and behavioral alterations in animals which closely resemble the changes observed in human petit mal epilepsy. Moreover, previous studies have also demonstrated that the neurophysiologic effects of GHB are alleviated by anticonvulsants which have shown clinical success as specific anti-petit mal agents (Godschalk, et al., Neurosci. Lett. 3:145, 1976; Snead, Neurology 28:1173, 1978). The present experiments indicate that within two minutes after the intraperitoneal injection of gamma butyrolactone (i.e. the lactone of GHB; 4 mmole/kg), intermittent bursts of hypersynchronous waves (300-500 μV, 3-6 Hz) coinciding with a behavioral depression are observed. This pattern is quickly followed by a continuous hypersynchrony, during which rats are flaccid and immobile. Similar electrical and behavioral changes, but with a more gradual onset, were observed when an equimolar dose of GHB (4 mmole/kg) was given as the acid salt. The hypersynchrony produced by GHB could be prevented or aborted by dipropylacetate (300 mg/kg, i.p.), a petit mal anticonvulsant. However, when given at the end of the GHB induced hypersynchronous phase, dipropylacetate did not reverse the effects of GHB. These findings corroborate previous observations, and provide additional quantitative evidence characterizing the neurophysiologic effects of GHB and petit mal anticonvulsants. Both the development of the GHB induced hypersynchrony, and the antagonism of the hypersynchrony by dipropylacetate were clearly observable by means of frequency analysis of the ECoG. The frequency analysis technique employed offers the advantages of simplicity and flexibility for studies of this type.

619 CHRONIC IRON-INDUCED EPILEPTOGENIC FOCI: AN ULTRASTRUCTURAL STUDY IN RATS. A. B. Butler, L. J. Willmore, and G. W. Sypert. Dept. of Neurosurgery, University of Virginia, Charlottesville, VA 22908, and VA Hospital and Dept. of Neurosurgery and Neuroscience, University of Florida College of Medicine, Gainesville, FL 32610.

Chronic focal epilepsy can be produced in rat sensorimotor cortex by subpial injections of 5 μl of 100 mM $FeCl_3$ (Wilmore et al., Science 200: 1501, 1978). Five weeks after subpial injections of ferric chloride in 10 rats (Sprague Dawley, 200-300 gm) transcardial perfusion fixation with aldehydes was performed, after which samples of sensorimotor cortex including the injection site and surrounding brain were prepared for electron microscopy using standard techniques. Light microscopic examination of plastic embedded thick (1-2 μ) sections of these tissues reveal a cavitary lesion (zone I) extending inferiorly from the subpial layer to include the majority of all cortical laminae. The neuropil immediately adjacent to the cavitary lesion (zones II,III) contains numerous swollen cell processes admixed with an increased number of glial cells and macrophages. Examining more peripherally (zone IV), the cortical neuropil gradually assumes the structural characteristics of normal cortex. Electron microscopic examination of the cortical neuropil immediately adjacent to the cavitary lesion reveals a narrow band of altered tissue (zone II) in which most cells and processes are either severly swollen or totally disrupted. Cell organelles are frequently present within the extracellular space. In the adjacent peripheral zone of tissue (zone III), the major cytopathologic alterations of cortical elements are confined to astrocytes and dendrites. These changes are characterized by dispersion of cytoplasmic organelles and a markedly electron-lucent cytoplasm. Dendrites exhibit increased intermicrotubular spacing, dilitations of smooth endoplasmic reticulum, and periodic increased swelling of the dendrite shaft (varicose swelling). Neuronal perikarya are relative less swollen whereas astrocytes exhibit a pale watery cytoplasm containing extensive bundles of glial filaments. Pleomorphic, electron dense inclusion bodies are numerous and are contained within astrocytes, macrophages and neuronal perikarya. Swelling of synaptic terminals in this tissue zone is uncommon. No alteration of myelinated or unmyelinated axons has been demonstrated. Within zone IV, a gradual transition in cytopathologic alteration is noted characterized by gradual diminutions of cytoplasmic swelling in astrocytes and dendrites associated with a decreasing number of pleomorphic electron dense inclusion bodies as more peripheral areas within this zone are examined.
(Supported by VA Hospital Medical Research Service).

620 RELATIVE ANALGESIC AND EPILEPTIC POTENCIES OF VARIOUS OPIATE DRUGS. J. T. Cannon, R. L. Nahin*, S. M. Ryan, A. S. Moskowitz, and J. C. Liebeskind. Dept. Psychol., UCLA, Los Angeles, CA 90024.

The discovery that the enkephalins, β-endorphin and morphine cause a characteristic pattern of epileptiform EEG seizures after intracerebroventricular (ICV) injection in rats has generated considerable interest in the possible role of endogenous opioids in certain seizure phenomena. That naloxone can block such seizures suggests that opiate receptor mechanisms are involved in their expression. The following experiments sought to provide additional evidence on this point.

Rats were chronically prepared with skull screw EEG electrodes and lateral ventricle guide cannulas. ICV injections (10 µl) of levorphanol (LEV) and dextrorphan (DEX) tartrate failed to cause seizures at any dose between 58-300 µg. All doses of LEV and 100-300 µg DEX produced naloxone reversible EEG synchrony. LEV and DEX caused tail-flick analgesia at 100-300 µg. LEV analgesia was more robust, longer lasting and naloxone reversible. Naloxone (164 µg, ICV) also caused brief analgesia. For some rats, 200-300 µg LEV proved lethal.

In preliminary work, ICV injections of 3 benzomorphans (cyclazocine, ketocyclazocine and WIN-35, 197-2) also failed to cause seizures throughout a dose range in which EEG synchrony, analgesia or death could all be observed. These drugs have been shown by others to be potent agonists for σ and/or κ opiate receptors. On the other hand, we have recently found that ICV levorphanol (100 µg) can cause seizures of the same sort previously seen with opioid peptide or morphine injections if sufficient potassium sulfate is added to produce an equimolar sulfate concentration to that present in a 100 µg dose of morphine sulfate. This preliminary finding is consistent with recent evidence of the opiate-like actions of sulfate ions (LaBella et al., 1979).

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621 INTRACORTICAL EVOKED RESPONSES RECORDED FROM PERI-FOCUS ELECTRODE SITES FOLLOWING PENICILLIN-INDUCED EPILEPTIC DISCHARGES IN CAT VISUAL CORTEX. A.B. Chatt and J.S. Ebersole. Neurology Service, V.A. Medical Center, West Haven, CT 06516 and Dept. of Neurology, Yale University School of Medicine, New Haven, CT 06510.

NaCl-filled microelectrodes were positioned in area 17 of the visual cortex of paralyzed, anesthetized cats at varying distances from a Na-penicillin filled microelectrode through which epileptic foci were induced by pressure-injection. In any one animal, only one pair of electrodes was positioned (a focus and peri-focus, or surround, electrode) and the distances between these electrodes was different for each animal. Recordings were taken from these electrodes to ON-OFF stimuli delivered to the preferred (center) receptive field positions of both electrodes before and after the microinjection of Na-penicillin.

Following drug injection at the focus electrode, variations in the normal visual evoked response (VER) recorded from the peri-focus electrodes were noted from those surround electrodes positioned nearest to the focus electrode (2 and 4 mm). No evidence of any influence from the epileptic focus was seen at the most distal peri-focus electrode position (7 mm). These alterations in VERs associated with the development of an epileptic discharge at the focus electrode, and recorded at the peri-focus electrode, were also dependent upon the receptive field (RF) site stimulated. Stimulation of the focus electrode RF center produced a secondary component (60-150 ms) at the peri-focus electrode simultaneous with the development of an epileptic discharge at the focus electrode with no change in the primary latency component (40-50 ms) at the peri-focus electrode, if one existed. Stimulation of the surround electrode RF center produced the following alterations at the peri-focus site in succession: 1) an enhanced secondary component as the epileptic focus developed at the electrode, followed by 2) an enhanced primary response.

Though it is generally agreed that the primary component of a VER is the result of local, summated, direct thalamo-cortical influences, it is hypothesized here that the secondary components reflect projected intra-cortical activity. The enhanced secondary responses, then, recorded at the peri-focus electrodes (always in synchrony with the occurrence of the epileptic focus at the focus electrode) may represent projected intracortical input from the cell population in the area of the focus electrode. The enhanced primary component recorded from the most proximal surround electrodes during peri-focus center stimulation probably reflect the actual physical spread of the convulsant drug to these recording sites. (Supported by the Veterans Administration)

622 ROLE OF FOREBRAIN CATECHOLAMINES IN AMYGDALOID KINDLING. Michael E. Corcoran and Stephen T. Mason. Dept. of Psychology, Univ. of Victoria, Victoria, B.C., and Div. Neurol. Sci., Univ. of British Columbia, Vancouver, B.C., Canada.

Previous evidence has suggested that central catecholamines play some role in kindling, a permanent increase in susceptibility to seizures after intermittent electrical stimulation of the brain. Specifically, it has been reported that amygdaloid kindling was facilitated by intraventricular injections of 6-hydroxydopamine (6-OHDA) that produced marked depletion of noradrenaline (NA) and dopamine (DA) throughout the neuraxis. The relative contribution of NA and DA to this effect is unclear, however, as is the anatomical locus of the critical amine. We therefore examined the effects on amygdaloid kindling of selective depletion of NA and separately of DA by bilateral intracerebral injections of 6-OHDA into the ascending axons of these neurons.

NA-depleted rats received bilateral injections of 6-OHDA into the ascending NA axon bundles; controls received vehicle injections. DA-depleted rats were pretreated with desipramine (DMI) and received bilateral injections of 6-OHDA into the nigrostriatal bundle; controls received DMI and vehicle injections. Electrodes were implanted bilaterally into the amygdala one week later, and kindling began two weeks later. The threshold intensity of stimulation for local amygdaloid afterdischarge was first determined, and all rats were then kindled at a fixed suprathreshold intensity of stimulation.

There were no differences in the thresholds for amygdaloid afterdischarge in NA-depleted, DA-depleted, or control rats. The rate of amygdaloid kindling was significantly enhanced in NA-depleted rats, however, but there was no difference from controls in DA-depleted rats. When stimulation was subsequently applied to the contralateral (secondary-site) amygdala all rats showed a facilitation of kindling rate (transfer), and once again this was significantly greater in the NA-depleted rats.

These results indicate that depletion of forebrain NA and not DA results in a marked facilitation in the rate of kindling, presumably by disinhibition of the spread of seizure discharge from the stimulated amygdala. Conceivably a lessening of seizure-suppressant NA activity in the forebrain is part of the mechanism underlying kindling.

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623 EFFECTS OF CORTICAL EPILEPTOGENIC ACTIVITY ON DEVELOPMENT OF RECEPTIVE FIELD PROPERTIES IN THE VISUAL CORTEX OF YOUNG RABBITS. John W. Crabtree*, K.L.Chow, Louis H. Ostrach, H. Dale Baumbach, and Bryann S. Bromley*, Dept. Neurol., Stanford Univ. Sch. Med., Stanford, CA 94305.

A previous study (Chow, et al., Brain Res., 146: 151, 1978) showed that normal development of receptive field properties in cells of the lateral geniculate nucleus in young rabbits was disrupted during chronic epileptogenic discharge in the visual cortex. In the present study, the effects of a chronic epileptogenic focus in one visual cortex on development of receptive field properties of visual cortical cells were examined.

In 7 to 8 day old rabbit pups a stainless steel cannula was implanted over each visual cortex so that the cannulae rested on dura which covered the monocular visual areas. Beginning on the day after surgery epileptogenic activity was induced unilaterally in each pup by the application of penicillin (200,000 µ/ml) to the dural surface through one cannula. Penicillin applications were administered twice a day for 17 to 23 consecutive days. Following each penicillin application, interictal spiking was observed within 10 minutes on the EEG monitor and lasted for 6 to 12 hours. Concurrent with the penicillin applications, a mixture of equal proportions of penicillin and penicillinase was applied through the other cannula. This second application served to control for possible physical damage to the visual cortex due to cannula implantation and the administration and presence of drugs. No cortical spiking was observed in the hemisphere which received the control drugs. Drug applications were terminated 24 hours before recording sessions. Responses of single units in the visual cortex were recorded on the rabbits' 25-31 postnatal day. This age range is after the time at which percentages of receptive field types of visual cortical cells have attained adult values in the rabbit (Mathers, et al., Exp. Brain Res., 19: 20, 1974). Interictal spike activity was not seen during recording sessions.

The percentages of receptive field types of cells in the visual cortex recorded up to 5 mm away from the cortical epileptogenic focus were clearly abnormal. Relative to adult values in the rabbit, a significant increase in the proportion of unresponsive cells and cells with indefinite receptive fields was found concurrent with a significant decrease in the proportion of cells with linearly oriented receptive fields. The cells sampled from the visual cortex contralateral to the epileptogenic focus showed normal percentages of receptive field types. (Supported by NIH grants EY 00691, EY 05176, NS 12151 and NS 07012).

624 KAINIC ACID ELICITS ELECTROGRAPHIC EPILEPTIFORM ACTIVITY AFTER CENTRAL AND PARENTERAL ADMINISTRATION TO AWAKE RATS. I.L. Crawford and W.C. Wooten*. VA Epilepsy Ctr. and Depts. Neurology and Pharmacology, Univ. Texas Hlth. Sci. Ctr. at Dallas, TX 75216.

Parallels between the neuronal excitatory actions and the neurotoxicity of kainic acid (KA) have not yet been fully delineated in concurrent experiments. We compared the histology and electrophysiologic effects of intracerebroventricular (icv) and systemic (i.p.) doses of the ascaricide. Adult male Sprague-Dawley rats (300±25 gm) were implanted with an icv cannula, and monopolar epidural electrodes were placed over the frontal (F) and parietal (P) cortex on each side. One week later, bipolar recordings were made bilaterally between F and P, and transcoronally from F to F and P to P. Low, threshold doses of KA (10-30 nanograms, icv; or 10 mg/kg, i.p.) shifted the predominant frequency to 20-30 Hz (β activity) and produced an active, alert behavioral state for 1.5 to 2 hr. A similar initial increase in β activity caused by 50 ng icv KA was followed within 4 min by spikes (<80 ms duration) and bidirectional sharp waves prominent in parietal areas. This electrical activity continued without motor convulsions, up to 2 hrs after injection then ceased. Severe electrographic seizures induced by KA (100 ng, icv; 30-50 mg/kg, i.p.) began within 2 min as spike-bursts lasting 40-80s separated by 30-50s interictal periods often associated with 'scratching' and 'wet dog shakes'. In the early stages of seizure development rats were immobile during the paroxysms, one hr later salivation, myokymia and myoclonus preceded head nodding, rearing and forelimb clonus. These ictal episodes continued to cycle and developed into prolonged (6-8 hr) electrographic seizures with occasional tonic-clonic motor convulsions. Abnormal slow wave activity (1-3 Hz) in the frontal leads and parietal spikes with afterdischarges or sharp waves persisted for 5 to 7 days after treatment; chromodacryorrhea and hematuria appeared one day after systemic injections. High doses (300 ng,icv) caused generalized seizures, status epilepticus and, without anticonvulsant treatment, death marked by tonic contracture within 1 hr. Histologically, CA3 hippocampal pyramidal cells were destroyed only in brains from rats given 100 ng or more. In addition to death of other CA pyramidal neurons, high doses (300 ng) also destroyed scattered cells in the thalamus, amygdala, and olfactory cortex. Our results provide support for KA as a potent excitatory agent, and suggest that its cytotoxic actions correspond more closely with subsequent long-lasting epileptogenicity than with acute, dose-related electrographic seizures. (Supported in part by VA Grant MRIS 1604).

625 TRIMETHADIONE AND SEIZURE SUSCEPTIBILITY IN EPILEPTIC FOWL. H.L. Davis*, D.D. Johnson and R.D. Crawford* Depts. of Pharmacology and Animal and Poultry Science, Univ. of Sask.,Saskatoon, Saskatchewan, Canada S7N 0W0.

Phenobarbital, phenytoin and primidone at plasma drug concentrations which closely parallel those required in human epilepsies will prevent seizures induced in epileptic fowl by stroboscopic stimulation. Dipropylacetic acid abolished seizures but the plasma concentrations required were approximately double those required for control of absence seizures in humans. However ethosuximide, the agent of choice in absence seizures, was without effect at plasma concentrations approximately 5 times those effective in humans. The present study was performed to further characterize epileptic fowl as a pharmacological model of human epilepsy and was designed to determine if the seizure process in epileptic fowl was sensitive to the anticonvulsant effect of trimethadione (TMO) and to determine the relationship of any observed reductions in seizure incidence to the plasma drug concentrations achieved. TMO dissolved in DMSO was administered to groups of 10 epileptic chickens in doses of 100, 200 & 300 mg/kg in a cross-over design. Chickens in the control groups received DMSO. All chickens were tested for seizure susceptibility in response to stroboscopic stimulation 1,3,6,8,12,24 & 48 h after TMO administration. Blood samples were obtained prior to each determination of seizure susceptibility for determination of drug concentrations by GLC. TMO produced significant reductions in the incidence of seizures 1,3,5 & 8 h following 200 mg/kg and for up to 48 h following the 300 mg/kg dose. As in other species TMO is metabolized to dimethadione (DMO), an active metabolite. Significant reductions in the incidence of seizures occurred when the total plasma drug concentration (TMO & DMO) exceeded 210 µg/mL. The maximum reduction in seizure incidence, without overt manifestations of acute neurological toxicities, was obtained at mean plasma drug concentrations of 442 µg/ml. DMO alone (200 mg/kg) produced a significant reduction in the incidence of seizures when the DMO plasma concentration exceeded 300 µg/ml. The data indicates that epileptic fowl are sensitive to TMO and DMO, and that plasma concentrations required to control seizures are somewhat lower than those required in absence seizures in humans. The finding that TMO has anticonvulsant activity in epileptic fowl indicates that epileptic fowl do not represent a specific pharmacological model of grand mal epilepsy.

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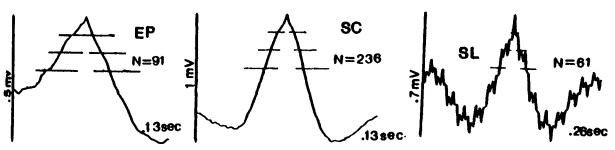
626 COMPUTER RECOGNITION OF EEG SPIKES FROM NORMAL, SCHIZOPHRENIC, AND EPILEPTIC SUBJECTS. David F. Dean, M.D., Herbert E. Longenecker, Jr., Ph.D., Valery Koss, Ph.D., Chi-Ming Huang, Ph.D. Departments of Neurological Surgery, Pharmacology, and Physiology, College of Medicine, University of South Alabama, Mobile, Alabama.

Reported methods for spike analysis in EEG have utilized Fourier analysis or correllation analysis. Both of these methods require complex instrumentation and large amounts of computer time. Neurologists examining epileptic EEG's will notice abnormality but obtaining a consensus from multiple encephalographers regarding all 'epileptic spikes' in the record is difficult. Computer alogrithms for high frequency analysis of monopolar scalp electrodes were developed which demonstrate epilepsy as well as in subjects with diagnoses of schizophrenia that were not seen in a population of normals. These waves also appear randomly in sleep in all tested subjects as well.

Monopolar electrode recordings were fed to a Grass (DC to 10 kHz) amplifier whose output was FM recorded in 17 subjects. The recorded output (DC to 3 kHz) was then fed to a Varian 73 Computer System for the analysis program. Data was sampled at 1 msec. continuously for up to 30 minutes and stored for analysis on magnetic tape. Arbitrary intervals of contiguous 330 msec. were analyzed for the occurrence of spikes.

Clustering techniques with multiple parameters demonstrated a high degree of success in identification of cortical potentials. A random spike wave activity occurred in both schizophrenics and epileptic patients. The average rate of occurence of the 'spike' potentials was 0.05 to 0.3 per second. Both interval and joint interval histograms of interspike intervals suggest a random order of potential occurrence. Muscle spikes were clearly differentiated from these potentials. The normals, however, did not show any of this degree of random spiking.

Examples of the clustering technique, alogrithms, and computer analysis will be shown. This study is part of an initial program in an on-line computer automated analysis system for inpatient continuous EEG monitoring.



627 ALTERATION OF INTRACORTICAL EVOKED POTENTIALS DURING FOCAL PENICILLIN EPILEPTOGENESIS IN CAT VISUAL CORTEX. J.S. Ebersole and A.B. Chatt. Neurology Service, Epilepsy Center, V.A. Medical Center, West Haven, CT 06516 and Dept. of Neurology, Yale Univ. School of Medicine, New Haven, CT 06510.

The interictal spike has been viewed as the basic field potential manifestation of the epileptic state. Its neuronal basis, the paroxysmal depolarization shift (PDS), is presumed to result from abnormal interactions among neurons of the epileptic aggregate. One would expect that evoked field potentials (EP), themselves a reflection of cell population activity, should show changes with epileptogenesis and perhaps help delineate its stages of development.

Potentials, recorded through micropipettes, were evoked from discrete regions of visual cortical area 17 in cats by 1° square ON-OFF stimuli before and after pressure-injection of Na-penicillin from the electrode. Stimulation at an optimal visual field location evoked a large amplitude potential of primary afferent latency (40-50 ms) with small or no secondary components. Stimuli presented progressively eccentric to the "receptive field" center (2°-10°) elicited increasingly smaller primary latency components while also recruiting longer latency secondary responses (60-150 ms). These secondary potentials appear to represent interactions of surrounding cortex with the recorded region, since the displaced stimuli evoking them preferentially activate adjacent cortical populations.

Micro-injection of Na-penicillin pathologically altered the normal visual evoked responses (VER) in a reliable and reproducible fashion. The initial alteration was an enhancement by 40-120% of its primary component to center stimuli which most likely is due to the direct effect of penicillin on neurons in the immediate vicinity. This was followed by the gradual development of a secondary component that exceeded the amplitude of the primary response and grew into a full-blown interictal spike potential. Although near complete reversal of this pathologic process followed a single injection within minutes, recurrent injections using controlled parameters could maintain a given stage of epileptic response abnormality.

During the period of penicillin action, eccentric stimuli produced altered VERs as well. Primary EP components were elicited by peripheral stimuli that were previously ineffective. The receptive field of the recorded neuronal population enlarged, as reflected by an increased range of stimulus displacement over which EP components could be elicited, particularly the pathologically enhanced secondary response. These later effects are probably a manifestation of more peripheral neurons being recruited into abnormal and enhanced interactions with the newly created epileptic focus. (Supported by Veterans Administration)

628 EXPERIMENTS ON HIPPOCAMPAL "KINDLING" IN THE RABBIT. C.L. Ehlers*, P.C. Chappus*, D.I. Whitmoyer* and C.H. Sawyer. Dept. Anat. and Brain Res. Inst., UCLA, Los Angeles, CA 90024.

The process of "kindling" is a phenomenon in which a progressive increase in neural and behavioral responsivity is produced by spaced and repeated epileptogenic stimulation of specific brain sites. In the present study we have investigated the development of kindling following electrical stimulation of the dorsal hippocampus (DHPC) of the rabbit and the ability of various drugs to modify kindled electroencephalographic and behavioral responses. Five New Zealand White rabbits were stereotaxically implanted with concentric bipolar electrodes aimed at two sites in the DHPC and one in the amygdala (AMY), as well as screws placed in the calvaria over frontal and limbic cortices (CTX). The kindling stimulus (1 msec biphasic square wave pulses, 60 Hz frequency, 200 μA current, 1 sec train) was applied once daily until 3 fully-kindled convulsions (chewing, rearing, falling) were noted. The initial stimulations produced only brief behavioral arrest and DHPC afterdischarge (AD) which did not spread to CTX or AMY. However, with repeated stimulations a sequela of "psychomotor" behaviors emerged which included sniffing, darting of the head from side to side, facial twitching, wet dog shakes, posturing, grooming of the face and violent hindlimb thumping associated with urination ($\bar{X}$ = 11.6 days). During this time ($\bar{X}$ = 10.4 days) independent interictal discharges in DHPC appeared which were enhanced during rest and slow-wave sleep, and were blocked during attention. With repeated stimulation AD was seen to spread to AMY and frontal CTX; following this the animals began to show typical rhythmic chewing characteristic of AMY kindling ($\bar{X}$ = 24.6 days). Animals showed fully-kindled responses soon thereafter ($\bar{X}$ = 29.0 days), although they remained "partial" in nature, i.e., the chewing and rearing were ipsiversive to the focus and animals would show brief falling and usually only contralateral forelimb clonus. First-trial stimulation of AMY or another DHPC electrode 2 or 4 mm from the focus in fully-kindled animals produced a similar seizure; however, the order of presentation of behaviors appeared different. Single doses of phenobarbital (30 mg/kg IP), carbamazepine (20 mg/kg PO) and Diazepam (5 mg/kg IP) all attenuated or blocked fully-kindled responses, although some of the "psychomotor" behaviors remained. Chlorpromazine (10 mg/kg IM) had no effect on fully-kindled responses, although it did block some of the "psychomotor" behaviors. None of the drugs blocked independent interictal discharges in DHPC. (Supported by NIH, the Ford Foundation and the Gianinni Foundation.)

629 ON THE SITE OF CONVULSANT-INDUCED ENHANCEMENT OF SENSORY RESPONSES OF RETICULAR NEURONS: IONTOPHORETIC STUDIES. C.L. Faingold, W.E. Hoffmann* and D.M. Caspary. Divisions of Pharmacology and Neurobiology, Dept. Medical Sciences, Southern Illinois University School of Medicine, Springfield, IL 62708

Our previous studies indicate that the responses of reticular formation (RF) neurons to sensory stimuli are greatly enhanced by subconvulsant doses of three very different convulsants. This phenomenon was suggested to be involved in the action of the 17 different convulsants which enhance sensory evoked field potentials (Faingold, Prog. Neuro-Psychopharmacology, 2: 401-422, 1978). Possible brain sites at which convulsants could act to produce RF response changes include 1) peripheral sensory receptors, 2) primary sensory neurons, 3) RF neurons, and/or 4) descending pathways which modulate RF activity. These studies utilized locally anesthetized paralyzed cats, or chloralose anesthetized white rats, and neuronal responses were evaluated using poststimulus time histogram analysis. Field potentials and single unit responses in the RF evoked by electrical stimuli in cochlear nucleus or lateral geniculate nucleus were greatly enhanced after administration of subconvulsant doses of pentylenetetrazol (PTZ). This suggests that the convulsant does not produce enhancement by acting predominantly on sensory receptors. PTZ administration results in only a modest increase in responsiveness of primary visual neurons in the lateral geniculate nucleus (mean 38%) (Faingold and Stittsworth, Neurosci. Abs., 3:139, 1977), while mesencephalic RF neuronal firing is increased by a mean of 310%. This suggests that effects of this convulsant on primary sensory neurons are not sufficient to explain the extent of RF response enhancement. The effects of microiontophoretic application of PTZ or strychnine directly onto mesencephalic or bulbar RF neurons were also examined. The sensory responses of RF neurons were enhanced extensively after iontophoretic application of strychnine (40-380 nA) or PTZ (65-190 nA). Following application of strychnine, the enhanced responsiveness often lasted for up to 30 min. However, the prominent and repetitive spike bursting pattern observed with systemic administration of strychnine (Faingold and Stittsworth, Soc. Neurosci. Abs., 4:142, 1978) was not observed with iontophoresis. In some cases the enhancement of RF neuronal firing by convulsant could be reversed by iontophoresis of the inhibitory amino acids glycine and GABA. Since iontophoresis of convulsants produces RF response enhancement, a major portion of the effect of systemic convulsant on RF neuronal responses may be exerted directly on the reticular formation. These data further support the theory that actions of convulsants on the reticular formation are important to the development of drug-induced generalized seizures. (Supported in part by the S.I.U. Foundation.)

630 REDUCTION OF THE EPILEPTOGENIC EFFECT OF INTRASTRIATALLY INJECTED KAINIC ACID (KA) BY TAURINE (TAU). Ruggero G. Fariello, Gregory T. Golden, Kathleen M. Kantak, James A. Black*. Dept. Neur. and Psych., University Wisconsin and Waisman Center, Madison, WI 53792

It has been suggested that symptoms of Huntington's Disease (HD) may partially result from the excitotoxic action of glutamate which is poorly counterbalanced by hypofunctioning inhibitory systems. Intrastriatal injection of KA offers at the present time the closest biochemical, and possibly pathogenetic model of HD. Seizures occurring in the first 5 hrs after intracaudate administration of KA are a behavioral index of the excitotoxic action of KA. Conceivably agents capable of reducing such excitotoxic effect may be candidates for the palliative treatment of HD. Taurine is a naturally occurring inhibitory amnioacid (AA), 3 aminopropane sulfonic acid (3APS) is a powerful gabamimetic agent that systemically injected can block focal epileptiform discharges (Fariello, Exp Neur in press). In the present study we have tested the effects of these inhibitory AA on the behavioral changes and the seizures induced by bilateral intracaudate injection of KA. Two groups of rats were pretreated for four days with IP 3APS or Taurine (500 mg/Kg/day). These two groups plus a third non pretreated group were then injected with 1 ul of 4.7 nM KA in both caudate nuclei. Behavior and seizures were then continuously monitored in all animals for 6 hrs. At 26 and 48 hrs after injections animals were sacrificed and their brains histologically examined. The mean number of seizures during the 5 hrs post injection are reported below for each group.

Control KA	94
3APS	73
TAU	36

In the taurine group seizures were also of less severe intensity and shorter duration. Hyperactivity, excessive grooming, jumping and wet dog shaking behavior were maximally prominent in the 3 APS group, followed by KA and only moderatly present in taurine rats. Taurine seems to offer significant protection against the epileptogenic and hyperactivity inducing effect of KA. The results are further discussed in light of the histological findings in the various groups at different times after KA administration.

631 NEOCORTICAL EPILEPTOGENESIS IS NOT REDUCED BY TRANSVERSE CORTICAL LESIONS. John H. Ferguson * and Howard Williams. Div. of Neurology, Case Western Reserve U. Sch. of Med. and Cleveland VA Hospital, Cleveland, Ohio 44106.

To test the hypothesis that transverse cortical lesions (TCL) would raise afterdischarge threshold (ADT), ADT and duration (ADD) were measured in suprasylvian gyrus (SS) acutely in 35 cats. ADT and ADD were determined in each half of each SS (4 quadrants) in 19 controls and 16 cats with various TCL made 0-48 days before testing. We had found no differences in ADT among control and TCL animals using surface stimulation (Williams et al, Neurosci Abstr 3:148, 1977) but found ADT was lower in controls using intracortical stimulation (0.13±.006 ma vs 0.63±.03 ma for surface stimulation). We repeated the TCL studies with intracortical stimulation.

The results are: 1. In cats with 4 parallel TCLs in one SS quadrant, ADT was significantly elevated (0.43±.05 ma) compared to the non-lesioned quadrants (0.17±.02 ma, $p < .001$). 2. In cats with an additional 4 TCLs (8 TCLs) in an adjacent quadrant, and in animals with 4 TCLs forming a box in one quadrant, a slight ADT elevation in the lesioned quadrants was not significant compared to non-lesioned quadrants but both were significantly elevated compared to controls, (0.38±.05, 0.19±.03 ma vs 0.13±.006 ma). 3. ADD as in previous experiments with surface stimulation, was elevated in lesioned quadrants compared to non-lesioned . Grouping all lesioned animals, it was significantly elevated in lesioned (13.8±5.23 sec) and non-lesioned (10.6±3.3 sec) quadrants in lesioned animals compared to control (4.5±0.4 sec). 4. The results occur immediately after the lesions (sham lesioned cats show no change) and are not time dependent within the 48 days.

Sectioning horizontal cortico-cortical connections affects epileptiform activity even in non-lesioned cortical areas. Although some support for the hypothesis is gained, epileptogenesis may be enhanced because of these lesions as shown by prolonged duration of afterdischarge.

632 ULTRASTRUCTURE OF THE MOSSY FIBER ENDINGS IN THE HIPPOCAMPUS OF THE CONVULSIVE MONGOLIAN GERBIL. I. Fried*, L. Paul*, K. Watanabe*and A. B. Scheibel (SPON: A. B. Harris). Dept. of Psychology, Neurology, Psychiatry and Anatomy, UCLA, Los Angeles, CA 90024.

The Mongolian gerbil (Meriones unguiculatus) has been proposed as a model for the epilepsies (Loskota et al., Epilepsia 15:109, 1974) and strains of seizure-sensitive and seizure-resistant gerbils have been established by several laboratories. Previous research has indicated structural differences between the two strains in CA3 zone of the hippocampus (Paul et al., Neurosci. Abs. 4:145, 1978). The main differences, as observed with the light microscope using Golgi techniques, were in the distribution of spines on CA3 pyramidal cells and in the shape and size of the mossy tufts of dentate granule axons. These changes have been now further studied with the electron microscope and several observations seem worthy of description. 1) Synaptic vesicles in mossy sacs in seizure-sensitive gerbils appear to be densely and homogeneously distributed throughout the tuft rather than clustered near a synaptic thickening as observed in seizure-resistant gerbils. We wonder whether the vesicle-crowded tuft in the seizure-prone gerbil may not reflect the disordered ictal state. 2) The seizure-sensitive gerbils exhibited evidence of ongoing degenerative changes in pre- and post-synaptic elements in CA3 and CA4 zones of the hippocampus. These changes appeared as electron dense profiles with most of the contained membranous components degenerated.

In view of similar degenerative changes observed in previous Golgi and electron microscope studies in temporal lobe epilepsy in man (Brown, UCLA Forum in Medical Sciences, 17:339, 1973; Scheibel et al., Epilepsia 15:55, 1974) further studies are underway to compare the structural changes in the latter disorder with similar changes in the convulsive gerbil.

633 DIPHENYLHYDANTOIN: INTERACTION WITH [^{3}H]DIAZEPAM BINDING SITE IN BRAIN AND ANTICONVULSANT ACTIVITY. Dorothy W. Gallager, Pierre Mallorga*, and John F. Tallman. Biological Psychiatry Branch, NIMH, Bethesda, MD 20205

Using extracellular recording and microiontophoretic techniques, the anticonvulsant agent diphenylhydantoin (DPH) was found to increase the efficacy of benzodiazepines when tested on dorsal raphe neurons in the anesthetized rat. This increased biological effect could be correlated with an enhanced specific binding of [^{3}H]diazepam ([^{3}H]DZ) in brain following pretreatment of rats with DPH. Enhanced [^{3}H]DZ binding could be demonstrated in an in vitro assay using cortical membranes from animals pretreated with DPH in vivo. In addition, using an in vivo labeling technique, DPH pretreatment significantly enhanced the amount of specifically bound [^{3}H]DZ in brain. Increased binding (of approximately 15% when measured at 0.25 nM [^{3}H]DZ) following treatment of rats with DPH is due to an increase in the affinity of the brain-specific benzodiazepine receptor for its ligand. However, this enhanced binding does not appear to be a direct effect of DPH on [^{3}H]DZ binding or due to an increase in brain GABA levels.

Increases in [^{3}H]DZ binding were found to be dose-dependent following anticonvulsant doses of DPH, while not significantly altered by subconvulsant doses of DPH. In developing rats, effects on brain-specific [^{3}H]DZ binding were found to parallel the time course for the development of the anticonvulsant activity of DPH. In rats younger than 3 weeks, treatment with DPH resulted in non-significant decreases in [^{3}H]DZ binding, while in rats 3 weeks of age and older, significant increases in [^{3}H]DZ binding were seen as compared to vehicle-injected littermates.

Since DPH, the benzodiazepines and several other agents which cause alterations in benzodiazepine binding site affinity are active as anticonvulsants, the data suggests that the benzodiazepine binding site in brain may be involved in some of the anticonvulsant properties of these drugs.

634 GLIAL DEPOLARIZATION AND EXTRACELLULAR POTASSIUM CHANGES EVOKED BY DIRECT CORTICAL STIMULATION AND AFTER DISCHARGE. Robert S. Greenwood*, Michiaki Takato*, and Sidney Goldring. Dept. Neurol. and Neurol. Surg., Washington U. Sch. Med., St. Louis, Mo. 63110.

Although invertebrate glial cells appear to respond to changes in extracellular potassium concentration, $(k^+)_o$, in a manner predicted by the Nernst equation, variable results have been obtained in studies of the response of mammalian glial cells to $(k^+)_o$ changes (Somjen, 1975; Futamachi and Pedley, 1976). In feline neocortex we simultaneously recorded intraglial potentials and adjacent $(k^+)_o$ changes induced by direct cortical stimulation (DCS). A series of stimulus trains, each train of greater intensity than the previous trains, was administered until after discharge (AD) was produced. We found that at the height of glial depolarization following DCS, the slope of the relationship between glial depolarization and $(k^+)_o$ changes was 52.9 mV membrane potential change for a 10 fold change in $(k^+)_o$. During AD, however, the relationship between glial depolarization and $(k^+)_o$, changed significantly, AD associated glial depolarization larger than that occurring during DCS was seen in only 3/14 instances despite $(k^+)_o$ rises which invariably exceeded the levels reached during DCS. These observations suggest that glial depolarization is related to $(k^+)_o$ in a manner approximating that predicted by the Nernst equation when measurements are made just after the termination of DCS, however during AD the relationship between glial depolarization and $(k^+)_o$ changes. Factors accounting for this change in relationship will be discussed.

635 DISTINCTION BETWEEN NORMAL AND EPILEPTIC RHYTHMS IN RODENT SENSORIMOTOR CORTEX. E.J. Hammond*, H.J. Villarreal*, and B.J. Wilder Medical Res. and Neurology Service, VA Med Ctr, Gainesville, FL 32602.

The rat is extensively used in experimental models of epilepsy, and several reports describe the appearance of induced electrographic seizures in rat cortex. We have recorded bursts of epileptiform activity over sensorimotor cortex in chronically-implanted rats and guinea pigs, in both brain-lesioned and unlesioned animals. In monopolar recordings these bursts consist of a negative wave followed by a higher amplitude negative spike at a frequency of 8-12 hz. Bipolar recordings produce quite dramatic spike activity. These bursts closely resemble those which are often reported as being electrophysiological correlates of epilepsy. These waves appear in fusiform "spindles" over sensorimotor cortex. They are concomitant with behavioral inactivity. They are accentuated by low doses of sodium pentobarbital and are abruptly terminated by a loud acoustical stimulus, and thus appear to be related to arousal mechanisms. These waves in unanesthetized, unlesioned animals appear similar in wave form to the artificially-induced recruiting and augmenting responses. Although thalamocortical valleys undoubtedly play a role in the genesis of epileptic brain rhythms, these particular epileptiform spindles do not appear to be a reflection of epilepsy. A comparison was made between these bursts and epileptic spiking arising from cobalt foci in the same animals. Although spindle waves and epileptic spikes look somewhat similar in waveform, the latter are aperiodic, particularly when unaccompanied by behavioral manifestations and do not present the 8-12 hz. fusiform burst configuration. Higher amplitude spikes from foci in sensorimotor cortex are synchronous with clonic limb movements.

636 THE USE OF HOMOCYSTEINE-INDUCED SEIZURES IN THE STUDY OF EXPERIMENTAL EPILEPSY. R.W. Hurd*, E.J. Hammond*, F.J. Thompson, and B.J. Wilder (SPON: A.J. Dunn). Dept. of Neuroscience, Univ. of Fla., and Neurology Service, VA Hospital, Gainesville, FL 32610.

Although the occurrence of homocysteine-induced behavioral seizures in animals has been reported, the use of this agent in the study of experimental epilepsy has not been fully exploited. We believe that this model has theoretical and practical advantages over models which rely on introduction of foreign substances into the brain, and systemic injections of substances with dubious clinical relevance. Homocysteine is structurally-related to methionine sulphoximine (MSO), a commonly studied convulsant, but unlike MSO, homocysteine occurs endogenously as a metabolite of methionine, and some patients with elevated plasma levels of homocysteine have been reported to have seizures.

With i.p. injections of homocysteine, dose-related electrographic and behavioral seizures are observed. The seizures are recurrent and semi-chronic. Doses of 1-2 mMoles/kg activate latent cobalt foci in rats. Focal spiking in the electrocorticogram (ECoG) is observed. At doses of 3 mMoles/kg activation of limb musculature is observed in synchrony with the ECoG spiking. At higher doses, electrographic and behavioral seizures are also seen in unlesioned animals. Doses of 5.5 mMoles/kg (ED_{50}) cause massive tonic-clonic seizures (latency 20-30 minutes) characterized by generalized high amplitude spiking in synchrony with clonic limb movements. The seizures recur within 30 minutes.

We have further studied a possible mechanism of these seizures in mice. A major pathway for homocysteine metabolism occurs via the methionine synthetase reaction which requires N^5-methyl tetrahydrofolic acid (N^5MTHFA) and vitamin B_{12}.

Pretreatment of animals with N^5MTHFA (.3 mg/kg), tetrahydrofolic acid (4 mg/kg) or methotrexate (4 mg/kg) decreased the latency to homocysteine-induced seizures ($p < .05$). Chronic pretreatment with vitamin B_{12} (5 µg/kg per day) increased the latency and decreased the severity of seizures.

These results have a clinical correlate, as previous reports have indicated a decrease in severity and number of seizures when epileptics were administered a combination of folate plus B_{12} rather than folate alone in treating the hematological complications of anticonvulsant therapy. Prophylaxis was also observed following pretreatment with therapeutic doses of the anticonvulsant drug phenobartital ($p < .01$); the anti-petit mal drug ethosuximide had no effect on seizure latency or duration. This paradigm therefore might serve as a useful animal model for assessing the efficacy of anticonvulsant drugs used in the treatment of convulsive seizures.

637 POSTWEANING HOUSING CONDITION AFFECTS SEIZURE ACTIVITY IN THE MONGOLIAN GERBIL. Harriett Kaplan. Inst. Bas. Res. in Ment. Retard. Staten Is., N.Y. 10314

Seizure prone Mongolian gerbils undergo epileptiform seizures when placed in a strange environment. These seizures appear to be triggered by stressful stimuli. I have previously shown that events occurring during the preweaning period can effect seizure latency and postseizure recovery time (Neuropsychologia 16: 649, 1978) as well as other seizure parameters. To determine whether postweaning manipulations would also affect subsequent seizure activity, housing condition was varied using a split litter technique. After weaning at 4 weeks of age, half of each litter was group housed. The other half was housed singly, so that these subjects could see, hear, and smell, but not touch other gerbils. Starting at 6 weeks of age, all subjects were tested for seizures, at first weekly and then biweekly, in our standard seizure test: placing the subjects into a large, empty cage for 3 minutes. Testing continued for 7 months. Results indicated that postweaning housing condition had a significant effect on seizure activity. Single-housed gerbils underwent seizures less frequently than group-housed gerbils. In addition, their seizures were less severe and of shorter duration. These rather surprising results are interpreted as indicating that housing gerbils alone may act as a mild form of stress, possibly increasing stress response threshold or affecting emotionality or arousal levels so that these gerbils can handle increased or stressful input more effectively. Further experiments are underway to determine more precisely the relationship between early experience, extra stimulation, and seizure activity in the gerbil.

638 DEPTH SPIKING RELATED TO SLEEP STATE AND SEIZURE FOCI IN PATIENTS WITH TEMPORAL LOBE EPILEPSY. Jeffrey P. Lieb, Jean P. Joseph*, Jeffrey Walker* and Paul H. Crandall. Reed Neurol. Res. Ctr. and Brain Res. Inst., Schl. Med., UCLA, Los Angeles, CA 90024.

The statistical properties of interictal EEG spike activity recorded from medial temporal lobe sites (amygdala, hippocampal gyrus and pes hippocampi) were evaluated using computer spike recognition techniques in all-night sleep records obtained from 10 patients with medically refractory TLE. For each patient, 1 all-night sleep record was chosen for analysis. Each sleep record was manually staged by 2 observers according to standard criteria. Sleep stages were classified into 1 of 4 groups: wakefulness, REM sleep, light sleep and deep sleep. Deep sleep was not observed in 1 patient and 5 patients did not demonstrate uniform periodic REM episodes due to many shifts from REM sleep to light sleep. An ongoing theta rhythm (4-8 c/s) was observed cortically in 1 or more states in 6 patients. Sleep spindles were not observed in 3 patients. Despite these abnormalities, the relative proportion of REM sleep, light sleep, and deep sleep was, on the average, close to that reported for normals.

Spike activity was observed to be most frequent during deep sleep in 7 of the 10 patients. Maximal spike rate was found to be most frequent during light sleep in the 3 remaining patients. In occasional sites, maximal spike rate was observed during waking or REM sleep.

All 10 of the patients reported in this study were considered to be suitable for temporal lobectomy as judged from the distribution of locus of seizure onset. In contrast to the results obtained in a previous study, the side with a site or sites demonstrating maximal mean spike rate during waking, light sleep, deep sleep, or REM sleep did not significantly correlate with the side chosen for lobectomy. However, the side with a site or sites demonstrating a minimal activation in mean spike rate in light sleep or deep sleep relative to waking did significantly correlate with the side chosen for lobectomy. In those patients in which spike properties yielded an apparently false lateralization with respect to side of seizure onset, decreased background activity was usually evident throughout the seizure-initiating side. Additionally, the mode of seizure initiation and propagation was found to differ in these patients. The results obtained here indicate that an analysis of sleep-induced changes in depth spike activity can be useful in improving predictions concerning the predominant side of seizure initiation but that a concurrent analysis of background activity would improve predictive power.

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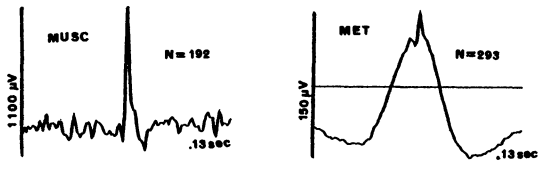
639 Computer Recognition of Spikes in the EEG of Metrazole Treated Rats using Fixed Interval Analysis and Clustering Techniques. Herbert E. Longenecker, Jr., Valery Koss, David F. Dean, and Chi-ming Huang. Departments of Pharmacology, Neurosurgery and Physiology, College of Medicine, University of South Alabama, Mobile, AL. 36688.

Classic methods for spike analysis in EEG records utilize Fourier analysis or correllation analysis. Both of these methods require either complex instrumentation or vast amounts of computer time. Even with the advent of microcomputers it does not appear feasible for recognition of spikes in an online and continuous multichannel mode with these methods. Here, we report a very simple computational technique that detects and facilitates classification and other analysis of spikes with a high degree of accuracy which can be implemented with only a minimal computer.

High frequency EEG (DC to 10KHZ) was FM recorded (DC-3kHZ) from monopolar leads from a cortical electrode. Data was presented to an A/D converter and sampled at 1 msec intervals. Contiguous 330 msec. epochs were examined, The following parameters were computed from each epoch: L, the length of the entire voltage excursion; T, the time between the min and max amplitudes; and D, the amplitude between min and max signals. Empirical observations were that clustering occured in either the L vs D plot or L vs T plot. By chosing windowed values of these parameters it was possible to detect a spike. The parameters Wn, the width the spike at n percent of the spike height were used for spike classification according to the following table:

Spike Type	D-uV	L-mV	T-msec	W86	W72	W57-msec
MUSCLE	180-650	00-30	00-65	$\leq$4.0	$\leq$9.5	$\leq$13.0
CORTICAL	180-650	00-30	00-65	$>$4.0	$>$9.5	$>$13.0

Once an epoch was found to contain a spike, the peak of the spike was aligned to mid screen and and the record could be signal averaged. The potentials below represent averaged muscle and cortical spikes as per the above selection algorithm.



10 OUABAIN CAUSES DIFFERENT MOTOR SEIZURE PATTERNS ACCORDING TO BRAIN REGIONAL SITE OF MICROINJECTION. James L. Minnich and +Theresa Page*. Dept. Physiol., Fac. Med., Univ. of Manitoba, Winnipeg, Canada R3E 0W3. +Biol. Dept., TWU, Denton, TX 76204.

Ouabain injection into brain ventricles induces reproducible motor seizure patterns (Canad. J. Biochem. 51: 198, 1973). In the present study ouabain was injected directly into selected brain regions of adult male Sprague-Dawley rats through indwelling stainless steel cannulas (26 gauge). Each rat had bilateral placement of cannulas into one brain region (N=9-11 for each region studied). Control injection of 1 µl of 0.3 M sucrose was done in the brain region on one side, and ouabain injection (10 µg in 1 µl of 0.3 M sucrose) was done on the contralateral side at 2 weeks and 3 weeks, respectively, after cannula implantation. The control injections of vehicle did not alter motor behavior. Histological verification of cannula tip location was done. The motor behavior of each animal was observed for 30 min after each intracerebral injection. Seizure activity was categorized as in a previous communication (ibid.). Analysis of variance (for repeated measures and unequal groups) was done between brain region injection sites for the occurrence of various seizure categories, and Duncan's multiple range test was applied to the mean difference obtained for each comparison between regions. The most severe seizure patterns were seen after injection of ouabain into the raphe nucleus and lateral thalamic nucleus. Less severe seizures (localized to head and neck) resulted from ouabain injection into sensorimotor cortex. The following table shows the seizure categories and the brain region where ouabain caused their highest occurrence. In every case the occurrence shown here was significantly ($P<0.05$) higher than in other brain regions.

Seizure category	Brain region with highest ouabain-induced seizure occurrence
Clonic (4 legs)	Raphe
Tonic flexion (legs)	Raphe
Clonic (hind legs only)	Lateral thalamic nucleus
Tonic flexion (body and head	Lateral thalamic nucleus
Running and leaping	Lateral thalamic nucleus
Clonic (head and face)	Sensorimotor cortex

Ouabain microinjection into the lateral septal nucleus caused vocalization. Brain regions in which ouabain caused seizure activity only at lower levels included dorsal hippocampus and caudate-putamen. Earlier work (ibid.) has shown altered serotonin levels associated with ouabain-induced seizures; the raphe region is the origin of ascending serotoninergic systems. The lateral thalamic nucleus is thought to project to the cortex in the rat, so that activation of widespread cortical areas might possibly result from ouabain stimulation of this diencephalic region.

12 PERSISTENT INCREASE IN HIPPOCAMPAL NEURONAL EXCITABILITY PRODUCED BY DAILY INJECTION OF PENTYLENETETRAZOL. A.P.Oliver, B.J.Hoffer, and R.J.Wyatt. Laboratory of Clinical Psychopharmacol., DSMHR, NIMH, Saint Elizabeths Hospital, Washington, D. C. 20032.

Repeated injection of chemical convulsants are reported to induce progressive lowering of seizure thresholds, a phenomenon known as "kindling". Epileptiform activity was studied in guinea pig hippocampal slices to examine the physiological changes which may be involved. Interictal spike (IIS) frequency was used as the primary parameter. Slices from normal guinea pigs, perfused with 7-8 mM K^+, will develop spontaneous IIS only when suitable concentrations of a chemical convulsant (i.e. 3000 U/cc penicillin) are added to the medium. In contrast, slices from animals injected daily for 5 days with 50 mg/kg pentylenetetrazol (PTZ) manifested clearcut IIS in 8 mM K^+, without addition of a convulsant drug. This increase in excitability was found for up to twelve days following the last drug injection. The rate of IIS discharge in the "kindled" animals perfused with 8 mM K^+, about 14 IIS/minute, is similar to that seen in normal animals perfused with 8 mM K^+ + 3000 U/ml penicillin. The configurations of the IIS are also similar in the two groups. The kindled animals convulsed about 80% of the time following PTZ injection. However, no spontaneous convulsions were seen during drug-free periods. Taken together, these results suggest that chronic administration of chemical convulsants induce long-term increases in neuronal excitability, which persist far beyond the time of drug exposure.

641 BRAINSTEM AUDITORY EVOKED POTENTIAL (BAEP) ALTERATIONS DURING INDUCED AND SPONTANEOUS GENERALIZED SPIKE-WAVE ACTIVITY IN ANIMALS AND HUMANS. Allan F. Mirsky, Boston University Medical Center, Boston, MA; James J. Stockard, Mayo Clinic, Rochester, MN; Barry F. Skoff*, Boston University Medical Center; & T.A. Jones, University of California (Davis).

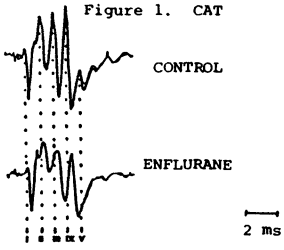
Preliminary data from animals and humans studied in our laboratories indicate that BAEPs are altered during certain types of seizures associated with generalized spike-wave activity.

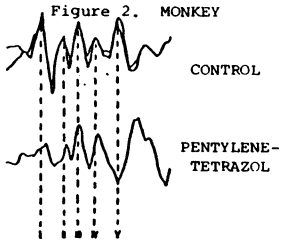
Figure 1 illustrates BAEP changes seen in the cat during administration of enflurane at a dose producing bisynchronous spike-waves. There is an increase in I-IV interpeak latency of 8 standard deviations. There was no significant change in interpeak latency with administration of equipotent doses of all non-epileptogenic anesthetics studied to date: halothane, thiopental, nitrous oxide, or isoflurane (the isomer of enflurane).

In the monkey, there is a significant increase in I-V interpeak latency, as shown in Figure 2, with administration of pentylenetetrazol in doses producing an electro-clinical model of petit mal epilepsy.

In addition to the animal data, there is evidence for BAEP alterations during spike-wave bursts in patients with "absence" seizures. For one of the patients seen (Boston), there was an increase of over 2 standard deviations in I-IV/V interpeak latency during spike-wave activity. In another patient (Rochester) induction of 3 per second spike-wave discharges with photic stimulation was associated with highly significant transitory prolongation of I-V interpeak latency that persisted for a short period after the spike-wave activity.

In summary, there appear to be alterations in functioning at the level of the rostral brainstem, as indicated by BAEP changes, during generalized spike-wave activity induced by drugs in animals, and accompanying absence seizures in humans.





643 ON STRUCTURAL SUBSTRATES OF EPILEPTIC BEHAVIOR IN THE MONGOLIAN GERBIL. L. Paul*, I. Fried*, K. Watanabe*, and A. Scheibel. (SPON: R. Schain). Departments of Physiological Psychology, Pediatric Neurology, Anatomy and Psychiatry, University of California, Los Angeles, CA 90024.

Extending work previously begun in our laboratory, we have investigated possible structural correlates of seizure behavior in the Mongolian gerbil (Meriones unguiculatus). A seizure-sensitive (SS) and a seizure-resistant (SR) strain have been selectively bred in the laboratory of Dr. R. Schain. Investigations were performed using anatomical, histochemical and lesioning techniques. Quantitative analysis of Golgi-impregnated material using improved tissue-blocking and staining methods enabled a clear visualization of hippocampal CA3 pyramidal cells. Dendritic spine counts performed on selected 45 µ segments (2 apical and 2 basilar segments per cell) in the cells and in neocortex revealed the following:

MEAN SPINE COUNTS
(Number of spines/45 µ dendritic segment)

	Hippocampal Pyramidal Cells[1]	
	SS (n=19)	SR (n=24)
Basilar	63.3	75.6 * $p<.025$
Apical	72.3	80.1 * $p<.05$
	Neocortical Pyramidal Cells	
	SS (n=14)	SR (n=14)
Basilar	71.8	76.5 N.S.
Apical	76.9	74.2 N.S.

[1]n=number of cells

The loss of spines in CA3 may indicate only that we are not observing a generalized metabolic deficiency. Results from transmission electron microscopy reported elsewhere at this meeting indicate differences between SS and SR animals in several parameters. Structural variations in mossy tuft terminals impinging on these pyramids have also been noted. We are examining the brains of developing animals, bracketing the age at which seizures first appear (about 2 months). Two other major approaches are being utilized: histofluorescence methods compare SS and SR animals, as well as brains before and after seizures in SS gerbils. Finally, based on a "deafferentation" hypothesis as explanation for the spine loss, seizure behavior in SR animals is carefully monitored following lesions in sites with heavy input into CA3. The spontaneous nature of seizure behavior in these rodents provides an interesting laboratory model of epileptic behavior. It is possible that our findings could illuminate some small portion of the human epilepsy picture.

Supported in part by BRSG grant #NW19, Neuropsychiatric Inst.

644 EVALUATION OF INTERTRIAL INTERVAL ON DEVELOPING AND DEVELOPED KINDLED SEIZURES. Steven L. Peterson*, Timothy E. Albertson and Larry G. Stark*. Dept. of Pharm., UCD, Davis, CA 95616.

The interval between each kindling stimulus and the presentation of massed stimulations has been shown to have an important effect on kindling. In this study, a grouped trial session (GTS) consisting of 5 daily stimulations, one hour apart, was used to determine the effects of a repeated massed stimulation paradigm on kindling. All rats were stimulated electrically in the right amygdala with a 60 cps, 400 μA stimulus of 1 sec. duration. Seizure intensity was determined by afterdischarge (AD) duration and behavioral response (BR) classifications characteristic of kindled seizures in rats. The effects of repeated GTS sessions on kindled rats were determined in Group I by presenting 6 consecutive days of GTS to rats previously kindled by one stimulation per day. These animals had kindled normally with consistent AD's and BR's when stimulated once daily, but exhibited reduced AD's and inconsistent BR's during the GTS days. Within 5 days of returning to daily stimulations all animals (Group I) responded with pre-GTS seizure intensity. The effects of GTS on the development of kindling were tested by stimulating the animals in Group II using only the GTS paradigm. These animals never showed the consistent AD duration or BR characteristic of rats kindled with just one stimulation per day. For comparison, Group III rats were stimulated once hourly for 32 hours during which the AD duration increased normally, but BR's were inconsistent with only 4 of 9 animals showing occasional Class 5 seizures. When reduced to daily stimulations, the group AD and BR response was greatly diminished compared to the hourly stimulation period, but all animals exhibited consistent kindled seizures within 11 days. These findings suggest that a prolonged inhibition of kindled seizures result from repeated massed stimulation and that animals kindled with repeated hourly stimulations may not share all the neurophysiological characteristics of those kindled with stimulations once a day.

645 **QUANTITATIVE EVALUATION OF PINOCYTOTIC ACTIVITY WITHIN BRAIN ENDOTHELIUM DURING EXPERIMENTAL SEIZURES.** **C.K. Petito* (SPON: D. Levy). Cornell University Medical College. New York, N.Y. 10021.**

During experimental seizures, increased vascular permeability to horseradish peroxidase (HRP) occurs via enhanced vesicular activity within the endothelial cells of small parenchymal vessels. In order to determine the relative contribution by small arteries, arterioles, and capillaries to the increased permeability, pinocytotic activity was assessed quantitatively in 6 paralyzed, ventilated rats whose blood pressure and arterial blood gases were monitored during the experiment. Intravascular HRP was allowed to circulate for 5 min and was followed by a series of 20 consecutive electroshocks (100 mamps, 1 sec duration) every 30 sec. The animals were anesthetized with ether and sacrificed by perfusion-fixation with paraformaldehyde-glutaraldehyde. A mid-coronal section through the third ventricle was incubated for peroxidase activity and prepared for light and electron microscopy. Light microscopy of the serial 1μm sections from 3 additional animals showed the presence of small arteries within each focus of HRP extravasation. Endothelial cytoplasmic area was determined by planimetry from electron micrographs taken at a constant magnification. The number of HRP-containing vesicles per 1 mm^2 endothelial cytoplasm was quantitatively evaluated. Mean pinocytotic activity within the endothelium of the different vessels was: capillaries (211), 83.71 vesicles/mm^2; arterioles (11), 159.87 vesicles/mm^2; and small arteries (37), 244.05 vesicles/mm^2. These results show that significant blood-brain barrier permeability occurs within small arteries and arterioles as well as within capillaries and that the small arteries may be the initial site of the increased vascular permeability that occurs during seizures.

646 LOCAL CEREBRAL GLUCOSE UTILIZATION IN LIDOCAINE-KINDLED SEIZURES. R. M. Post, C. Kennedy, M. Shinohara,* K. Squillace,* M. Miyaoka,* S. Suda,* D.H. Ingvar,* and L. Sokoloff. NIMH, Bethesda, MD 20205

Repeated daily injections of subconvulsant doses of lidocaine in the rat eventually evoke major motor seizures resembling those seen following repeated electrical (kindling) stimulation of the amygdala (Life Sci. 17: 943, 1975). The lidocaine seizures progressively increase in severity and duration. Animals also undergo progressive behavioral changes, becoming omniphagic and extremely aggressive following repeated lidocaine seizures. In the current study the ^{14}C-2-deoxyglucose method was used to measure local cerebral metabolic rates and to identify neural systems involved in the progressive lidocaine effects on behavior and development of seizures. Twenty-one male Sprague-Dawley rats weighing 250-300 gm. were administered lidocaine (65 mg/kg, i.p.) or saline once daily (5 times a week) for 12 to 43 days. Eleven of the 13 lidocaine-treated animals eventually exhibited 1 or more seizures (maximum = 28; median = 9) lasting several seconds to as long as 30 minutes with salivation, chewing, and perioral movements followed by intermittant clonic movements of head, trunk, and forepaws; Straub tail phenomenon; and rearing and falling. Lidocaine and saline treated animals were prepared and studied in pairs. One femoral artery and vein were catherized under light halothane anesthesia and the animals were partially immobilized in loose fitting pelvic plaster casts and allowed to recover at least 2 hours from anesthesia. The animals were then injected with lidocaine or saline i.p., and approximately 10 minutes later the measurement of local glucose consumption with ^{14}C-2-deoxyglucose was carried out, as previously described (J. Neurochem. 28: 897, 1977). Compared to chronic saline treated controls, all lidocaine-treated animals had 15%-25% lower rates of metabolic activity in all areas of brain including cortex, striatum, brainstem, and cerebellum. In animals without or with only mild seizures lidocaine treatment also reduced the metabolic rate throughout limbic structures. In animals exhibiting prolonged lidocaine seizures, however, striking increases in glucose utilization were observed in hippocampus, dentate gyrus, amygdala, septal nuclei, and entorhinal cortex. The increase in average glucose utilization over the 45 minute period of measurement correlated positively in all these limbic areas with seizure duration ($p < .05$). Inverse correlations between metabolic rate and duration of seizures were observed in several other regions, such as the thalmus and medial and lateral geniculate bodies ($p < .05$). The current studies demonstrate that limbic structures, particularly hippocampus, amygdala, septal nuclei, and entorhinal cortex, are intimately involved in the convulsive and behavioral aberrations associated with lidocaine-kindled seizures which appear to offer an experimental model for temporal lobe epilepsy.

647 ELECTROPHYSIOLOGY OF HUMAN EPILEPTIC NEURONS. D.A. Prince and R.K.S. Wong. Dept. of Neurology, Stanford Univ. Med. Sch., Stanford, CA 94305

We have recently examined data obtained from eight human cortical biopsies using the in vitro slice technique in order to determine whether abnormal patterns of neuronal activity were present in biopsies from patients with focal epileptiform discharges. A total of 28 neurons were analyzed. These cells all had stable high resting potentials with overshooting spikes and input resistances which were as high as 50 Mohms, suggesting that they were relatively "healthy." Intracellular current pulses as well as orthodromic activation were applied in each case. Six neurons from two biopsies of cortex which was normal, (i.e., remote from sites of cortical pathology or abnormal EEG epileptiform discharge), showed patterns of activity similar to those recorded in guinea pig neocortical cells. Single orthodromic stimuli evoked EPSP-IPSP sequences usually associated with single spikes. Synaptic events could be graded with the intensity of the stimulus. Six cortical biopsies were studied from areas which were known from prior EEG studies or electrocorticography to be capable of generating focal epileptiform activities. Twenty of 22 neurons from such cortex generated bursts of 3-7 spikes with orthodromic stimulation. Intracellular recordings revealed that depolarizing potentials with amplitudes of up to 25 mV or more and durations as long as 100 msec served as generators for bursts of fast spikes. Occasionally spikelets resembling fast prepotentials were intermixed within the fast spike bursts. The slow depolarizations had many of the characteristics of depolarization shifts (DSs) described in acute neocortical foci. They were often triggered at long latency (ca. 50-60 msec), and arose abruptly in an all-or-none fashion following threshold orthodromic stimuli. With increasing stimulus intensity, latency became shorter, but the DS configuration remained unchanged. In some cells DSs could be regularly evoked by low frequency stimuli (ca. .75-1 Hz) but would "alternate" when stimuli of higher frequencies (2 Hz) were used. DSs increased significantly in amplitude during hyperpolarizing current pulses and were never blocked by this maneuver. DSs and bursts could not be evoked in any neuron by direct depolarization (up to ca. 1.0 nA). These limited data suggest that human epileptic neurons generate DSs which are very similar to those in other models. In light of recent findings in guinea pig cortex and hippocampus, we would speculate that these all-or-none depolarizations are intrinsically generated. Neurons of chronic epileptogenic cortex appear to maintain abnormal features in the in vitro environment, a finding which will be important in further studies of the pathophysiology of epilepsy. (Supported by NS 06477 and NS 12151 from the NINCDS).

648 HEMOGLOBIN INDUCED EPILEPSY. Arthur D. Rosen and Natalie V. Frumin*. Dep't. Neurology, State Univ. of New York, Stony Brook, New York 11794.

Recent reports have demonstrated that both the di-and-trivalent forms of iron are potent epileptogenic agents when in contact with the cerebral cortex. The present study was carried out to test the hypothesis that ionic iron released from hemoglobin plays a role in the development of seizures following intracortical bleeding. Adult Sprague-Dawley rats were anesthetized with sodium pentabarbital and secured in a stereotaxic apparatus. A 2mm hole was drilled through the skull over the left occipital cortex and with the aid of a micro-injection syringe fitted with a 30 gauge needle, 10 ul of a 13 mg% solution of purified hemoglobin in saline injected 1.5mm below the cortical surface. The animals were permitted to recover and maintained for periods of up to two months. EEGs were recorded, using needle scalp electrodes, daily for the first week and twice weekly thereafter. All recordings were done under sodium pentabarbital anesthesia and continued until the animal showed signs of awakening. At varying intervals after hemoglobin injection animals were anesthetized and killed by transcardiac perfusion with neutral buffered formalin. Histological verification of the extent of the lesions was carried out. High amplitude focal spike activity was recorded from 24 of the 27 rats injected with hemoglobin (89%) and in none of the four animals injected with saline alone. 50% of the animals showed spikes within 48 hours. The spike focus, when present, became relatively stable within two days after it was noted and in all cases persisted until the animal was sacrificed. No clinical manifestations of seizures were seen.

649 EPILEPSY: A PREDICTIVE MODEL OF SEIZURE BASED ON THE TIME DISTRIBUTION OF EEG SPIKING. Bernard Saltzberg, Texas Research Institute of Mental Sciences and Baylor College of Medicine, Houston, Texas; Peter Kellaway, Baylor College of Medicine and Methodist Hospital, Houston, Texas; William D. Burton, Jr.*, Texas Research Institute of Mental Sciences, Houston, Texas; and James D. Frost, Jr., Baylor College of Medicine and Methodist Hospital, Houston, Texas.

A mathematical model is developed which accurately approximates the cumulative distribution of the focal EEG spike discharges occurring during a night's sleep. This model provides a quantitative as well as an intuitive phenomenological basis for understanding the relationship between focal EEG spiking and the state of the epileptic patient. The EEG spiking events in this model are considered the realization of a stochastic point process; that is, a process which is characterized by the points in time at which such spiking events occur. Using the model, it is postulated that a single descriptive parameter of the cumulative distribution of EEG spiking events provides a predictive measure of risk of convulsive seizure or a measure of the degree of control which is produced by anti-epileptic drugs. A two parameter model characterizing the distribution of spiking events is derived and regression equations are developed to fit the model to empirical data. The initial findings are based on EEG sleep recordings obtained from a population of ten patients (a total of thirty-one sleep nights). The spiking profiles which are derived by visual analysis of each of the thirty-one sleep nights were subjected to regression analysis to derive numerical values for the regression parameters. The curvature of the cumulative distribution is represented by one of the regression parameters and it is shown that this parameter alone provides a predictive measure of seizure risk.

650 EFFECTS OF VENTROBASAL THALAMIC LESIONS ON KINDLED SEIZURES AND ASSOCIATED SLEEP DISTURBANCE IN CATS. M.B. Sterman, P. Hauri* and M.N. Shouse*. Sepulveda VA Med. Ctr., Sepulveda, CA and Depts. of Anatomy and Psychiatry, UCLA, Los Angeles, CA 90024

Lesions placed in ventrobasal thalamus (VB) have been shown to reduce susceptability to seizures both in man and in various experimental animal models of seizure disorders. In previous studies we have shown that such lesions protected cats against drug-induced seizures and facilitated sleep, which is typically disturbed with the development of seizures. In the present study the effect of VB thalamic lesions was examined in relation to chronic seizures induced by amygdaloid kindling and the disturbance of sleep which also accompanies the development of seizures with this model. Two groups of cats were prepared for basolateral amygdala kindling and standard sleep-waking state evaluation. Animals in both groups were monitored polygraphically during 12-hr recordings of EEG, EOG and EMG activity before and at intervals after daily stimulation of amygdala with a one-second train of biphasic square waves (1 msec pulse duration, 60 Hz, at currents just sufficient to elicit afterdischarge). After initial afterdischarge thresholds were established animals in one group were subjected to bilateral electrolytic lesions of ventrobasal thalamus. Both groups were then studied until generalized tonic-clonic seizures were obtained with kindling stimulation or for a maximum of 75 days.

Animals in the non-lesioned group demonstrated kindled seizures within 19-25 days and a progressive decrease in both slow wave and REM sleep. In contrast, animals with VB lesions required a minimum of 61 days for kindling or showed no generalized seizures within the 75 day period of study.

Both groups of animals showed a disturbance of sleep during initial afterdischarge threshold determinations. This disruption of sleep was extended and sustained in animals without lesions during subsequent kindling but recovered completely within 30 days in animals with VB lesions. Animals in the latter group which failed to develop generalized seizures showed both remarkably stable afterdischarge thresholds and sleep state distributions.

These findings support the body of data indicating that thalamic lesions protect against seizures. Further, they suggest that conduction of abnormal discharge through sensorimotor thalamic circuits is involved in the propogation of seizure activity and the development of generalized seizure disorders of subcortical origin.

(Supported by the Veterans Administration)

651 A QUANTITATIVE STUDY OF THE REDUCTION OF INHIBITION NECESSARY TO CAUSE SPINAL SEIZURES. G.L. Tribble*, P.C. Schwindt*, W.E. Crill (SPON: H.D. Patton). Dept. Physiol. & Biophysics, & Medicine, Univ. Washington, Seattle, WA 98195.

Several recent studies using a variety of model neural systems have shown that convulsive drugs such as penicillin, pentylenetetrazol, and others reduce inhibitory postsynaptic potentials (IPSP's). These results suggest that the epileptogenic action of these agents may depend on a common mechanism, reduction of inhibition. To evaluate this mechanism in the mammalian CNS, it is essential to know how much inhibition must be reduced in order for a seizure to occur. As a first step, we have investigated the amount by which postsynaptic inhibition must be reduced by the well-known glycine antagonist, strychnine, in order for spinal seizures to occur. Experiments were conducted on cats anesthetized with pentobarbital or chlorolose, or decerebrated (unanesthetized). In each case, the spinal cord was transected at the L4 or C1 segments. In each cat, a motoneuron was recorded before and during incremental I.V. injections of strychnine. Reduction of the disynaptic group Ia IPSP or the Renshaw IPSP was observed as doses of strychnine were incremented until seizures developed. Seizure initiation was characterized by the appearance of high frequency bursts of action potentials occurring synchronously among many motoneurons in the recorded segment. Although the strychnine dose producing a seizure varied among the preparations, in all cases the IPSP's were reduced to at least 25% of their control values before the first sign of seizure activity appeared. These results suggest that if other drugs such as penicillin cause seizures in the mammalian CNS by decreasing postsynaptic inhibition, then a large, easily observable reduction in IPSP amplitude should be seen prior to seizure onset. Equivalent experiments on a GABA-mediated IPSP are in progress. Supported by VA Research Grant MRIS 1610.

652 ENDORPHIN EVIDENCE AGAINST THE SYNAPTIC BARRIER MODEL OF KINDLING. Roy A. Wise and Anita Rackham Center for Research on Drug Dependence, Psychology Department, Concordia University, Montreal, Canada H3G 1M8.

Kindling refers to the progressive lowering of local afterdischarge (AD) thresholds and the progressive development of convulsive responses to initially innocuous electrical stimulation of limbic and other structures. It has been traditionally thought to reflect the breakdown, by repeated bombardment, of synaptic barriers between the site of stimulation and the motor systems. Studies of the epileptigenic effects of intraventricular injections of β-endorphin do not fit with this model, however. Injections of β-endorphin (10 μg) caused limbic epileptiform activity in rats which was not expressed in behavioral convulsions even when given to animals that had had their presumed limbic-motor synaptic barriers broken down by prior electrophysiological kindling. This was true whether amygdala, hippocampus, caudate, or dorsomedial thalamus was kindled to the stage of full clonic convulsions. The electrophysiological AD in the kindled hippocampus had greater amplitude than the ictal episodes caused by β-endorphin in kindled animals, but the biphasic pattern of response was the same; thus the two epileptiform events seem to have had qualitatively similar limbic effects. That the difference in amplitude might account for their differential effectiveness seems unlikely since the initial ictal episodes had greater amplitude than ADs at the begining of kindling. That the amplitude of the ADs increased as a result of electrical kindling while the amplitude of the ictal episodes from β-endorphin did not indicates that there must be some difference between the two events which is more than a simple difference in amplitude. These data indicate that if synaptic barriers account for the failure of β-endorphin to cause behavioral convulsions in association with limbic ictal episodes, then the synaptic barriers involved must differ from those that block similar behavioral expression of ADs elicited by limbic electrical stimulation.

EVOKED POTENTIALS AND EEG

653 EVALUATION OF WIENER FILTERING APPLIED TO BRAINSTEM AUDITORY EVOKED POTENTIALS. J. R. Boston, C. A. Younger*, and P. J. Ainslie*. Biomedical Engineering Program, Carnegie-Mellon University, Pittsburgh, PA 15213.

The application of Doyle's version (ECN 38:533, 1975) of a Wiener filter for averaged evoked potentials to the brainstem auditory evoked potential (BAEP) was evaluated as a means of reducing the number of stimulus-response pairs necessary to obtain a consistent estimate of the BAEP. Averaged responses based on 256 individual evoked potentials were processed using the Wiener filter and a simple narrowband filter (200-1600 Hz). (This narrowband filter had previously been found to improve the identification of waves in the BAEP without distorting the waveform.) The resulting waveforms were compared to both the unfiltered averaged potential based on 256 individual potentials and an averaged potential (from the same subject) based on 2048 individual potentials. Subjectively, the Wiener filtered potentials were much more consistent from run-to-run than the narrowband filtered responses based on 256 individual potentials. The Wiener filter reduced the mean square error of the waveform, and the reduction was greater than that obtained with the narrowband filter. (The mean square error was estimated as the difference between the filtered waveform and the averaged potential based on 2048 responses.) The variability of latency and amplitude (peak-to-following trough) measures of waves I through V obtained from averaged potentials based on 256 individual responses was also reduced by the Wiener filter. Although the resulting variability was larger than the intersession variability of averaged potentials based on 2048 responses for a given subject, it was about the same as intersubject variability. The "goodness" of the spectral estimate of the evoked potential signal was observed to be an important factor in reducing the variability in the latency and amplitude data, as has been discussed in the literature. Finally, the Wiener filter appeared to severly distort the waveform in many cases; although waves I through III were usually enhanced, wave V was often significantly reduced in amplitude. This problem appeared to be due to the magnitude of the signal spectral estimate in the frequency range of 200-300 Hz., but the cause of the problem has not been identified.

654 RELIABILITY OF THE HUMAN AUDITORY EVOKED POTENTIAL AMPLITUDE-INTENSITY FUNCTION. Carl P. Browman, Helen T. Sullivan*, and Marie A. Fullerton*. Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL 60612 and St. Louis University, St. Louis, MO 63103.

This study was designed to assess the test-retest reliability of auditory evoked potential (AEP) amplitude-intensity functions. Male and female volunteers were tested on consecutive and nonconsecutive days. Averaged AEP waveforms were obtained from bipolar recordings. Ten levels of stimulus magnitude, ranging from 60 to 87 dB SL in steps of 3 dB SL, were presented at each session. AEP amplitude-intensity functions per session were computed by the method of least squares. F tests were used to compare intersession residual variances, regression coefficients, and amplitude measures.

There was no statistical difference in residual variances across sessions. Similarly, individual regression coefficients were statistically unchanged from day to day. Periodic significant differences in AEP amplitude thus indicated a shift of the entire function. The duration of the test-retest interval had minimal effect. These results demonstrate the relative reliability of AEP amplitude-intensity functions.

655 MUSCLE INPUT TO HUMAN MOTOR-SENSORY CORTEX. William F. Brown. Dept. Clin. Neur. Sci., University Hospital London, Ontario.

There is clear evidence in sub-human primates for projection of muscle length receptors to the sensori-motor cortex. This sensori-motor input may function as part of the transcortical system to adjust motor output from cortex in response to unexpected changes in muscle length or load. In the human, there is a strong body of evidence to support the presence of such a 'transcortical reflex' but muscle afferent specific inputs to sensori-motor cortex have not been identified. To investigate the latter, a method has been developed to excite muscle stretch afferents, exclude the cutaneous input and measure the ipsilateral and contralateral voltage distribution for the evoked scalp potential. Healthy subjects have been instructed to contract the first dorsal interosseous (IDI) muscle in an isometric manner at various force levels. Measurements of the IDI EMG, ulnar, median and radial nerve action potentials and the evoked potentials from the ipsilateral and contralateral scalp in response to mechanical taps delivered to stretch the IDI were made. The cutaneous input from the skin over the IDI muscle and the 1st, 2nd and 3rd digits could be removed by local anaesthetic block of the median and radial nerves. The anaesthetic block was proven complete by a combination of nerve stimulation and the absence of radial or median but preserved ulnar nerve action potentials in response to the taps. A low voltage initial surface positive potential could be detected, localized primarily to the contralateral scalp hand area. The shortest latencies to the initial positive voltage deflections were 20-22 ms and were correspondingly shorter for taps delivered to more proximal muscles. This method has, therefore, provided evidence for muscle, probably length afferent, input to the human sensori-motor cortex, a measure of the latencies for this input and a possible method for investigation of this input in relation to changes in muscle length and contraction force in the periphery.

656 RESEARCH APPLICATIONS OF A QUANTITATIVE STANDARDIZED EEG AND EVOKED POTENTIAL ANALYSIS SYSTEM (MINICEARS). Donald G. Brunet*, Reginald G. Bickford, Kenneth R. Hanson*and Virgil Rochowansky*, Dept. Neurosci., Sch. Med., UCSD, La Jolla, CA 92093

The field of CNS macro potential analysis is largely characterized by fragmented and unstandardized approaches to computer analysis. An impetus towards standardization has come from the clinical field with the development of the ACE test (Automated Cerebral Electrogram) which combines EEG and evoked potentials into a single procedure. Efficient processing of test data has been made possible by use of the MINICEARS computer system (LSI-11, 32k memory, 2 floppy disks, 100k sample A-D, X-Y and graphics printer output.) Apart from clinical uses this system has wide ranging research applications. The functions include: 1) spectral programs producing 16 channel spectral analysis displays (compressed spectral arrays) sampled over 2 minutes; 2) plots of spectral averages in the form curves for each electrode; 3) bin microvoltages (delta, theta, alpha, beta, or selectable ranges) are generated and statistical programs provide significance tests for homologous electrodes and for normal populations matched for age; 4) area display programs make voltage contour plots of bin data (delta, etc.) or "time slices" of evoked potential fields at selected latencies following the stimulus; 5) recognition of patterns (i.e. spikes) is performed on 16 channels and the data assembled as "spike pack" graphics. By aligning primary spike data (recognized as "probable spike" by the computer algorhythm) for final visual validation; 6) evoked potential averaging proceeds on 16 channels (brainstem response 4 channels) and computer programs recognize peaks and assign latency and amplitude automatically; 7) monitoring of EEG or evoked potentials over long periods and the construction of a "somnogram" or "comagram" can be achieved. The system formats reports on the printographic terminal and can be used for data gathering, word processing, etc. Single unit processing algorhythms are under development and customized software development will be available. Cost economy has been the major consideration in developing the system which is manufactured by Plessy Peripherals of San Diego. (Supported by NIH Grant USPHS/NS 08962-11.)

657 Computer Classification of Somatic Evoked Potentials Using Euclidean Distance and Cross Correlation.
E. Carlton, S. Katz, and L. Martin.* Department of Physiology, Medical University of South Carolina Charleston, South Carolina 29403

Computer classification of somatic evoked potentials (SEP) obtained from monkeys subjected to different spinal cord lesions was based on two different discriminant functions: cross correlation and Euclidean distance. Prototype SEPs were chosen arbitrarily from each lesion class. Each unknown SEP was classified according to its relationship with the prototype. In the minimum distance classification procedure, the Euclidean distance between the unknown and each prototype is calculated. The unknown is then assigned to the class of the prototype for which this distance is minimum. In the cross correlation classification, the correlation coefficient between the unknown and each prototype is calculated. The unknown is then assigned to the class of the prototype for which this coefficient is maximum.

In a series of experiments, monkeys were subjected to 3 different spinal cord lesions (anterolateral, hemisection, and central). Prior to the lesion three control SEPs were recorded from electrodes implanted in the skull overlying upper limb and lower limb SSI. A week after the lesion three SEPs were again recorded from each of these areas. For each lesion, the SEPs from three different monkeys were used for computer classification. Two of the three recorded SEPS from each monkey were taken as prototypes from each class. The third SEP was the unknown to be classified. Thus, the classification programs were asked to assign each of 18 unknowns to one of four classes: normal, anterolateral lesion, hemisection lesion, and central lesion. Both procedures classified over 90% of the unknowns correctly. The Euclidean distance program made more correct classifications than the cross correlation program, indicating the significance of both size and shape parameters in the decision-making process. (Supported partly by research grant NINDS NS11066).

658 VISUALLY EVOKED POTENTIALS AND AFTER-DISCHARGE AS A FUNCTION OF AROUSAL AND FRONTAL LESION IN RATS.
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Differences in tendencies to augment or reduce, defined as the increments or decrements in amplitude of visually evoked potentials (VEP) to increasing intensities of light stimulation (Lukas & Siegel, Science, 198: 73, 1977) are believed to be related to level of arousal. Since frontal cortex has been implicated in control of arousal, two experiments were conducted to determine augmenting/reducing patterns in primary VEPs, N1-P2, and the 1st component of the photic after-discharge, N3-P4, preceeding and following lesions to area 8. In addition, behavioral responsiveness of lesioned and control Ss was measured and compared with the electrophysiological data.

In Experiment 1, Ss were implanted with electrodes over area 17, and following recovery were tested on two separate occasions. Five intensities were presented in random blocks of 30 flashes (1 per 5 sec.) and computer averaged. Slopes for peak to peak amplitude of the first (N1-P2, 30 msec) and third (N3-P4, 115 msec) components were computed and compared. In Experiment 2, all Ss were lesioned bilaterally in area 8 (2 mA for 20 sec.). Rats were then tested on the 1st, 7th and 37th day post lesion as described in Exp. 1. Subjects were found to demonstrate either augmenting or reducing of the first component. However, following lesion, VEP reduction was reversed such that all Ss yielded characteristic augmenting of the primary component. In addition it was found both pre- and post-lesion in response to increasing stimulus intensities and thus arousal, that the after-discharge component of the VEP showed corresponding decreases in amplitude, and in most Ss completely dropped out at the highest intensities.

In Experiment 3, to assess behavioral correlates of the electrophysiological effects of frontal lesion, 12 additional rats received either lesion or sham operations as described for Exp. 2. For 4 days beginning on the 7th and again on the 37th days following surgery Ss were tested in a closed field apparatus (Joseph, et al. Behav. Biol. 24: 364, 1978) to assess differential responsiveness and activity in a complex, novel environment (Joseph & Gallagher, Devel. Psychobiol. 1979, in press). It was found that the lesioned rats were significantly more active and responsive than the sham operated rats on the first 4 and last 4 testing days. Surprisingly, no subsequent decreases were found in activity as a function of testing sessions. Viewed together the electrophysiological and behavioral data suggest that lesions to area 8 result in or maintain the augmenting VEP and results in increased behavioral responsiveness. These findings suggest that VEP augmenting/reducing and degree of behavioral responsiveness are both functions of frontal pole control of arousal level.

659 FREQUENCY RESPONSE CHARACTERISTICS OF THE SOMATOSENSORY EVOKED RESPONSE SYSTEM.
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Somatosensory evoked responses to transient stimuli (SERs) have received widespread application in diagnosis of somesthetic disorders. Such disorders frequently alter SER latency, amplitude or waveform. However, this is not always the case, and it is often difficult to correlate SER changes with the underlying neurophysiology (Hume, A.L. and Cant, B.R., EEG. Clin. Neurophysiol., 45:361-875, 1978). One reason for this is the difficulty in describing the complex SER waveform.

Recently, Namerow, et.al., (EEG. Clin. Neurophysiol., 37:11-21, 1974) introduced a method utilizing high-frequency stimuli which produces a sinusoidal SER (steady-state SER or SSER) quantifiable by two parameters: amplitude and latency or phase. They report a relation between stimulus frequency and response amplitude (frequency-response curve or FRC) which changes with diseases such as multiple sclerosis.

We have utilized a variation of this method to study the FRC for the somatosensory system, as well as FRC changes with disease. Five neurologically normal adults were tested on three occasions. Pulsatile stimuli at frequencies from 5 Hz to 50 Hz were delivered transcutaneously to either median or peroneal nerves at current levels between 2 and 6 mA. The SSER was recorded bipolarly from electrodes at Fz and Pz using a reference at Cz (10/20 system). Potentials were amplified by $2x10^5$ with a passband of 1 Hz to 1 kHz, and stored on FM tape for off-line analysis by digital computer (DEC PDP 11/10).

Recorded data were digitized at 200 pts/sec. Ensemble averages were computed for 50 responses with a sample epoch of 400 msec. The ensemble averages were then filtered with a high-Q, narrow-band digital filter centered at the stimulus frequency. The SSER magnitude was estimated from the root-mean-square value of the filtered ensemble average. The FRCs were then made by plotting SSER magnitude vs. stimulus frequency.

In the frequency range tested, for normals, the FRCs for median nerve stimulation show bandpass characteristics with a center frequency near 20 Hz. The FRCs for peroneal nerve stimulation show lowpass characteristics with a cut-off frequency between 5 and 10 Hz. Three patients with sciatica were then tested. Their neurological exam was unremarkable. The transient SER for one patient showed increased onset latency to L_5 stimulation. For all patients, the FRCs from the affected side were abnormal. With peroneal stimulation there was a marked decrease in the magnitude of the low-frequency portion. In one case, this change correlated with surgical findings. For median nerve stimulation, the FRC peak shifted to lower frequencies. Our results suggest the FRC may be an important measure of somatosensory function.

660 DEVELOPMENTAL CHANGES IN NEGATIVE COMPONENT (N2) OF EVENT-RELATED POTENTIALS RECORDED DURING REACTION TIME TASK.
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Event-related-potentials have been recorded from children (6-8 yrs), preadolescents (10-13 yrs), and adolescents (16-18 yrs) engaged in a variety of information processing tasks, as part of a project investigating the changes in brain activity which accompany normal mental development and retardation. We report here on data obtained during a reaction time task.

A light flash presented at random intervals between 10 to 18 sec served as the stimulus. The subject responded by pressing a button with his dominant thumb. EEG was recorded DC from a vertex lead referred to linked ears. Eye movements were monitored by electrodes placed above and below the left eye. The data was digitized (250 pts/sec) and edited offline using a PDP 11/10.

Individual subject averages were computed using the ten trials with the fastest reaction times. For each subject's waveform it was possible to identify a prominent negative peak preceeding the maximum positive peak evident during the first 500 msec of the epoch. This negative component resembles the N2 component observed by Ritter et al. (Science,203:1358,1979) and may thus reflect a decision process related to sensory discrimination or stimulus identification.

Reaction time, measured from stimulus to thumb EMG, showed an inverse relation to age. The N2 component had the longest latency for the children ($\bar{X}$=246 msec,SEM=10,N=23). Its latency did not differ for the preadolescents ($\bar{X}$=177 msec,SEM=10,N=17) and the adolescents ($\bar{X}$=183 msec,SEM=05,N=22). N2 always preceeded the response for both the children and the preadolescents, but not the adolescents. Correlations between N2 and reaction time were as follows: children ($r=.74$, $p<.01$), preadolescents ($r=.65$, $p<.01$) and adolescents ($r=-.24$, n.s.).

The findings suggest that during a simple reaction time task the older subjects can dissociate response initiation from the sort of stimulus identification reflected in the N2 component. In contrast, the younger subjects must identify the stimulus before responding. This highlights the changes in subjective task demands with cognitive and motor development.

(Supported by NICHD grant HD 08265)

661 EFFECT OF ENVIRONMENTAL SCALE OF SUBJECTS ON SPECTRAL EEG OUTPUT. Alton J. De Long* and Joel F. Lubar. Sch. Arch., Dept. Psych., Univ. Tenn., Knoxville, TN 37916

Three studies were conducted varying the environmental scale of subjects. The purpose was to assess changes in neurological functioning as postulated by a theory of experiential space-time relativity.

Environmental scale was manipulated three-dimensionally, two-dimensionally, and perceptually through color. Each study involved a comparison between large and small scale conditions. In the first study scale contrast consisted of exposure to the full-size laboratory environment, and a 1/12 scale-model replica of this space. In the second study, subjects viewed different image sizes of projected slides, the smaller being 1/3 the scale of the larger. In the third study scale contrast was provided perceptually by presentation of receding(cold) and approaching(warm) colors.

Neurological functioning was measured through absolute and percent power output of the left and right central cortex across seven EEG spectral bands comprising the range of 0-27 Hz. Scalp electrodes were placed bilaterally over the motor cortex with active electrodes placed 10% and 30%, measured from the vertex to the ipsilateral ear with an ear-lobe ground. Data were analyzed according to a spectral program employing the Fast Fourier Transform.

Results of all three studies indicate two principal effects of reduced environmental scale on spectral EEG output: 1)an increase in absolute power output across all spectral ranges, and 2) a selective increase in percent power output across the higher frequencies (16-27 Hz).

These findings are consistent with the theory of experiential space-time relativity which postulates that the rather precise mediation of temporal experience by spatial scale (De Long,1978, Technische Universität Berlin--Dokumentation, 6:344-358) should exhibit neurological correlates. The selective increase in percent power output for higher frequency ranges seems compatible with the experience of time passing more swiftly for subjects under scale-reduced conditions. The increase in absolute power output across all spectral ranges still eludes interpretation, but appears consistent with reports by subjects that the compression of temporal intervals under scale-reduced conditions is experienced as being intense -- the temporal intervals feeling densely packed.

When considered in conjunction with the space-time theory,these findings suggest environmental scale may play a central role in mediating changes in neurological functioning associated with maturational development. Current studies are examining the potential role of environmental scale in maturational development and the etiology of hyperkinesis. [Research supported by The Office of Graduate Studies and Research, University of Tennessee]

662 THE SOMATOSENSORY EVOKED POTENTIAL IN THE NORTH AMERICAN OPOSSUM: EFFECTS OF INTERACTION OF PERIPHERAL NERVE INPUTS. R. M. Dom*, J. G. Blackburn, R. K. Simpson and S. Katz. Depts. Anat. and Physiol., Medical Univ. South Carolina, Charleston, SC 29403.

Alterations of the somatosensory evoked potential (SEP) resulting from interaction of peripheral nerve inputs were studied in adult opossums anesthetized with alpha chloralose. In all cases, the averaged SEP was recorded from the forepaw area of the cerebral cortex (Lende, J. Comp. Neur., 121: 395-404, 1963) with the superficial peroneal nerve being stimulated (conditioning stimulus) at different time intervals prior to stimulation of the superficial radial nerve (test stimulus). Maximal interaction was present at stimulus delay intervals ranging from 100 to 200 msec. The primary component of the SEP is most attenuated at 100 msec, whereas the secondary responses remain depressed for a longer time interval.

The similarity of the interactive responses in the opossum to prior observations in the cat and monkey indicates that interaction may be a cortical phenomenon characteristic of mammals. Because of the potential usefulness of interaction studies in evaluation of spinal trauma, the relatively nonspecialized central nervous system of this pouched mammal may prove to be a useful model for spinal cord injury studies.

663 ADVANCES IN CLINICAL NEUROPHYSIOLOGY. PSYCHIATRIC RELEVANCE. B. Dubrovsky. Allan Memorial Institute, McGill University, Montreal, Quebec, Canada.

Recording of event-related slow potentials (ERSP) during activities that require the active engagement of subjects has turned out to be a useful, atraumatic method of exploration of brain activity in psychiatric populations. From the various ERSP, the contingent negative variation, CNV, has been one of the most extensively studied. While in normal control populations the resolution of the CNV, that is the return of the negative wave to the baseline level occurs immediately after S_2, a significant number of psychotic patients show an apparent prolongation of the negativity which extends for more than 2 sec. after S_2. Different brain reactivity to peripheral stimuli during persistence negativity suggest that neural activity generating it is at least partly independent from the CNV per se. Dongier (In Biol. Diagn. of Brain Disord. S. Bogoch Ed. p. 47; 1973) has named this prolongation post-imperative negative variation (PINV). Moreover, metabolic changes (varying concentrations of CO_2 measured in the expired air) selectively affect PINVs (Dubrovsky et al. Biol. Psychiat. 11 535; 1976) without significantly affecting amplitude and duration of CNV waves per se. In other context, significant differences viz. larger CNV amplitude, abnormally prolonged PINVs and shorter reaction times were found when using phobogenic stimuli as S_2 in patients suffering from specific phobias. After successful treatment with relaxation desensitization therapy, the CNVs of the patients were within normal limits. These results led us to propose a neural mechanism for relaxation desensitization therapy. Regions in the hypothalamus and limbic system mediate, at least in part, certain global emotional reactions such as fear. Most animals form associations as long as the modality to which the association is made is a limbic modality (Gershwind, Brain 88; 237, 1965). We propose that the pairing of a pleasurable condition (relaxation) with phobogenic stimuli establishes a new association, presumably in limbic structures involved in these emotional states. Limbic frontal connections (Nauta, Psychiat. Res. 8; 167, 1971) can then act in part as modulators of frontal regions from where CNVs are mainly generated. The functional connections between the relaxed condition and phobogenic stimuli established through the desensitization treatment, possibly involving limbic regions, would then affect the gain of the threshold mechanism for CNV generation in frontal regions (Loveless and Sanford, Biol. Psychol. 2; 217, 1975) and consequently affect CNV amplitude and duration. For us mental pathology is the result of regional disorganization of neural activity rather than "dissolution" or "regression".

664 NEUROPHYSIOLOGICAL LOCALIZATION OF DYSLEXIA. Frank H. Duffy, and Martha B. Denckla* (SPON: M. Duchowny). Seizure Unit and Department of Neurology, Children's Hospital & Harvard Med. Sch. Boston, MA 02115.

EEG and evoked potential data were recorded from 8 dyslexic and 10 control children aged 9-11. Data were collected during test situations designed to activate the left hemisphere (reading, listening to speech), right hemisphere (recognizing geometric forms, listening to music), both hemispheres (sound-symbol association, phonemic discrimination), or neither hemisphere, (resting EEG and evoked response). EEG spectra and evoked potentials were formed from 20 electrodes and the resultant waveforms were processed into topographic maps of brain electrical activity. Separate topographic maps of the average (mean) electrical activity for the control and dyslexic groups was formed for each test state. Topographic maps of group difference were formed following statistical tests and were used to delineate regions of the brain where the 2 groups differed. In addition to a large area of group difference overlying the left temporal and parietal regions, prominent differences were found over both hemispheres, in the frontal lobe overlying left Broca's area and the bilateral medial supplementary motor area. Thus, dyslexia appears to involve more cortex than would be predicted by analogy to adult-onset aphasia. The areas implicated above are analogous to regions shown by others to be active in normals during reading and speech (isotope radiography). We suggest that dyslexia represents a "system deficit" rather than an isolated "lesion."

665 SELECTIVE ATTENTION, P300, AND POSSIBLE REGIONAL CEREBRAL BLOOD FLOW CORRELATES. Dragoslav Ercegovac*, Richard C. Josiassen*, Richard A. Roemer and Charles Shagass*. Eastern Pennsylvania Psychiatric Institute and Temple University Health Sciences Center, Philadelphia PA 19129.

There is growing acceptance that selective attention to task-related sensory stimuli which resolve a subject's uncertainty elicit a cerebral evoked potential (EP) of 250-500 msec peak latency, namely the P300. The first section of this report examines somatosensory EPs elicited by random sequences of electrical stimulation of four different fingers, where the subject is instructed to attend only to the number of stimuli delivered to one finger which has been designated the "target finger". Electrical stimulation of non-target fingers is to be ignored. Following the work of Desmedt et al (1977), the authors have replicated the finding that reliable P300 components may be obtained using this selective attention task. Robust P300 components were recorded over the central-parietal region contralateral to target finger stimulation.

The experimental group was comprised of 25 normal adult volunteers. All subjects were screened for disorders of skin sensation, bone fractures, head trauma, and brain disease.

It is the hypothesis of this report that these obvious somatosensory EPs are accompanied by systematic changes in regional cerebral blood flow. This hypothesis is based on the general observation that mental activity increases circumscribed regional cerebral blood flow. Using impedance plethysmography procedures (rheoencephalography), it is possible to calculate a blood flow index which is highly correlated with cerebral blood flow. The rheoencephalographic method used in this study follows the procedure of Jacquay et al (1974).

Within the context of the identical selective attention paradigm, rheoencephalographic data were obtained to test the hypothesis that mental activity which elicits robust cerebral electrical potentials is accompanied by measureable cerebral blood flow changes.

666 AUDITORY EARLY, MIDDLE AND LONG LATENCY EVOKED POTENTIALS IN CAT. Glenn R. Farley* and Arnold Starr. Depts. of Psychobiology and Neurology, Univ. of Calif., Irvine, Irvine, CA 92717.

We are studying early, middle, and long latency (1-650 msec) averaged evoked potential (AEP) components of muscle-paralyzed cats to define their relationship to evoked potentials recorded from man. We are using behavioral paradigms designed to control arousal and attentional state. In behavioral "habituation," 500 msec noise bursts are presented once every 25-65 sec against a background of continuously presented (1/sec) clicks. In "sensitization," clicks and noise are presented as above, along with a tail shock once every 25-65 sec. The shock has a random temporal relation to the noise. "Conditioning" is similar to sensitization except that the tail shock invariably follows noise onset by 5 sec. The behavior indicating learning of this association is a pupillary dilation that is temporally related to the noise. Averaged evoked potentials are recorded from each of 17 skull electrodes to clicks immediately preceding and following the noise, and to the noise itself, during each of the behavioral paradigms. Multivariate statistical analyses are applied to these waveforms for determination of AEP components. Factor scores of the AEP waveforms for each component are derived, and subjected to further analysis to find which experimental manipulations control each component. Results show that various components vary differentially with the physical parameters of the stimuli (clicks, noise), the behavioral context in which the stimuli are presented, and the topographical distribution of the potentials across the skull surface. These results suggest that some of these components may be useful models of some human auditory evoked potential components which show similar controlling factors.

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667 EEG CORRELATES OF TEXT THEMATIC STRUCTURE. E.Françozo* and A.F.Rocha* (SPON:C.Timo-Iaria) Dept.Phys.Bioph., UNICAMP, 13100 Campinas, SP, Brasil.

Text structures can be studied through the notions of *theme* (information on which the text is built up) and *rheme* (what the text informs about its theme).This conceptual pair can be viewed as the counterpart of the pair *topic* and *comment* at the level of sentences. Linguistic stimuli have already been demonstrated to evoke EEG responses correlated to functions and properties of natural languages. Our purpose here is to report the existence of correlations between evoked responses and thematic structures of texts, the dynamics of such correlations and their linguistic relevance.We presented a previously recorded text to the subjects while their EEG was recorded and, immediately after, asked them to reproduce it in written and then to point to its theme. The whole procedure was repeated twice within an interval of 30 minutes. Results can be thus summarized: i) two context dependent strategies were used by the subjects during text reproduction: a *divergent* one, in which the theme was given in the first sentences of the reproduction, and the rheme followed, and another, *convergent*, in which a set of arguments was given first, and theme followed; ii) the thematic structure defining elements evoked a characteristic electrical activity pattern; iii) the EEG activity reflected accurately the syntactic or semantic performance of the subjects; iv) in the subject's convergent approach to the text, only comments evoked marked patterns in the EEG, while when a divergent strategy was used both topics and comments evoked responses.The above data seem to indicate: a) the existence of responses evoked in the EEG by the thematization and topicalization structures of natural languages; b) that the relationship between such structures depends on a context dependent strategy; c) that relevant linguistic data can be obtained from the EEG by means of an adequate methodology which takes into account pertinent theoretical parameters.

668 EFFECT OF CHRONIC HALOTHANE EXPOSURE ON SENSORY EVOKED POTENTIALS IN FREELY BEHAVING RATS. G.N. FULLER, B.M. RIGOR*, R.C. WIGGINS and N. DAFNY. Depts. of Neurobiol. & Anat. and Anesthes., Univ. of Texas Med. Sch., Houston, TX 77025.

Chronic halothane exposure has been implicated in pathologic changes in several peripheral organs. The present study was initiated to determine if chronic halothane exposure has any effect on bioelectrical activity recorded simultaneously from two different deep brain nuclei. Sensory evoked field potentials were employed as a measuring tool to assess halothane induced alterations in bioelectrical activity. The target nuclei in the present study were the mesencephalic central gray and the nucleus parafascicularis, two deep structure mediators of analgesia and anesthesia. Permanent semimicroelectrodes (60μ diameter) were implanted stereotaxically under pentobarbital anesthesia several days prior to experimentation. The averaged acoustic evoked response (AAER) following 32 repetitive click stimuli was recorded from both regions in halothane naive freely behaving rats on day 1. The rats were subsequently exposed to 0.25% halothane delivered through a vaporizer (Fluotec 3) with compressed air for 3 hrs per day, 5 days a week. AAER recording was resumed at 28 and 56 days after the initial daily exposure of halothane. The AAER consists of a positive (P_1)-negative (N_1) spike followed by a positive-negative-positive (P_2-N_2-P_3) wave. The last three components were consistently observed within and between animals and were therefore quantitated and compared to naive values. After 28 days of chronic exposure, no significant changes were observed in the recording obtained from the central gray as compared to the day 1 recording. In contrast to this, in the nucleus parafascicularis the P_2 and P_3 components showed a statistically significant ($p<0.01$ and 0.05 respectively) increase in amplitude (paired t test). Following 56 days of halothane exposure, the N_2 and P_3 components of the central gray AAER were statistically increased ($p<0.05$ and 0.005 respectively) over naive values. In the nucleus parafascicularis, the P_2 and P_3 components remained increased as at 28 days but with a more significant value for P_3 ($p<0.005$). Thus although a general increase in AAER amplitudes is seen in both regions examined, the time course and individual components affected vary markedly. These data demonstrate a pronounced alteration of sensory evoked potentials following chronic halothane exposure. (Supported in part by USPHS grant no. NS-14355.)

669 EEG CORRELATES OF SEX AND SCHIZOPHRENIA. Duilio Giannitrapani. Veterans Administration Medical Center, Perry Point, MD 21902.

EEG sex differences, with particular reference to power, phase angle and coherence spectra, were compared to those found in schizophrenia. Eighteen males and 18 females, 11 to 13 year-old right preferents, were selected. Subjects were administered an EEG during which spectra were obtained from 16 brain areas between 2 and 34 Hz in 16 frequency bands, each 2 Hz wide.

Power spectra show overall greater power in the males except in the left occipital area in the 2 - 10 frequency range where the opposite was true for the females. The areas in which the females show greater power slowly broaden with increasing frequency. The broadest distributions occur in the 19, 21, 29 and 33 frequency bands, possibly indicating a broader distribution of the 2nd and 3rd harmonic of dominant activity in females, also found in schizophrenics by this investigator.

In the phase angle analysis in the 13 Hz activity, males show predominant significant anterior leading while females show significant posterior leading. A similar pattern has already been shown by this investigator for schizophrenics and normal adults respectively.

Coherence in general is higher in females in anterior areas and in males in posterior areas bilaterally. An exception is the 29 and 33 Hz activity, showing higher coherence in females in all comparisons, also found by this investigator in schizophrenics.

The 13 Hz phase angle finding and the 29 Hz power and coherence spectral finding are in contradiction in regard to the hypothesized sex-relatedness of schizophrenia. The 13 Hz finding is consistent with greater vulnerability toward schizophrenia in males while the 29 Hz data for the females correlates with higher power and coherence found by this investigator among schizophrenics. An attempt is made to coordinate the data from the three parameters studied and relate them to the known unequal distribution of psychopathological incidence among the sexes.

The data points to a physiological substratum of schizophrenia. Inferences could be made for the genesis of the underlying morphological differences which would have to go back to the early ontogenetic development of the brain when it still consists of a neural tube. The manner in which the ablateral holosphere becomes bilateral and develops into lobes as we know them would be at issue.

670 INTRACRANIAL DISTRIBUTION OF SOMATIC AND AUDITORY EVOKED POTENTIALS DURING SLEEP IN MAN. W.R. Goff, T. Allison, G.D. Goff*, C.C. Wood* and P.D. Williamson*. Neuropsychology Lab, West Haven VAMC and Dept. Neurol., Yale Sch. Med., New Haven, CT.

Analysis of morphological and topographical relationships between scalp and intracranially recorded evoked potentials is fundamental to inferring the cerebral origins of scalp activity. One approach is to record from the two areas under conditions known to alter scalp potentials and to determine the effects of these conditions upon intracranial potentials. Scalp recorded somatic (SEP) and auditory (AEP) evoked potentials undergo dramatic changes during sleep. Therefore we are comparing scalp and intracranial recordings during waking and different stages of sleep. To date five patients have been studied; they had up to six multicontact depth probes chronically implanted bilaterally in frontal, central, and occipital regions as part of an evaluation for possible neurosurgery to relieve intractable epilepsy. Simultaneous recordings were made from 16 contacts selected from up to 108 available contacts on the probes. In all patients, the gross morphological changes from waking to sleep seen in scalp potentials were also observed intracranially. In four patients, long latency SEPs and AEPs had different scalp-depth relationships during waking whereas scalp-depth relationships were similar for the two modalities during sleep. Emphasizing the tentative nature of data from a few patients with abnormal brains, these results are consistent with our previous suggestion (Goff et al., 1966) that long latency potentials occurring during waking and sleep reflect activity of different neural generators.

671 A MICROPROCESSOR SYSTEM FOR CORTICAL AND BRAINSTEM EVOKED RESPONSES. Steve H. Graham and M. E. Miner. Division of Neurosurgery, University of Texas Medical School, Houston, Texas 77030.

Recent advances in large scale integration have brought computer technology within the budget of almost every electrophysiological laboratory. The microprocessor, due to it's programability may be used to replace an assortment of electronic devices at a fraction of their cost. We have developed a microcomputer system to signal average up to 14 channels of cortical evoked potentials and one channel of brainstem evoked responses.

The system consists of a commercially available Z80 microcomputer with 64K RAM, dual 8 inch floppy disk, a CRT conversion, interval timers, interupt logic, and a parallel port. Using the interrupt timers, the system is able to perform three tasks simultaneously: acquire signal averaged data, display waveforms on an oscilliscope, and run the BASIC program that controls the sampling interval, number of points and number of repetitions to be averaged. Maxima and minima of the evoked potentials may be identified and their latencies from the stimulus computed. The peaks determined by the computer are displayed on the oscilliscope; manual review of the waveform and positioning of a cursor allows for correction if necessary. The waveforms and other data are stored on the floppy disk for later review and analysis. The A/D converter utilized has 8 bit resolution; therefore, one must compromise between maximal resolution and the possibility of the A/D converter being saturated. We have partially resolved this dilemma by not including any data in the sum if any of the channels contain a saturated data point. The parallel port is used to control external devices such as stimulators, allowing the microprocessor to supercede delay timers. Stimuli intervals may be randomized to prevent habituation.

672 EFFECTS OF GENOTYPE, AGE, INTERSTIMULUS INTERVAL (ISI), AND BINAURAL SUMMATION ON COCHLEAR AND AUDITORY BRAINSTEM EVOKED RESPONSES (BSER's) IN THE LABORATORY MOUSE. Kenneth R. Henry. Dept. Psychol., University of California, Davis, Ca. 95616.

Several recent studies have shown that, as the ISI is decreased, the amplitudes and latencies of the various components of the BSER show differing recovery rates. These responses also differ in the human neonate, adult, and geriatric adult (Fujikawa and Weber, 1977). A binaural occlusive effect also occurs with P_{IV} in the cat (Huang and Buchwald, 1978) and P_V in man (Blegvad, 1975).

Mice of the C57BL/6 and CBA/J strains, from 20 to 380 days of age, were used for these studies. The former genotype expresses a progressive postpubertal hearing loss, while the latter maintains its hearing throughout its life (Henry, 1979).

As the ISI was decreased from 100 to 50 to 25 to 12.5 msec, latencies increased by 66, 141, 163, 188, and 356 μsec for P_I-P_V, respectively, regardless of age and genotype. For the CBA genotype, decreasing the ISI reduced all amplitudes by approximately 45%. The C57BL/6 mouse also had this same 45% amplitude decline for P_I, P_{II} and P_{IV}, but its P_{III} and P_V responses only declined by 25% with decreasing ISI.

When monaural and binaural responses were compared, all ages and genotypes showed the same effect. For P_{I-III}, binaural stimulation produced the same responses as did summing the responses obtained by monaurally stimulating the two ears. For P_{IV} & $_V$, however, binaural stimulation reduced the amplitude by 25% and 60%, respectively.

673 SPATIAL AND TEMPORAL DISTRIBUTIONS OF NARROW AND WIDE BAND SOMATICALLY EVOKED FIELDS IN HUMANS. A. Joseph Lipton*, Lloyd Kaufman*, Samuel J. Williamson*, and Douglas Brenner. Neuromagnetism Laboratory, Depts. of Physics and Psychology, NYU, New York, NY 10003.

A Superconducting Quantum Interference Device (SQUID) demonstrating high spatial resolution in measurements of evoked cortical activity was used to elucidate the spatial and temporal distributions of somatically evoked fields (SEF) associated with median nerve stimulation in humans. Reliable narrow and wide band measurements were made over the somatosensory areas with both contralateral and ipsilateral stimulation. 1 msec transcutaneous pulses were delivered at 20 Hz for the mapping aspects of the study. Detectable evoked fields were clearly distributed over the contralateral cortex only. Within the 1 cm spatial resolution of the system, activity was well modeled by a single current dipole oriented normal to the central gyrus, although evidence for other nearby sources was found. For both steady-state and transient measurements a 180^{o} polarity reversal of the field occurred within a 3 cm scan through the hypothesized dipole with response amplitudes reduced to 50% of peak values within 2 cm. Simultaneously recorded narrow and wide band magnetic responses at stimulation frequencies between 1-12 Hz allowed the comparison of waveform and latency derived from wideband somatically evoked potential (SEP) measurements with those derived from wide as well as narrow band SEF measurements. Although the basic components of the wideband SEF, characterized by early components followed by a much longer and slower component, were similar to the earliest cortical components of the SEP their respective onset latencies of 70 and 30 msec did not agree. No unique latency could be derived from narrow band measures, as derived latency varied over different ranges of measurement.

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674 THE RELATIVE CONTRIBUTIONS OF PERIPHERAL NERVES TO THE SOMATOSENSORY EVOKED POTENTIAL. Joel Myklebust*, Joseph Cusick. Med. Coll. of Wis. and Wood V.A. Med. Ctr., Milw., WI.

The relative contributions of cutaneous and muscle afferents to the somatosensory evoked potential (SSEP) are not well established. In this study, the SSEP elicited by mixed nerves (the common peroneal at the fibular head, and the posterior tibial at the ankle) and primarily cutaneous nerves (the deep peroneal nerve distal to the bifurcation of most muscular branches, and the sural nerve at the ankle) were compared in man and in the stumptail macaque monkey.

In the human, stimulation was applied through stainless steel disc electrodes, and recordings were made from needle electrodes placed in the scalp over the somatosensory cortex. In the monkey, the nerves were surgically exposed and stimulated with hook electrodes. Recordings were made with platinum disc electrodes placed epidurally over cauda equina (CE), conus medullaris (CM), thoracic spinal cord, and sensory-motor cortex (SMC).

In the human, the response to stimulation of common peroneal (mixed) nerve at levels below the motor threshold was almost identical to that obtained with supramaximal stimulation of the sural and deep peroneal (cutaneous) nerves. With increased stimulus intensity, the common peroneal response appeared at a shorter latency and, in some cases, with an altered waveform. In contrast, no threshold effects were observed with stimulation of the sural or deep peroneal nerves. Stimulation of the tibial nerve at levels below motor threshold produced a response at approximately the same latency as that for the sural and deep peroneal nerves. Increased stimulus intensity produced an earlier response with an altered waveform.

In the monkey, low level stimulation of all four nerves elicited a complex tract response at CE. Increased stimulus intensity on the common peroneal and tibial nerves produced an earlier, large triphasic potential. At CM and the thoracic cord, the tibial and common peroneal responses had shorter latencies and were more compressed temporally than with sural nerve stimulation. At SMC, the responses from the tibial and peroneal nerves were shorter in latency than the sural nerve response.

These results indicate that, with mixed nerve stimulation, the fast, early portions of the SSEP are probably due to muscle afferents, while the later, slower components result from cutaneous fibers.

675 RELATIONSHIP BETWEEN INTRACRANIAL PRESSURE AND LATENCY SHIFT IN VISUAL EVOKED POTENTIAL. Morris Pulliam, Donald H. York, Clark Watts and John G. Rosenfeld*. Dept. of Physiology and Section of Neurosurgery, School of Medicine, University of Missouri, Columbia, MO 65212. Previous studies have suggested that increases in intracranial pressure (ICP) as a consequence of hydrocephalus may compress the visual radiation fibers as they course through the lateral ventricle. The present study was designed to test the hypothesis that increases in ICP would cause consistent shifts in latency of waves constituting the visual evoked potential (VEP). An initial pilot study was undertaken on a 16-year-old head injury patient who had a pressure transducer placed epidurally to monitor ICP. Visual evoked responses were conducted over a 10-hour period to provide 18 independent assessments. The VEP was measured by differential recording from surface electrodes at O_1, O_2 (International 10-20 system). The stimulus was a strobe flash delivered to the eyes at a rate of 1/sec. Each flash generated a VEP which was averaged in a computer of average transients for 75-100 successive responses. The peak of each major positive and negative going wave component was identified by subsequent analysis in a microprocessor which assigned latency values to each peak. Latency values for eight component peaks of the VEP up to 256 msec were then correlated at 21 different intracranial pressures over the 10-hour time period. The results show a linear correlation (r=0.68) of ICP with increased latency of the N70 peak. This would suggest that VEP measurement may provide a non-invasive estimate of intracranial pressure. The second part of the study sought to examine this relationship by studying the VEP response in children with hydrocephalus or symptoms of raised ICP. Using age matched controls of normal children as baseline, 9 of fourteen children demonstrated an increase in latency of N71 wave, whereas all 14 also showed an increase in latency of N138 wave. These findings taken together with the pilot study conducted with ICP monitoring suggest that the VEP may provide an important clinical tool for the assessment of raised ICP.

676 BRAINSTEM AUDITORY EVOKED RESPONSE AS A TOOL IN NEUROTOXICOLOGY AND OCCUPATIONAL MEDICINE. Charles S. Rebert. Neuroscience Laboratory, SRI International, Menlo Park, CA 94025

Event related potentials are becoming increasingly useful in assessing the integrity of the nervous system, and so are of potential benefit in evaluating the effects of exposures to neurotoxins--perhaps especially in indexing sub clinical deficits. Advantages of ERP methods include their objectivity, situational versatility, unique information content, cultural nonspecificity and usefulness in both animals and humans. The brainstem auditory evoked response (BAER) is most useful because of its consistency, ease of recording, relatively well known genesis, need for minimal patient cooperation, homology of animal and human responses, demonstrated sensitivity to clinical dysfunction and separability of peripheral and central components.

Little use has been made of BAERs in the evaluation of chemical toxicity although it has been shown to be modified by alcohol (Squires et al. 1978) and by triethyltin (Shah et al. 1978). To further evaluate the efficacy of the BAER as an index of neurotoxicity, chronically implanted young Fischer rats were administered misonidazole, a neurotoxic radiation-enhancing compound of possible use in cancer therapy.

Five rats were given ip injections of 300 mg/kg 5 days/week and 5 were controls. No changes in BAERs occurred in controls for 22 days so they were subsequently injected with 250 mg/kg. Two animals died after the fifth high dose and no changes in BAERs were seen. In the other 3 rats peak conduction times increased to 2.55, 2.58 and 2.48 msec from an average baseline of 2.03 msec. As expected, there was a consistent lengthening of latency for all components as stimulus intensity was reduced. The low dose produced no obvious behavioral toxicity before two weeks. The latency of components 3 and 5 exceeded 1.96 SDs of the baseline mean from the 14th through 35th day. There was a significant change in component 1 only at the lowest intensity. These results suggest that the peripheral mechanisms were relatively spared. For all three intensities the .025 probability level for central conduction time was exceeded by the 14th day. Average conduction times increased from a baseline of 2.09 msec to a peak of 2.33 msec. Recovery began soon after injections terminated but were not fully normal after 13 days.

These results indicate that the BAER can be a robust index of neurotoxicity. Clinical utility of the BAER in this context is suggested by our findings (Rebert, Schaeffer and Dahlgren) that a large proportion of industrial workers exposed to heavy metals and solvents have prolonged central conduction times.

677 THE GENERATORS OF THE AUDITORY BRAIN STEM POTENTIALS AS STUDIED BY DEPTH RECORDING IN THE CAT. Foster K. Redding, Richard J. Meltzer*. Dept. Neurol, Wayne State U. Sch. Med. Detroit, MI.

Short latency evoked potentials were first recorded from the intact skull of the cat, using averaging techniques. Subsequently, an amazingly similiar series of positive deflections were recorded following click stimuli in the intact human. Evoked potentials have proved clinically useful in patients with lesions of the brain stem.

Methods: A laminar analysis of the auditory evoked potential waves was made in 19 barbiturate-anesthetized cats. Fine stainless steel wire electrodes were placed stereotaxically at a calculated oblique angle into the superior olivary nucleus(SO), the lateral lemniscus (LL), and the inferior colliculus(IC). Conventional responses were recorded from an electrode at the vertex, all recordings using a reference in the pinna. Clicks were delivered through hollow ear bars. The recording parameters and averaging and read-out routines were the same as those used by other workers.

Results: A series of 4 to 6 positive waves were recorded from the vertex, identical to those reported by others. These ranged up to 6 or 7 ms. in latency, and had amplitudes in the 1 microvolt range.

Evoked potentials recorded directly from the depth in the brain stem were of much greater amplitude, so that they could clearly be seen on the oscilloscope face even without averaging. At each stereotaxic target point, a series of 2 or 3 positive or biphasic waves were recorded. As the electrode was advanced gradually, the waves remained of uniform configuration over a length of 4 or 5 mm. A locus of maximum amplitude could easily be found. This was presumed to be the center of the neural activity.

Latency of the first of the series of waves of each evoked potential, was shortest at SO. The first wave occurred progressively slightly later at LL and at IC. The amplitude of the waves was clearly greatest at SO, and successively somewhat lower at LL and at IC.

The precise latencies of the peaks of the waves recorded from the several brain stem electrodes did not all exactly correlate with each other, nor with the exact latencies of the peaks recorded from the vertex.

These results agree with the preliminary descriptions of Starr and Achor. It is clear that a simplistic "generator" theory of the origin of the vertex-recorded auditory evoked potential is untenable. A more complex analysis, analogous to that of the electrocardiogram recorded from the surface of the body, is needed.

678 PREDICTION OF CLINICAL PSYCHIATRIC CHARACTERISTICS BY MEANS OF CLUSTER ANALYZED EVOKED POTENTIAL MEASURES. Richard A. Roemer and Charles Shagass*. Temple University and Eastern Pennsylvania Institute. Philadelphia, Pa. 19129

This presentation reports the initial results of a rational, stepwise analysis of evoked potential (EP) measures conducted for the purpose of predicting clinical psychological characteristics by electrophysiological methods. Subjects were a diagnostically heterogeneous, consecutively tested group of 145 unmedicated psychiatric inpatients. Somatosensory (SEP), visual (VEP) and auditory (AEP) EPs were recorded in one session, using pseudorandomly presented left (LSEP) and right (RSEP) median nerve shocks, checkerboard pattern flashes and binaural auditory clicks. Recordings were made from one EOG and 14 scalp leads. Clinical criteria were as follows: (a) psychiatric diagnosis--discharge diagnosis independently made by two senior psychiatrists; (b) symptom ratings--16 Brief Psychiatric Rating Scale (BPRS) items rated by a psychiatrist; (c) intelligence--Raven's Progressive Matrices.

Visual detection of EP peaks in key leads provided estimates of the latency of each of 11 or 12 consecutive peaks (depending on the type of stimulus). The estimates were used to automatically determine the amplitude of each peak in all scalp and EOG records.

The first stage of analysis was to reduce the dimensionality of the data by extracting the factors involved in the spatial distribution of each peak. A varimax-rotated factor analysis of the amplitudes for a given peak at all leads usually resulted in the extraction of five factors, one of which often reflected EOG activity. The resulting factor scores for all peaks of a given type of EP for the 145 patients were subjected to hierarchical cluster analysis. Using RSEP factor scores as an example, six distinct clusters of patients were identified, and the results so far obtained indicate that two of these clusters contained a large weighting of depressive patients. Other RSEP clusters were significantly related to the intelligence measure and to ratings of anxiety.

The encouraging results justify further research aimed at classifying psychiatric patients by means of EPs to identify clinically meaningful subgroups that transcend conventional diagnostic categories.

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679 CONTRIBUTIONS TO THE SEP BY THE MAJOR ASCENDING SENSORY PATHWAYS. Richard K. Simpson Jr., John G. Blackburn, Henry F. Martin and Sidney Katz. Department of Physiology., Medical University of South Carolina, 171 Ashley Avenue, Charleston, South Carolina. 29403.

The contributions of various spinal cord pathways to the somatosensory evoked potential (SEP), was investigated in monkeys anesthetized with 70% N_2O and 30% O_2. SEPs were recorded in response to stimulation of exposed superficial peroneal nerves. Stimulus intensities were sufficient to excite either large diameter nerve fiber groups or all nerve fiber groups. While applying maximal stimulus intensities, the large diameter nerve fiber groups were blocked using a combination cooling and electrical polarization, (Blackburn, J.G. and Katz, S. 1976., J. Electrophysiol. Tech. 5:14-17.). This procedure allows selective small diameter nerve fiber input to continue. SEPs were recorded in response to stimulating various peripheral nerve fiber groups following either bilateral dorsal or anterolateral transection, left or right hemisection, and central cord lesion or complete cord transection at levels T3-T4. The results indicate that only the dorsal column system, (including the spinocervical tract), contributes to components P1 and N1. Only the anterolateral column systems contribute significantly to components P3 and N3. Both the dorsal and anterolateral column systems contribute to components P2 and N2. It appears that small diameter nerve fibers and the anterolateral columns contribute significantly to the later wave components of the SEP. The observation that both major ascending spinal cord pathways contribute to various components of the SEP enhances the possible utility of the SEP as a method of evaluation and treatment of human spinal cord injury. (Supported by NINDS grant P-5P81-NS-11066).

680 AUDITORY BRAIN STEM EVOKED POTENTIALS IN AUTISM. Barry F. Skoff*, Allan F. Mirsky. Laboratory of Neuropsychology, Boston University Medical Center, Boston, MA 02118.

Current theories of childhood autism suggest that the dysfunction is an organic one, primarily affecting perceptual and language mechanisms. Furthermore, it has been suggested that pathology involving the brain stem could explain many of the symptoms commonly seen in autistic children. The auditory brain stem evoked potential (ABSEP) is a simple, non-invasive method of assessing the integrity of the brain stem areas through which the auditory system courses.

In the present study, ABSEPs were acquired in a group of 20 children who had been diagnosed as autistic, or who exhibited autistic-like behaviors. Click stimuli (10 Hz., 60 dBSL) were presented monaurally to each ear, while the filtered (300-3K Hz.) EEG was recorded. Signal averaging was done off-line with artifact rejection.

Data from 3 children could not be analyzed. Of the remaining 17, 6 (or 35%) had abnormal ABSEPs when compared to a group of normal control subjects. One child had a prolonged latency in the components (I-III) said to reflect transmission from VIII nerve to pons. Four children had prolonged interwave III-V latencies (pons-midbrain) on at least one side, and 2 of these children apparently had no response when the other ear was stimulated. The remaining child had early waves I through III, and no detectable wave V (midbrain/inferior colliculus).

It appears that some form of brain stem pathology exists in at least some children that are diagnosed as autistic or exhibit autistic symptoms. The next step in elucidating the etiology of the syndrome is to determine whether there exists a correlation between brain stem pathology as shown by the ABSEP and specific behaviors or symptoms. The ABSEP may be eventually a simple and painless method of screening for early detection of childhood autism.

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681 LUMBOSACRAL REPRESENTATION IN HUMAN CORTEX: SOMATOSENSORY EVOKED POTENTIALS RELATED TO DISCRETE TACTILE SENSATIONS IN THE ANOGENITAL REGION. H. Stowell, ERBP Unit, Central State Hospital, Milledgeville GA, USA.

Cortical representation of the body surface is of intrinsic interest to neurologists. Correlation of easily replicable, recognizable, natural sensations with cerebral electrophysiology is useful for understanding the functional significance of event related brain potentials (ERBP). Marginal mucocutaneous skin of anogenital regions has these advantages over other peripheral sites for recording somatosensory evoked potentials (SEP): (1) Transcutaneous electrical minipulses (0.03-0.08 ms), suitably entrained, elicit only tactile, superficial, and spatiotemporally discrete sensations, without shock overtones, for a wide range of qualities and magnitudes; (2) SEP so elicited are amplitude-focal at vertex, without polarity reversal laterally or evidence of electro-ocular interference; (3) risk of local muscular reflex is low (contrasted to median and other nerve-trunk stimulation); and (4) stimulation and recording sites are distant (compared to lip and orodontal sites, where sensation also tends to radiate).

For formal Informed Consent reasons, this intensive study was confined to two volunteers: an experienced male who self-paced his stimulus time-window, and a relatively naive female who received conventional, regularly repetitive stimulation programmed by the experimenter. Unequivocal results: (1) Anogenital SEP has later cortical processing times (peak latencies, 30-250 ms) than SEP for comparable stimulation, electrical and mechanical, eliciting comparable sensory magnitudes but different qualities, on hand and foot; also higher mean frequency in the intermediate time segment (50-175 ms) than is recordable for stimulation at other extremities; (2) its dipole origin seems not to be oriented normal to the gross medial surfaces of the hemispheres, as expected from the classical neuroanatomy.

It is hoped the above advantages will outweigh possible psychosocial disadvantages. This form of input might be a more sensitive probe than median nerve shock for diagnostic SEP recording in prospective surgical cases.

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682 TRANSIENT WAVE ADJUSTMENT: AVERAGING IN THE FREQUENCY DOMAIN. Andrea Lee Thompson* and Donald O. Walter. BRI, UCLA, Los Angeles, CA 90024.

In those situations where one or more components of activity vary in latency, averaging in the time domain underestimates their amplitude and smears their shapes. The Fourier transform separates amplitude and phase information, allowing us to estimate those parameters separately for each record. One use of these parameters is to estimate their average for each frequency. The inverse transform of this function is a latency-adjusted average wave in the time domain with well estimated amplitude and shape.

Applications of transient wave adjustment (TWA) to event-related potentials, and to produce a "typical" page of EEG will be displayed.

683 BRAINSTEM AUDITORY EVOKED POTENTIALS (BAEPs): WHEN IS WAVE-2 A ROSE AND WHEN IS IT A CARNATION ? Peter L. E. van Kan*, Rob McLain*, Gwen Gale*, Al Seacord*, W. Jeffrey Weidner*, and Timothy A. Jones. Department of Animal Physiology,UCD,Davis,CA. 95616

It is often the case that multiple electrode derivations are used to record and identify specific peaks of BAEPs. This usually entails recording from two vertex-mastoid pairs, one from each mastoid side and a common vertex as "G1". In cat both derivations produce a series of positive peaks which can be labeled 1 through 5 with activity in primary afferents producing wave-1 of both records. For the first three peaks slight differences in latencies and amplitudes are commonly seen between the two records even though they are recorded simultaneously. Ordinarily, the generators for each peak have been taken to be the same for both derivations, that is, the generator for wave-2 in the ipsilateral derivation is thought to be the generator for wave-2 in the contralateral perspective. In a series of experiments designed to identify BAEP generators in cat, we have established that this latter point of view may be missleading; that is for example, in the case of wave-2 the brain region generating this component in ipsilateral records may not be the same region which generates wave-2 in the contralateral record. This multiple generator concept is suggested since cooling in two putative generator regions differentially affects the two records, where the contralateral wave-2 can be delayed independently of the ipsilateral wave -2. A similar phenomenon can be demonstrated for waves 3 and 4 suggesting a generalized bilateral representation where the generators of each respective side can be physiologically and anatomically distinguished although the respective far-field potentials may be labeled the same. In the case of wave-2, ipsilateral cooling in caudal pontine regions, as would be expected, slows activation of both generators whereas contralateral cooling affects contralateral wave-2 to a greater extent than the ipsilateral wave-2.

684 BINAURAL INTERACTION IN AUDITORY BRAINSTEM POTENTIALS. Kathy S. Wrege* and Arnold Starr (SPON: C. E. Ribak). Depts. of Psychobiology and Neurology, Univ. of Calif., Irvine, Irvine, CA 92717.

Binaural interaction was examined by recording auditory brainstem responses to clicks from scalp electrodes (vertex-neck) in human subjects. Deviations of the binaural response from the sum of the monaural potentials were observed at the time of occurance of waves V-VII. Both amplitude and latency of the interactions were dependent on click polarity: rarefaction clicks produced interactions of larger magnitude relative to the monaural sums (38.2 ±9.3%) and longer latency (6.26 ±.65 ms.) than did condensation clicks (25.4 ± 4.4%; 5.34±.63 ms.). These latency differences cannot be accounted for by any concurrent latency shifts in the monaural or binaural evoked potentials. The relationship of binaural interaction to stimulus intensity, interaural time and intensity differences, and audiograms were examined.

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685 PSEUDORANDOM BINARY SEQUENCE (PRBS) STIMULATION FOR VISUALLY EVOKED POTENTIALS (VEP). Weldon W. Wright, Richard Srebro and Barry A. Sokol*, Department of Ophthalmology, Southwestern Medical School, Dallas, Texas 75235.

PRBS stimulation permits a rapid measurement of the power spectrum of the VEP with an input that approximates bandlimited white noise but which is amenable to signal averaging techniques. We have applied the method to both luminance modulation of a 30^{0} homogeneous red field and to pattern reversal of sinusoidal and square wave gratings. Power spectra were obtained in less than 2 minutes of recording time. These spectra are similar to those obtained using an array of single sinewave frequencies each presented separately but the PRBS technique requires much less recording time and has the additional advantage that separate epochs are not required for each frequency tested. The PRBS consists of a repeating sequence of N equally likely binary states. It simulates a true coin toss experiment in that the number of runs of consecutive identical states of length γ is proportional to (1/2) State changes are allowed only at equally spaced time intervals, Δt, and the sequence has a periodicity of $N\Delta t$. In the frequency domain, the PRBS is represented by a set of discrete equispaced pulsed of constant amplitude in the frequency band from $1/N\Delta t$ to $1/3\Delta t$. The PRBS can be conveniently generated using a shift register with appropriate feedback, An n stage shift register generates a PRBS whose length is $N=2^{n}-1$. Selecting n and Δt, permits adjustment of the frequency resolution and bandpass appropriate to the system being studied. We have generated PRBS stimuli from both an inexpensive hardware shift register and by simulation using a small computer. For luminance modulation the binary states were made to coincide with 2 adjustable luminance levels so that average luminance and % modulation were precisely and independently adjustable. For pattern reversal experiments the position of the grating on an oscilloscope was shifted by 1/2 cycle left or right in accordance with the binary state of the PRBS. The VEPs were recorded both by using a conventional signal averager and were later digitized, or were averaged directly by the lab computer, A Blackman-Harris spectral window was applied to the VEP before taking the Fourier transform.

686 EVOKED POTENTIALS TO SHIFTS IN FREQUENCY OF A CONSTANT TONE Charles D. Yingling. Langley Porter Neuropsychiatric Institute, University of California, San Francisco, CA 94143.

Much recent research in visual evoked potentials has used patterns (such as checkerboards) or pattern transitions (ie., checkerboard reversals) as stimuli rather than simple light flashes. In the pattern reversal EP, there is no change in the total stimulus flux, the effective stimulus being rather the change of pattern in a field of constant average illumination. The EPs thus produced are different in both waveform and scalp distribution from those produced by flashes or even flashed checkerboards, suggesting that a different process is involved in their generation (Allison et al, EEG Journal 42:185-197,1977).

In the auditory modality, the simplest example of such a shift in pattern is a sudden shift in frequency of a tone of constant amplitude. Given the tonotopic mapping of auditory cortical areas, one might predict that a greater shift in pitch would cause a greater shift in the neural population activated by the stimulus and that this would be reflected in the amplitude of the cortical EP. To test this hypothesis, six adult subjects listened to a constant 1250 Hz tone which changed unpredictably by an amount ranging from a barely detectable 20 Hz to a shift of 1050 Hz, nearly an octave higher. Subjects were instructed to count the number of occurrences of the smallest shift. 48 trials at each shift were averaged from Cz referenced to A_1.

All subjects displayed clear EPs to the transitions, with an "N100" component whose latency ranged from 150 msec (20 Hz shift) to 120 msec (1020 Hz shift) and an "N200" component of relatively constant 215 msec latency. The N100-P200 peak to peak amplitude increased almost monotonically as a function of the amount of shift, ranging from 4.58 μV (20 Hz shift) to 13.42 μV (1020 Hz shift). The P300 component, defined as the most positive point between 300 and 500 msec, averaged 5.67 μV to the target shift of 20 Hz, and only 2.94 μV to the nontarget shifts which had however produced larger N100-P200 components. Thus the earlier components depend primarily on the physical parameters of the stimulus (ie., amount of shift) whereas the P300 component is more a function of the cognitive response of the subject (target detection).

In a second experiment, five subjects listened to a narrow range of shifts, from 20 to 120 Hz above the same base tone of 1250 Hz. These shifts were so similar that subjects could not accurately tell them apart, so no detection task was performed. Nevertheless, the N100-P200 amplitude again varied as a function of the amount of shift, being within one point of a monotonic function for three of the five subjects. In this case, the EP was apparently more sensitive at detecting differences among these small shifts than were the subjects themselves.

FEEDING AND DRINKING

687 THE EFFECT OF RESTRICTED FOOD AVAILABILITY SCHEDULES ON CIRCADIAN RHYTHMS. Norman T. Adler and Rodney J. Pelchat. Dept. Psych., Univ. of Penna., Phila., PA.

Much recent work has suggested that food restriction schedules (FR) can entrain or synchronize circadian rhythms. We performed a series of experiments to compare the entraining effects of food availability cycles to the known effects of light cycles on two rhythms - wheel-running activity and feeding as measured by an operant response which led to food delivery. Although some effects seem best explained by entrainment of an oscillator, others clearly do not fit the classic effects of entraining agents.

First, although there is an anticipatory wheel-running response to FR which can persist for two days of total food deprivation, FR does not entrain free-running activity rhythms of either blinded animals or animals in constant light. The free-running rhythm and the food-related activity seem to be independent.

Second, the effect of a shift from FR to ad libitum food while concurrently on a light-dark cycle is different for the feeding and activity rhythms. If food availability were entraining an oscillator, then residual effects of this entrainment might be expected at the old feeding time. Residual effects for the operant feeding response but not for wheel-running were observed.

Third, extra access to food either as an extension of the normal feeding time or as a separate feeding seems to affect the expression of the anticipatory food-related responses of both wheel-running and the food-getting operant.

The results suggest that the food-availability cycles are not acting as classic Zeitgebern and that mechanisms other than entrainment of an oscillator are at work.

688 INSULIN-INDUCED ELEVATION OF HYPOTHALAMIC NE TURNOVER PERSISTS AFTER GLUCORESTORATION UNLESS FEEDING OCCURS. Steven I. Bellin and Sue Ritter. College of Veterinary Medicine, Washington State Univ., Pullman, WA 99164.

Both food intake and norepinephrine (NE) turnover are increased during cerebral glucoprivation. R.C. Ritter, et al. (Am. J. Physiol. 234(6):E617-E621, 1978) have demonstrated that if food is returned to rats 6 hours after regular insulin administration, intake will still be enhanced even though normoglycemia has been restored. In other words, feeding can be temporally dissociated from other signs of glucoprivation. This paradigm, therefore, provides an opportunity to assess the possibility that a glucoprivation-induced increase in brain NE turnover is specifically related to feeding behavior and not to other effects of insulin or glucoprivation. To evaluate these relationships, we measured turnover of NE and dopamine (DA) in selected brain regions immediately and 6 hr after insulin administration in the absence of food. In a second study, catecholamine (CA) turnover was again monitored 6 hr after insulin injection, but food was present during the delay. Sixty-three adult male rats were used in these studies. In the first experiment, rats were treated with saline or regular insulin (3U/kg, s.c.) and α-methyl-p-tyrosine (AMT, 350 mg/kg, s.c.) was given 30 min or 5 1/2 hr later. At 1, 1 1/2 and 2 1/2 hr after insulin, turnover of hypothalamic NE and caudate DA were enhanced compared to saline-AMT controls. Cortical, midbrain and brainstem CA turnover were unaffected. At 6, 6 1/2 and 7 1/2 hr following insulin, however, when blood glucose levels had returned to preinjection baseline values, caudate DA turnover did not differ from control, but NE turnover was still significantly enhanced in the hypothalamus. In the second experiment, rats were given the same dose of insulin but were allowed to eat during the 5 1/2 hr postinsulin period prior to AMT injection. In this study, where food was consumed by insulin-treated rats, the insulin-induced increase in hypothalamic NE turnover was abolished. These results suggest that insulin-induced increases in NE turnover in the hypothalamus may be specifically related to food intake and not to other signs of glucoprivation.

689 OVARIECTOMY (OVX) ALTERS BODY FAT BUT NOT THE LOWERED BODY WEIGHT (BW) 'SET POINT' OF RATS WITH DORSOMEDIAL HYPOTHALAMIC LESIONS. Larry L. Bellinger and Lee L. Bernardis. Dept. Physiol., Baylor Col. Dent., Dallas, TX 75246 and VA Med. Ctr. and Depts, Surg. and Path., SUNY at Buffalo, Buffalo, NY 14215.

Withdrawal of ovarian steroids by OVX results in a temporary increase in food intake with a concomitant increase in BW. Food intake generally returns to control levels when BW reaches a new, higher level. The primary effect of hyperphagia may be to alter the level at which body fat is regulated, i.e., the 'body fat set point.' Recent data shows that rats with DMNL have experienced a lowering of the 'set point' for BW but not for body fat (Bellinger, et al, Neurosceince, in press). The present study was designed to investigate whether OVX would be able to raise the body fat 'set point' in DMNL. Rats received DMNL at the age of 28 days, sham-operated rats served as controls. At the age of 84 days some DMNL and control animals were OVX while others were sham-OVX. BW, food intake and obesity index were monitered and the experiment terminated when the rats were 140 days old. Both OVX and sham-OVX caused precipitous drops in BW that lasted only three days; however, the BW drop was greater in the sham-lesioned than in the DMNL rats. Subsequently, the patterns of BW increase were identical in the DMNL-OVX and sham-lesioned-OVX rats, the curve of the former being merely shifted to a lower absolute BW. The data allows the inference that OVX causes the same degree of BW gain in DMNL and sham-DMNL rats and thus cannot override the BW-lowering effect of DMNL. Deposition of body fat as evidenced by the Lee Index was greater in DMNL-OVX rats than in any of the other three groups of rats. The data suggests that DMNL, although lowering BW on an absolute basis predisposes the rats to the lipogenic consequences of OVX. Similar to BW, the post-operative drop in food intake was smaller in DMNL rats than in sham-lesioned rats when OVX was performed. Subsequently, for six post-operative days DMNL-OVX rats ate more than DMNL-sham-OVX animals, but thereafter food intake normalized. By contrast, sham-lesioned rats responded to OVX by increasing their food intake for 27 days, whereupon they too normalized food intake. The data are analogous to recent findings that DMNL lowers the 'set point' for BW but not for body fat. Furthermore, while DMNL have lowered the BW 'set point,' the neural structures involved in regulating the body fat 'set point' seems intact and more responsive to manipulation, i.e., OVX. Finally, as reported by others OVX may lead to obesity by means other than solely affecting food intake as illustrated in the DMNL-OVX group.

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690 TAIL PINCH (TP)-INDUCED HYPERPHAGIA AND MACRONUTRIENT PREFERENCE IN YOUNG-MATURE RATS MADE HYPOPHAGIC BY LESIONS IN THE DORSOMEDIAL HYPOTHALAMIC NUCLEI (DMN). Lee L. Bernardis and Larry L. Bellinger. Veterans Administration Medical Center and Depts. of Surgery and Pathology, SUNY at Buffalo, 14215 and Dept. of Physiology, Baylor Coll. Dent., Dallas TX 75246.

Sprague-Dawley rats received bilateral electrolytic lesions in the DMN at the age of 49 days. Sham-operated rats served as controls (CON). The rats were originally used in various feeding studies. At the age of 153 days they were subjected to TP for five minutes per day for eight days. Three equicaloric diets, complete in all nutrients and accessory food factors, with one macronutrient higher than the other two, were available ad libitum during the TP sessions in color-coded pellet form. They were a high-carbohydrate (HCD), high-fat (HFD) and a high-protein (HPD) diet. In their home cages, the rats received Charles River Rat Mouse Hamster Formula ad libitum. Rats with DMN lesions (DMNL rats) ate significantly more than their sham-operated controls from each of the three diets during TP sessions, but particularly from the HCD and the HFD diets. Nevertheless, in terms of per cent intake, the DMNL rats and the CON rats ate similar amounts of food from each of the three diets. In their home cages, the DMNL rats showed the previously reported profound hypophagia. Indeed, food intake in the home cages showed over time a trend to decrease in the DMNL rats and a trend to increase in the CON. The data show that the mature DMNL rat, as its weanling counterpart, is grossly hypophagic in its home cage but shows stimulus-bound hyperphagia when subjected to TP. Similarly the mature DMNL rat shows preference for a HCD and HFD diet, as does the weanling DMNL rat. The data also show certain similarities to the stimulus-bound hyperphagia in rats with lateral hypothalamic lesions (LHA rats) which have been shown to be capable of increased food intake during the critical post-operative time until recovery, i.e. spontaneous food intake, supervenes. However, DMNL rats never go through stages of aphagia and adipsia. The possibility is entertained that DMNL cause a partial disruption of some inhibitory tracts, possibly part of the noradrenergic bundle, thus disinhibiting it so that on TP the dopaminergic (DA) system is more active in DMNL than in CON rats.

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691 METHYSERGIDE STIMULATES RHYTHMIC AND ARRHYTHMIC EMG-RECORDED SUCKING IN 20-DAY-OLD RATS. Stephen C. Brake* and Christina L. Williams. Dept. Psychiat., Montefiore Hosp., Albert Einstein College of Medicine, Bronx, N.Y. 10467 and Institute of Animal Behavior, Rutgers Univ., Newark, N.J. 07102.

The likelihood that a rat pup will attach and remain attached to a dam's nipples declines between 10 and 20 days of age, as the pup begins to eat solid food. Methysergide, a putative serotonin receptor blocker, administered i.p. increases the probability of nipple attachment in pups 20 days of age and older without altering pups' control of milk or solid food intake. In this study we asked: 1) Does sucking behavior of rat pups also decline between 10 and 20 days? and 2) Does methysergide stimulate sucking as it does nipple attachment, or does it have no effect on sucking as if sucking were related to intake control?

We injected 10- and 20-day-old, short (4-6 hr) and long (20-24 hr) deprived pups with methysergide (20 mg/kg) or the saline vehicle (10 ml/kg). Twenty min later we recorded their sucking for 1hr using an EMG recording technique designed to measure the frequency, duration, and intensity of dry sucking (sucking from an anesthetized dam who provides no milk).

It has previously been shown that rats less than 14 days of age suck in 2 different patterns: rhythmically and in arrhythmic bursts. Pups deprived of food, water, and sucking for 20-24 hr show both sucking patterns, while nondeprived pups display only arrhythmic bursts at a lower frequency than deprived pups. The present results indicate that 20-day-old pups, in contrast to 10-day-old pups, spend little of the time they are attached to the nipple actually sucking, and rarely suck rhythmically, even after 24 hr of deprivation. However, methysergide stimulates arrhythmic sucking activity and reinstates the rhythmic sucking pattern characteristic of deprived, 10-day-old pups. This effect was not caused by indirect drug effects on activity, since overall EMG intensity and nipple shifting (both indexes of activity) were not altered by the drug. Methysergide had no effect on sucking duration or intensity in 10-day-old pups.

These data show that: 1) Sucking declines between 10 and 20 days of age and, in fact, rhythmic sucking disappears completely, and 2) Because methysergide has previously been shown not to alter pups' control of milk or solid food intake, it is interesting that in 20-day-old pups this drug induces vigorous and prolonged sucking normally displayed only by pups younger than 14 days of age.

692 ANGIOTENSIN-INDUCED SODIUM APPETITE: EFFECTS OF SODIUM SOLUTION CONCENTRATION, DELAYED ACCESS TO SODIUM, AND DIET. Richard W. Bryant, Steven J. Fluharty*, and Alan N. Epstein. Dept. of Biology, Univ. of Pennsylvania, Philadelphia, PA 19104.

Continuous intracerebroventricular infusion (cICV) of angiotensin II (AII) evokes massive intakes of both 3% NaCl solutions and water (Bryant et al., Fed. Proc. 37:1978; Avrith & Fitzsimons J. Physiol. 282:1978). The salt intake is dose-related, specific for Na^+, not secondary to the large water intake and temporally unrelated to it. The appetite appears with a latency of several hours after initiation of the AII infusion and persists after its termination. When 0.9% is substituted for 3% NaCl massive intakes of both 0.9% NaCl and water occur during cICV of AII (6µg/µl/hr), however, unlike the 3% NaCl and water intakes, more 0.9% than water is drunk (116.8±21.7 water, 147.2±15.4 ml 0.9% NaCl;216.1± 33.5 water, 74.5±14.6 ml 3% NaCl). Total intake is therefore the sum of the preference for dilute salt solutions and the induced appetite for salt. The excess intake does not appear more quickly despite access to a preferred concentration, and intakes of 0.9% NaCl persist after termination of AII infusion.

After establishing that cICV of AII produces Na^+ appetite, it was of interest to determine whether salt ingestion was necessary for its induction. Therefore, after rats had access to water and 3% NaCl for 4 days, the 3% NaCl was removed and 4 days of AII (6µg/µl/hr) were begun, and then when cICV of AII was stopped, either no delay or a 24hr delay was imposed before the 3% NaCl was returned. The long delay was imposed to assure that exogenous AII could not be affecting the salt intake when the 3% NaCl was returned. In addition, other groups of rats were fed normal Purina rat pellets (containing approximately 1% Na^+) or a Na^+-free diet either throughout the experiment or only during infusion.

Rats that had normal diet before and during infusion drank immediately and avidly when given access to 3% NaCl (17.1±2.1 ml, 1st day), and it persisted. Avid acceptance also occurred in rats receiving normal diet for the first 4 days then Na^+-free diet throughout the experiment. The 24hr delay did not alter initial salt intake, but made persistence less likely. Rats given Na^+-free diet for 5-13 days with or without cICV of AII also drank immediately, but persistence occurred only in those receiving cICV during Na^+ deprivation.

Thus, 1) it is not necessary that the animals drink salt solutions during AII infusion in order for the hormone to evoke salt appetite, and 2) salt ingestion, either in solution or in solid food, during chronically elevated AII, produced either by cICV or by Na^+ deprivation, appears to be necessary in order for the excess salt intake to persist. Supported by MH07177-02, MH07753-01, NINCDS03469.

693 VMH OBESITY REDUCED BUT NOT ABOLISHED BY SCOPOLAMINE METHYL NITRATE. Richard G. Carpenter* and S.P. Grossman. Univ. Of Chicago, Chicago, IL 60637.

A recent review by Powley (Psych.Rev.84:89, 1977) suggests that VMH obesity may be entirely explained by increased vagally mediated insulin secretion. Friedman and Stricker (Psych. Rev. 83:409, 1976) agree but suggest that vagal (or other neural) influences on the liver may also contribute to the obesity. Vagal influences should be abolished by antimuscarinic drugs. Therefore, food intake and body weight were measured daily for controls and rats with ventromedial (VMH) obesity. Chronic treatment with the antimuscarinic drug scopolamine methyl nitrate (ScMN; 0.15mg/Kg 4 times per day) which does not enter the CNS, reduced food intake initially to about 10gm/day in both control and VMH obese group. Intake recovered to normal during the next 5-10 days in the controls; body weight loss was less than 10gm and was recovered in 10 days. VMH rats recovered normal food intake more slowly and lost 35% (80gm) of their obesity in 15 days. Both VMH and control rats doubled their water intake during ScMN treatment, probably to compensate for the absence of saliva. The dose of ScMN was doubled at 20 days, again at 30 days, and again at 35 days. By 40 days food intake was again essentially normal in both groups; the VMH group had lost only 20gm since day 20, the controls had not changed. With the highest dose, gastrointestinal motility was obviously decreased in both groups; the stomach and gut were distended with food. In a second experiment, rats were maintained on the highest dose (1.2mg/Kg 4X/day) for 20 days, then given VMH lesions. Food intake increased greatly in the first days after VMH lesions (day 3: VMH=40gm, control=18gm); body weight increased by 38gm in 10 days. However, food intake returned to normal after 10 days, with no further weight gain, probably because of gross distention of the stomach and gut.

Vagally mediated insulin secretion has been shown by Henderson et al (Acta Endo.83:772, 1976) and Bergman and Miller (AJP 225:481, 1973) to be abolished by low doses of antimuscarinic drugs. Secretory effects of parasympathetic stimulation are more easily blocked than effects on smooth muscle; this differential sensitivity explains why ScMN treatment causes much less weight loss than vagotomy. These results suggest that a substantial fraction of VMH obesity is independent of vagally mediated insulin secretion.

694 THE EFFECTS OF HEPATIC VAGOTOMY ON SALT INTAKE AND BODY WEIGHT IN RATS. Robert J. Contreras. Yale Univ., Dept. Psychol., New Haven, CT. 06520.

The mammalian liver is believed to contain neural receptors that are sensitive to changes in the osmolarity, and ionic and metabolic composition of the blood. These receptors might play a role in maintaining steady state levels of fluid, electrolytes and glucose by activating physiological and behavioral processes that act to correct for deviations from homeostasis. Sensory functions of the liver are believed to be mediated by the hepatic branch of the vagus nerve. The present study was carried out to ascertain the effects of hepatic vagotomy on salt intake, body weight and food intake in rats.

Six rats received hepatic vagotomies and another six rats received sham operations under sodium pentobarbital anesthesia. All animals were examined postoperatively for two months. The two groups of rats were given two-bottle preference tests between water and solutions of NaCl (.03 M, .1 M, .3 M) or glucose (.1 M). Each test solution was presented for two days and 24 hr intakes were measured. Hepatic vagotomized (HV) rats ingested less NaCl solution (.1 M, .3 M) than sham controls (SC) but there were no differences in glucose intake. The animals were given a second drinking test following 9 to 16 days of sodium deprivation. Both groups showed an increased salt appetite and salt intake compared to rats fed a sodium-replete diet. Nevertheless, HV rats tended to consume less NaCl (.1 M, .3 M) solution than SC, although these differences were nonsignificant. The differences in NaCl intake may be attributable to the lessened ability of HV rats to excrete electrolytes in conditions of osmotic stress (Adachi, Niijima and Jacobs, 1976).

HV rats gained weight at a faster rate than SC. These differences in weight gain were modest, yet were apparent soon after the operation and continued for the two month period of examination. The preoperative weight of SC was 4.8 g more than that of HV rats; two months later HV rats weighed 37.4 g more than SC. The relative obesity of HV rats was apparently not due to differences in food intake. For six days daytime (7 am - 7 pm) and night-time food intakes were measured. The two groups of rats did not differ in the amount of food consumed over 24 hr nor in their relative intakes between day and night periods. The differences in average body weight agree with the results reported by Sawchenko and Friedman (personal communication) although they also report that HV rats ingested more food than SC. The relative obesity of HV male rats may be due to disturbances in peripheral metabolic processes rather than due to hyperphagia. (Supported by a NIH Biomedical Research Support Grant 5-507-RR-07015).

696 SEX DIFFERENCES IN RAT OBESITY PRODUCED BY HIGH-FAT DIETS. D.V. Coscina, J.W. Chambers*, and G.H. Anderson*. Sect. Biopsychol. Res., Clarke Inst. Psychiat., and Dept. Nutr. Food Sci., Fac. Med., Univ. Toronto, Toronto, ONT. M5T 1R8

Under fixed environmental conditions, rats closely regulate their bodyweight (BW) by eating constant quantities of energy in response to caloric dilution or concentration of single diets. Therefore, the relative overeating and obesity which can develop on diets high in fat is an interesting model of apparent breakdown in caloric intake metering. However, a confound exists in that such caloric enhancement simultaneously lowers protein concentration. This latter factor is rarely considered despite abundant data demonstrating behavioral and physiological regulation of protein intake in this species. Our experiment addressed this issue by comparing the development of obesity on high-fat (HF) diets in which the concentration of energy in the form of protein was either low or equal to that found in standard maintenance diets. Adult (250-280g) male (n=30) and female (n=30) Wistar rats were housed separately in a temperature (22°C±1°) and light (12 hrs on commencing 0800 hrs) controlled colony. Following 2 wks acclimatization to these conditions and ad lib access to Teklad chow (4% fat) and tap water, rats were randomly assigned to one of three (n=10 each) dietary conditions: (1) stock diet (18% protein energy in the form of casein; 5% fat; 4 kcal/g), (2) HF diet (7.2% protein energy; 43% fat; 6 kcal/g), (3) HF diet (2) + casein, vitamins and minerals at the expense of cornstarch (18.1% protein energy; 43% fat; 5.75 kcal/g). Tap water and diets (in non-spill food cups) were freely available for 9 wks during which BWs and food intakes were recorded every 2-4 days. At the end of this time, all rats were lightly etherized for measurement of naso-anal (NA) length. Clear sex differences in magnitude and expression of obesity were found as a function of HF diet type. Female rats showed equal linear growth on all diets. Both HF diets produced equivalent enhanced BW gain compared to controls (+220%) and equivalent obesity (316 or 318) by the Lee index (cube root of total g BW X 10^4/mm NA length; 300-310 is considered normal). Male rats showed slight but significant linear growth decrement (-2%) on HF but enhanced linear growth (+3%) on supplemented HF. BW gain was significantly enhanced only on supplemented HF (+60%). However, the Lee index showed males to be obese on both HF diets (314 or 316). These data show that (1) the continued linear growth of adult male vs female rats confounds assessment of obesity if only BW is used as an index, and (2) unsupplemented HF diets create nutrient deficiencies which impair growth of lean body mass in male rats.

697 BEHAVIORAL AND NEUROCHEMICAL EFFECTS OF NEONATAL ADMINISTRATION OF MONOSODIUM L-GLUTAMATE IN THE MOUSE. Ralph Dawson, Jr. and Joan F. Lorden. Dept. of Psychology, Univ. of Alabama in Birmingham, Birmingham, AL 35294.

Mice treated during infancy with monosodium L-glutamate (MSG) suffer neuronal degeneration and exhibit both obesity and neuroendocrine abnormalities as adults. The present study evaluated the behavioral, physiological and neurochemical effects of MSG treatment. Albino mice were injected with MSG from days 4-13 postnatally in increasing doses from 2.8 mg/g to 4.8 mg/g. Controls received equivalent volumes of 0.9% saline. MSG-treated mice showed significant increases in body weight and adiposity and significant decreases in nasoanal length. Animals were housed in either individual or group cages. Many characteristics of the MSG syndrome were found to differ under these conditions. When mice were caged individually, body weight declined to control levels; however, at sacrifice the MSG-treated mice were found to have a significantly higher percentage of body fat than controls. Activity levels were significantly lower in group-housed MSG animals than in controls; but no differences were observed in the individually-housed mice. Body temperature was also affected by housing condition. At 22°C the group-housed MSG-treated mice maintained their body temperatures at a significantly lower level than any of the other groups. No differences in mean body temperature were observed between MSG and saline-treated mice housed individually. Measures of food intake indicated that MSG-treated mice were hypophagic in both housing conditions and consumed a significantly larger percentage of their food during the light part of the light-dark cycle than did controls. In addition, 24h food deprivation produced a significantly smaller increase in food intake in the MSG groups than in the controls. A glucoprivic challenge induced by injection of 250 mg/kg of 2-deoxy-D-glucose (2DG) caused a significant suppression of food intake in both MSG and control mice that were caged individually. In group-housed animals, 2DG significantly increased food intake in MSG and saline treated mice; however, the increase was significantly larger in the controls. The data suggest the importance of housing condition as a variable; but the mechanism by which housing alters the MSG syndrome is unknown. It is also apparent that hypophagia and adiposity are relatively enduring aspects of the syndrome.

Assay of hypothalamic norepinephrine (NE) and dopamine (DA) indicated that in the mouse MSG treatment significantly reduced DA but did not alter NE levels. The behavioral effects of MSG cannot as yet be attributed to a loss of DA; however, a change in dopaminergic control of the pituitary may account for some aspects of the syndrome. (Supported by NINCDS Grant 14755-01)

696 WEIGHT LOSS CAUSED BY INTRAVENTRICULAR INFUSION OF SUBSTANCES INVOLVED IN CARBOHYDRATE AND FAT METABOLISM. John D. Davis, Karen Asin, and David Wirtshafter, Dept. Psychol., Univ. of Illinois, Chicago, IL 60680.

Body weight and food and water intake were measured in rats before, during and after continuous intraventricular infusion of a variety of substances which can be metabolized by the brain. Unincumbered infusion was achieved by means of a subcutaneously implanted Alzet osmotic minipump which was connected to a ventricular cannula by a polyethylene tube. The pumps delivered fluid to the ventricular space either at a rate of 1.0 µl hr^{-1} for 7 days or 0.5 µl hr^{-1} for 10 days. The substances infused were glycerol, propylene glycol, glucose, fructose and β-hydroxybutyrate, all at a rate of 0.15 µM hr^{-1}. All solutions infused were adjusted to be isoomolar with cerebrospinal fluid by adding appropriate amounts of NaCl to the solution.

The infusion of all of these substances resulted in a significant reduction in body weight to approximately 90 per cent of the initial weight. The weight loss resulted in part from a hypophagia which occurred principally during the dark phase of the 12:12 light dark cycle. Food intake during the light phase was largely unaffected. Infusion of 0.15 M NaCl resulted in a transient weight loss due probably to trauma associated with pump implantation, but after this brief period of weight loss the rats gained weight at a normal rate during the remainder of the infusion period. Infusion of 0.15 M glycerol into the fourth ventricle had effects similar to those obtained with NaCl in the third ventricle. The amount of weight lost during infusion of the nutrients was correlated with the body weight at the beginning of the infusion period ($r = +.77$, $p < .01$). When the infusions were terminated, all of the animals gained weight rapidly and returned toward their normal body weight. When body weight was reduced by food deprivation prior to the infusion of 0.15 M glycerol no further reduction in body weight was observed during a 10-day infusion period.

Neither the total weight lost during the infusion perior nor the accelerated weight gain after the infusions were terminated could be accounted for solely in terms of the magnitude of changes of food intake. For example, the correlation between weight loss and reduction in food intake during the infusion period was +.30.

These results are interpreted as indicating that a variety of substances involved in carbohydrate and lipid metabolism are also involved in the body weight and food intake control mechanisms of the brain. Brain tissue in the vicinity of the third ventricle may be monitoring the availability of carbohydrates and products of lipid metabolism in its regulation of body weight and food intake. Supported by NSF Grants BMS75-17091 & PCM78-09602.

698 AREA POSTREMA LESIONS CAUSE INCREASED INTAKE OF HIGHLY PALATABLE FOOD. Gaylen Edwards and Robert C. Ritter. Dept. of Veterinary Medicine, Univ. of Idaho, Moscow, ID 83843, and College of Veterinary Medicine, Wash. State Univ., Pullman, WA 99164.

The area postrema (AP) is a circumventricular organ located in the caudal fourth ventricle. This structure, which is a known chemoreceptor for certain drugs and toxins, has been shown to be involved in drug-induced vomition and formation of conditioned taste aversions (McGlone et al., Neuroscience Abst cts, 1979). In addition, the AP is in close anatomical proxim ty to cranial nerve nuclei and integrative centers for gustatic and gastrointestinal function. These anatomic and physiologi al features recommend the AP as an area through which control of feeding behavior might be exerted.

We have made lesions of the AP in adult male rats. After recovery from surgery, AP lesioned rats consumed normal amounts of laboratory chow and water ad libitum. Following 21 hours of food deprivation, AP lesioned rats increased their food intake by amounts which were statistically indistinguishable from sham operated rats and unoperated controls. However, when non-deprived AP lesioned rats were permitted to consume a highly palatable liquid food (vanilla instant breakfast) during a thirty minute test, they consistently ate more than double the amount consumed by sham operated or control rats. For example, in a typical experiment involving 10 lesioned rats and a pooled group of 5 sham operated and 5 control rats, the AP lesioned rats ate 27.5 ± 2.5 mls whereas shams and controls averaged only 12.1 ± 1.0 mls. This increased intake by AP lesioned rats appeared shortly after surgery and persisted for at least 6 months.

Overeating of the highly palatable liquid food was suggestive of a satiety deficit in AP lesioned rats. Therefore, we tested the possibility that AP lesion-induced overeating resulted from reduced sensitivity to the putative satiety hormone cholecystokinin (CCK). Intraperitoneally injected CCK octapeptide (20 or 40 Ivy dog units/kg) produced similar percentage decreases in liquid diet intake by all groups.

Our data suggest that the AP is involved in termination of feeding induced by highly palatable liquid foods. The elevated liquid food intake by AP lesioned rats cannot however be attributed to decreased sensitivity to CCK. Therefore, destruction of the AP may impair satiety by interfering with detection of, or response to, some other satiety signal.

699 ELEMENTAL HYPOTHALAMIC OBESITY AFTER DISCRETE LESIONS OF THE PARAVENTRICULAR NUCLEUS. Ricardo Eng, Richard M. Gold, and Antonio Nunez*. Dept. Psychol., Univ. Mass., Amherst, MA 01003.

The large electrolytic lesions of the ventromedial hypothalamus (VMH) which produce hyperphagia and obesity in the rat typically damage many nuclei and fibers and thus are not useful for the localization of neural substrates mediating satiety. More discrete lesions have shown that the critical area which must be damaged lies rostral to the ventromedial nucleus (Gold, 1973). Using asymmetrical knife cuts Gold et al. (1977) revealed the coronal plane of the paraventricular nuclei (PVN) as the rostralmost terminus of the satiety neurocircuit. The PVN is the most sensitive brain site for norepinephrine-induced eating (Leibowitz, 1978). However, knife cuts of the tractus filiformis, a major noradrenergic tract to the PVN, paradoxically produce a transient reduction in food intake, despite a significant depletion of PVN norepinephrine (Crowley et al. 1978). In the present study we report that discrete lesions of the PVN produce hyperphagia and rapid weight gain in the rat without the several additional deficits traditionally associated with VMH lesions. We thus suggest a specific role for the PVN in satiety.

Bilateral anodal lesions were made in 40 female rats using a platinum electrode (1 MA x 10 sec.). The lesions were aimed in a grid pattern centering on the PVN.

The greatest weight gains (7 g/d) were obtained when the PVN was completely and selectively destroyed. This rate is comparable to that produced by large VMH lesions. Partial damage to the PVN produced a partial effect (3-4 g/d) while lesions sparing the PVN showed weight gains comparable to that of shams (0-2 g/d). Thus no single compact fiber bundle entering or leaving the PVN can be implicated. The normal nocturnality of eating and drinking was not significantly reduced by PVN lesions (70% vs. 80-90% for controls). The PVN lesions also did not produce polydipsia (water to food ratios were normal). Rats showed normal estrous cycling and wheel running activity. In contrast, large obesity-producing VMH lesions or hypothalamic knife cuts usually disrupt all 4 of those measures. We conclude that traditional VMH lesions disrupt PVN afferents and/or efferents.

700 INTRAVENTRICULAR 2-DEOXY-D-GLUCOSE CAUSES FEEDING IN THE ABSENCE OF HYPERGLYCEMIA. Robert M. Engeset* and Robert C. Ritter. College of Veterinary Medicine, Washington State University, Pullman, WA 99164.

Previous work from our laboratory demonstrated that feeding in response to 2-deoxy-D-glucose (2 DG) or insulin persists after the sympatho-adrenalmedullary response to glucoprivation has abated (American Journal Physiology 234-236, 1978). This result suggested that the stimulus for 2 DG and insulin induced food intake might persist in the absence of ongoing glucoprivation, i.e., postglucoprivically. In order to determine whether postglucoprivic feeding is mediated by central or peripheral receptors, we investigated feeding induced by intracerebroventricular (ICV) 2 DG infusions. Sequential blood samples for glucose analysis collected after ICV 2 DG doses of 3.5 or 5.0 mg confirmed that 2 DG-induced hyperglycemia had abated by six hours post 2 DG infusion. Feeding tests were performed in which food was withheld until six hours after the ICV 2 DG infusion (delayed feeding test). When food was subsequently returned for two hours, rats infused with ICV 2 DG (3.5 or 5.0 mg) ate 1.2 ± .3 and 1.5 ± .5 grams respectively above control intakes even though the 2 DG-induced hyperglycemia had abated. Since both ICV 2 DG doses were below the peripheral threshold dose for feeding, these results suggest that at least part of the delayed feeding after 2 DG resulted from activation of cerebral receptors.

It is interesting that the magnitude of feeding induced by ICV 2 DG is only 50% of that which is induced by subcutaneous 2 DG. This finding suggests that either part of the feeding response is not mediated by receptors accessible from the cerebral ventricles or that the kinetics of 2 DG presentation to cerebral receptors are less effective via the ICV route than via the subcutaneous route. We are currently pursuing experiments to determine which of these explanations is correct.

701 TAIL-PINCH BOUND FOOD-DIRECTED BEHAVIOR EXHIBITED BY LATERAL HYPOTHALAMIC RATS DEPENDS UPON LESION SIZE. Barry Fass and Jeffrey M. Greenspon*, Psych. Dept., Clark U.,Worcester, MA 01610

Feeding and sensorimotor impairments characteristic of Stage 1 of the lateral hypothalamic (LH) syndrome are hypothesized as resulting from a loss of endogenous tonic-arousal (Levitt & Teitelbaum, 1975; Wolgin & Teitelbaum, 1978). If this hypothesis is correct, then exogenous sensory-stimulation which increases LH rats' arousal should restore feeding behavior (Stricker & Zigmond, 1976). Confirmatory evidence previously was reported; for example, noninjurious tail-pinch (TP) allegedly resulted in "basic feeding behavior" when administered to LH rats presented with dry food (Mufson, Balagura, & Riss, 1976).

We now report contrary evidence; namely, that TP given to LH rats in Stage 1 does not elicit feeding, but rather food-directed behavior (FDB) which easily can be mistaken for feeding upon casual observation. Two groups of 40-day-old male albino rats (N=8 per group) were subjected to 1.0 mA anodal-current passed through LH. Current passage lasted for 10 sec for one group (after Mufson et al., 1976) and 20 sec for the other (after Levitt & Teitelbaum, 1975); coordinates for lesion placement were held constant across groups (after Lytle & Campbell, 1975). Every subject was aphagic and adipsic on the second day after surgery; however, 10-sec rats were noticeably less somnolent and cataleptic than 20-sec rats. Noninjurious TP administered to 10-sec rats for 2 min on the second day after surgery resulted in biting and dropping of dry-food chunks, with little or no chewing and swallowing (mean ingestion=0.15 gm, mean spillage=0.35 gm). In contrast, TP administered to 20-sec rats for 2 min resulted in mouthing of food pellet, with infrequent or no biting (mean ingestion=0.03 gm, mean spillage=0.05 gm). TP-bound behaviors exhibited by 10-sec rats were 1) different from food-deprivation induced behaviors (i.e., normal feeding) exhibited by intact rats (mean ingestion=1.86 gm, mean spillage=0.22 gm), and 2) reminiscent of TP-bound behaviors exhibited by intact rats, albeit less frequent (mean ingestion=0.73 gm, mean spillage=0.98 gm). Histological analysis of lesions confirmed that damage sustained by 10-sec rats was significantly less extensive than damage sustained by 20-sec rats (p=.01).

The present findings are taken as evidence that arousal resulting from noninjurious TP is not sufficient to restore feeding behavior lost after LH damage, regardless of whether the damage is extensive enough to produce sensorimotor impairments. Rather, TP results in FDB superficially similar to feeding in that both involve biting of pellet. FBD differs from feeding, however, in that FDB involves dropping of food-chunks with little or no chewing and swallowing. Funds provided by Clark Univ. and by grant 5 ROl AG00295-03 awarded to D. Stein.

702 SODIUM APPETITE INDUCED BY CONTINUOUS INTRAVENOUS INFUSION OF ANGIOTENSIN II. A.L.R. Findlay* and A.N. Epstein. Dept. Biol. and Inst. Neurol. Sci., Univ. of Pa., Phila., PA 19104.

It has been shown that continuous infusion of angiotensin II (AII) into the anterior cerebral ventricles provokes a large increase in the ingestion of strong NaCl solutions (Avrith and Fitzsimons, J Physiol 282: 40P, 1978; Bryant, Fluharty and Epstein, Fed Proc 37: 323, 1978). If the hormone participates in the arousal of the appetite for sodium that arises during sodium deficiency, then it should induce excess sodium chloride intake when its level is increased in the blood as well as in the cerebral ventricles.

Adult male rats that had been maintained on dry food and both water and a 3% solution of NaCl received continuous infusion of AII through right atrial catheters. AII was delivered in 0.315 ml hr^{-1} of isotonic saline at doses of 15, 30 and 60 ng min^{-1} rat^{-1}. Infusions were maintained (except for brief interruptions once a day) for 3 to 5 days. During the first night of infusion, intakes of salt increased above preinfusion levels in rats infused at 30 and 60 ng min^{-1}. Surprisingly, at the 30 ng min^{-1} dose, water intake did not increase until the second day of infusion. The intake of saline and water remained elevated for so long as the infusion continued. In most rats there was no tendency for persistence of high levels of salt intake after the cessation of AII infusion. The results (mean intakes in ml ± SEM) for the middle dose of AII are shown below:

	Day before infusion (5)	Days after infusion Day 1 (5)	Day 2 (5)
Water	36.6±7.3	44.4±3.9	43.8±2.9
3% NaCl	0.0±0.0	3.8±2.6	1.8±1.8

	AII Infusion Days (30 ng^{-1} min^{-1} rat^{-1}) 1 (5)	2 (5)	3 (5)	4 (3)	5 (2)
Water	36.8±7.3	70.0±7.3	78.2±12.7	77.3±19.6	73.5±24.5
3% NaCl	15.2±4.5	21.0±3.6	20.0± 7.2	22.3±11.9	21.0±20.0

(Numbers in parentheses = number of animals)

Mean daily intakes (ml) at 60 ng hr^{-1} were 103.6±4.1 (water) and 30.4±2.6 (3% NaCl); at 15 ng hr^{-1} were 34.3±6.6 (water) and 4.4±2.1 (3% NaCl). The animals did not gain excess weight and there was no sign of edema at any dose.

Elevated blood-borne levels of AII are clearly capable of inducing appetite for salt, and this can occur without necessarily increasing water intake.

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703 EATING AND DRINKING FOLLOWING CENTRAL CHOLINERGIC AND ADRENERGIC STIMULATION OF THE GENETICALLY OBESE RAT. Judith A. Finkelstein and William T. Chance. Depts. Anat. and Pharmacol., N.E. Ohio Univs. Col. Med., Rootstown, Ohio 44272.

The genetically obese Zucker rat represents a possible animal model for the experimental analysis of human obesity. The obesity is transmitted as a Mendelian recessive trait and appears to result primarily from hyperphagia. Although food intake of the fatty rat (fafa) and its lean littermate (Fa-) has been studied with a variety of drug and dietary manipulations, no reports of eating following chemical stimulation of hypothalamic areas have been made. The literature concerning the neuropharmacology of eating has typically focused upon noradrenergic systems as principle mediators of the response. Recent research has indicated that cholinergic stimulation of perifornical hypothalamic areas (PFH) also elicits dose-dependent eating in satiated rats (Chance et al., Physiol. Psych. 5: 440, 1977). In this experiment, we examined eating and drinking following cholinergic and noradrenergic stimulation of the PFH in 6 fatty and 6 lean littermate Zucker rats. The rats were anesthetized (chloral hydrate) and cannulae were implanted into the PFH. After at least one week to recover from surgery, the rats were placed on ground chow and water ad lib. An additional 7 day period of adaption to the new diet was allowed before any intracranial injections. Food and water intake was determined in satiated rats 1 hr. following the initial control injection of normal saline (1 µl) as well as 1 hr. following subsequent administration of carbachol (CARB; 4.0 nmol) or norepinephrine (NE; 24.0 nmol) with intake being expressed as mean differences between these two periods. In the initial experiment CARB was administered daily for 5 consecutive days. Following a 2 day drug-free period, NE was administered for 3 consecutive days. Although there was no difference between groups, NE elicited significant eating which increased from an initial value of 1.8 g to 3.1 g by day 3. Following the injection of CARB, significant drinking was observed in both groups (overall mean = 6.2 g) with no group differences being apparent. Food intake following CARB was dramatically different between the two groups, with the lean littermates consistently showing significant eating across the 5 days (overall mean = 0.9 g). The fatty rats ate more during the control period than following CARB on the first 2 days, however, this pattern changed by day 4 when there was no difference between the obese and lean rats. These data suggest that the adrenergic systems may be similar in fatties and normal rats. Although the physiological significance of eating elicited by cholinergic stimulation is at present unknown, genetically obese rats appear to exhibit an initial refractoriness to this stimulus. Supported by NSF Grant BNS77-19302, NIH Grant 5 R01 NS14344 and NIAAA Grant 5 R01 AA03157.

704 DOPAMINERGIC MODULATION OF FEEDING IN HUNGRY RATS. Harriet R. Friedman* and Edgar E. Coons* (Spons: Samuel M. Feldman). Dept. of Psychology, New York University, New York, N. Y., 10003.

Rats intrahypothalamically injected with dopamine (DA) while 22 hrs food deprived and measured on consumption for 1 hr immediately thereafter demonstrated facilitated food intake compared to baseline saline controls not only on that day but on similarly conducted saline injection tests administered thereafter every 6 days. This after-facilitation declined significantly as the temporal interval between drug and subsequent saline test increased. By contrast, no facilitated food intake was observed in rats injected with DA while satiated and then tested. Nor did these rats show facilitated intake if they were retested on their subsequent saline injection tests while 22 hrs food deprived. These data imply that DA-facilitated feeding is strongly linked to the state of the animal at the time of the DA injection and not to the state on subsequent saline retests. Given, but only given, that a reward mechanism is activated, as it is when a rat encounters food while hungry, does it appear that DA can enhance this activation. But once the enhancement occurs this facilitated reinforcement of food intake is reflected on similar saline tests for some time thereafter.

In the preceding experiments the rats tested 22 hrs deprived nevertheless had free access to food between test dates; a further experiment placed severe constraints upon the feeding situation by routinely allowing the rats only 2 hrs daily access to food. After weight and intake stabilized, DA was intrahypothalamically injected prior to the presentation of food. Food intake was not significantly increased. However, intake was not attenuated as has been reported by Heffner, Zigmond and Stricker (J. Pharm. Exp. Ther., 201: 386-99, 1977) after i.p. injections of DA agonists in a similar paradigm. These data suggest that under such temporal restraints the pressure to feed until satiated superceded at all times any expression of DA-facilitated intake. A role of DA in modulating the rewarding consequences of food intake will be discussed further.

705 MEAL PATTERNING IN INTACT AND VAGOTOMIZED ANIMALS. Paula J. Geiselman, James R. Martin, Dennis A. VanderWeele, and Donald Novin. Dept. Psychol. & Brain Res. Instit., UCLA, Los Angeles, CA 90024; Dept. Beh. Sci., Swiss Fed. Instit., Zurich, Switzerland; Dept. Neurol., NYU Sch. Med., New York, N.Y. 10016.

In Experiment 1, meal patterns of intact female rabbits were measured throughout a 12:12 light-dark cycle. Changes in food intake across the cycle were reflected differentially in other variables, depending upon the specific time of the cycle at which the animals were tested. The increase in food intake occurring during the hours immediately following light offset was attributed to increased meal frequency and feeding rate, while that found during the latter hours of darkness was attributed to increased meal duration and meal size. Meal patterning was also nonhomogeneous within the various portions of the light-on period. The differences in meal patterning of intact animals from one period to another would suggest that this source of variation has been problematic in developing an understanding of short-term regulation of food intake.

Experiment 2 was conducted to study the disruption of feeding patterns following vagotomy. This study demonstrated the times of the light-dark cycle when feeding patterns of vagotomized rabbits were different from those of intact rabbits and, further, delineated the relevant variables that were altered by vagotomy. During the first portion of the dark cycle, vagotomized rabbits were distinguished from intact rabbits by slower feeding rate and decreased total food intake. Interestingly, the meal patterning of vagotomized animals was not only significantly different from that of intact animals during the initial portion of the dark cycle but, furthermore, bore a resemblance to the feeding patterns of both intact and vagotomized animals during the latter portion of the dark cycle. During the period immediately following light onset, vagotomized animals were distinguished from intact animals by decreased feeding frequency, increased meal duration, and increased satiety ratio. During the periods immediately preceding either light onset or light offset, the feeding patterns of vagotomized animals could not be distinguished from those of intact animals.

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706 BOMBESIN SUPPRESSES SHAM FEEDING IN RATS. J. Gibbs and C.F. Martin*. Dept. Psychiatry, Cornell Univ. Medical College and E.W. Bourne Laboratory, The New York Hospital, White Plains, N.Y.10605.

We have previously reported (Abstr.Soc.Neurosci.4:174,#529, 1978) that the gut-brain peptide bombesin (BBS) suppresses food intake in rats, and have suggested that BBS may play a role as a satiety signal when it is released by food during a meal.

This previous test was performed in intact rats feeding normally, in which BBS had the opportunity to interact with other food-contingent satiety signals. In order to analyze the satiety power of BBS alone in a situation in which other effective food-contingent satiety signals were minimized, we tested its satiety action in a sham feeding paradigm. (In this situation, rats eat almost continuously throughout a 60 min test--thus, no effective satiety signals operate.) Thirteen adult male Sprague Dawley rats were each surgically equipped with a chronic gastric stainless steel cannula; these cannulas could be temporarily opened to provide drainage and complete collection of an ingested liquid food. After recovery from surgery, rats were injected intraperitoneally with different doses (2,4,8,16 or 32 $\mu g kg^{-1}$) of synthetic BBS (the gift of R. de Castiglione, Farmitalia Carlo Erba, Milan) or equivolumetric 0.15M NaCl control just prior to presentation of liquid food (25% EC116, GIBCO) following a 17h overnight food deprivation. Tap water was always available.

BBS had a clear and rapid satiety action during sham feeding:

Percent Suppression of Food Intake 15 min after BBS Injection

	Dose of BBS ($\mu g kg^{-1}$)				
	2	4	8	16	32
cannulas open	-6	6*	14*	15*	25**
cannulas closed	16	22*	25**	23*	47**

*$p < .05$; **$p < .01$, statistical difference from control injections

Throughout the 60 min test, BBS not only reduced the rate of sham feeding, but it also elicited prolonged periods during which rats stopped sham feeding entirely, groomed, and appeared to sleep. The incidence of these periods of behavioral satiety increased as the dose of BBS increased.

Comparisons of percent suppressions produced by each dose of BBS between the closed and open cannula conditions (see table) revealed a consistent tendency for BBS to produce a more pronounced satiety action when cannulas were closed, but this tendency did not usually reach statistical significance.

We conclude:(1) BBS alone is sufficient stimulus for the induction of satiety under these sham feeding conditions; (2) the satiety action of BBS does not depend upon the presence of gastric distention.

This study was supported by USPHS grant AM17240 and RSDA MH70874.

707 **HYPOPHAGIA-INDUCING LATERAL HYPOTHALAMIC LESIONS AND KNIFE CUTS: EFFECTS ON MOBILIZATION OF FREE FATTY ACIDS AND BLOOD GLUCOSE.** Carlos V. Grijalva, Donald Novin, and George A. Bray*. Depts. Psychol. and Med., UCLA, Los Angeles, CA 90024.

Ventromedial hypothalamic (VMH) lesions suppress the mobilization of free fatty acids (FFA) but do not affect the rise in blood glucose typically elicited by 2-deoxyglucose (2-DG) (Nishizawa & Bray, J. Clin. Invest. 61: 714, 1978). Because VMH lesions are associated with hyperphagia and obesity, and also with a decrement in sympathetic activity, the present study examined the possibility that hypophagia-inducing lateral hypothalamic (LH) lesions or parasagittal knife cuts (placed lateral to the LH) differentially alter FFA and blood glucose levels.

In the first experiment groups of rats were given bilateral electrolytic LH lesions, knife cuts (KC) or control operations and sacrificed 24 hr postoperatively. The KC group had lower FFA levels than either the LH or control groups, however, the KC and LH groups had higher blood glucose levels than the control group.

In a second experiment 3 groups of rats similarly were given LH lesions, knife cuts, or control operations and allowed 25 days to recover feeding behavior. Two additional groups were pair-fed to the LH and KC groups to maintain equivalent body weight. The results showed that the LH group and the 2 pair-fed control groups had significantly higher FFA and lower triglyceride levels than the KC or control (ad-lib) groups. Because the KC and control group appeared to eat in a similar manner, these differences in lipids probably were related to eating patterns rather than to CNS intervention. The LH group also had lower blood glucose levels than either the KC or control group.

In the third experiment groups of rats were similarly treated as in Expt. 2 except that after 25 days of recovery, all groups were given 2-DG 30 min prior to sacrifice. The results showed that the LH and KC groups were less capable of mobilizing FFA when compared to the pair-fed or ad-lib-fed control groups. Interestingly, the KC group did not show the hyperglycemic response to 2-DG that was seen in the LH group or the control groups.

The present results and previous findings indicate that hypophagia-inducing as well as hyperphagia-inducing hypothalamic damage suppresses the mobilization of FFA, but does not interfere with the hyperglycemic response to 2-DG. However, parasagittal knife cuts placed lateral to the LH additionally block the hyperglycemic response to 2-DG. This unique finding suggests that these knife cuts interfered with the sympathetic activation of the adrenal medulla.

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708 LACK OF INGESTIVE COMPENSATION TO DEHYDRATIONAL STIMULI IN DECEREBRATES. H. J. Grill and R. R. Miselis. University of Pennsylvania, Phila. Pa. 19104.

The traditional view of the forebrain, particularly the hypothalamus, as the site of the requisite neural circuitry for regulatory behaviors has been effectively challenged in recent years. Hypothalamically lesioned preparations have demonstrated compensatory responses to a range of regulatory challenges suggesting to some a more general arousal deficit. Furthermore, it has been shown that the chronic decerebrate rat can regulate its intake of an orally presented sugar solution as a function of food deprivation and repletion (Grill and Norgren, '78). We reasoned that a parallel regulatory system for water intake in response to dehydrational stimuli could exist caudal to the forebrain. The present experiments sought to determine whether decerebrate rats which others have shown to have a functioning osmo-ADH system and which would compensate for food deprivation by increasing their ingestion of a sucrose solution would also compensate for deficits in body water by increasing their intake of orally presented water. Decerebrates (n=11) and their controls (n=18) were examined using a simple behavioral test for their ingestion of orally presented water as a function of 3 dehydrational challenges and were allowed extended periods of time to demonstrate their capacity to compensate. Supracollicular decerebration was performed in two stages with a hand-held spatula; a minimum of 10 days was allowed before testing commenced. Decerebrates and their controls were fed exclusively by gavage. To assess their compensatory response each rat was injected intraorally with 50 µl of distilled water every 5 sec. via fistulas until water was actively or passively rejected. The volume ingested of this constant stimulus was examined under two conditions: sated baseline, 1 hr. post gavage; and challenged, 24,30 or 48 hrs. post gavage; 1, 4, 8 and 24 hrs. post sodium adulterated meal and 4, 8, 10 and 24 hrs. post s.c. hypertonic saline. Subsequent to these experiments the volume of 0.03M sucrose ingested was compared in sated and deprived conditions. Unlike controls, decerebrates failed to increase ingestion of water following intracellular dehydration regardless of the means of producing dehydration or the duration of the time permitted for a response. Since the same rats showed increased ingestion of sucrose as a function of deprivation it is unlikely that general debilitation or lack of arousal would account for these data. The lack of increased water intake in response to dehydration favors the existence of at least some aspects of the regulatory circuitry in the forebrain. Relevant sensors and integrative mechanisms may both reside in the forebrain or one or the other could exist within the caudal brainstem. In summary, when physiologically challenged decerebrates demonstrate regulatory responses to orally presented sucrose but not water.

709 ANALYSIS OF NORADRENERGIC PROJECTIONS MEDIATING FEEDING RESPONSE ELICITED THROUGH PARAVENTRICULAR HYPOTHALAMIC DRUG STIMULATION. Ronnie Halperin, Lucy L. Brown, and Sarah F. Leibowitz. Rockefeller Univ., New York, NY 10021; Albert Einstein College of Medicine, Bronx, NY 10461.

Noradrenergic stimulation of the hypothalamic paraventricular nucleus (PVN) causes an increase in feeding behavior in the rat. This response can be observed after injection of the receptor agonist norepinephrine (NE), as well as after injection of tricyclic antidepressants (such as desipramine and amitriptyline) which produce their effect through activation of endogenous NE stores. In the present study, the ascending noradrenergic projections which innervate the PVN and mediate these drug effects on feeding were examined through the combined use of three techniques, involving hindbrain electrolytic lesions, drug-injection cannulas aimed at the PVN, and fluorescence histochemistry. Tests examining drug effects on feeding were conducted before and after lesion. The functional integrity of mediating postsynaptic receptors and efferent fibers was evaluated through injection of the receptor stimulant NE and the functional integrity of afferent noradrenergic projections from the hindbrain was evaluated through injection of the presynaptically-acting drugs desipramine and amitriptyline. Anatomical analysis of catecholamine (CA) fiber damage was conducted using fluorescence histochemistry.

Dorsal midbrain tegmental lesions, which damaged fibers coursing immediately ventrolateral to the central gray, selectively abolished the feeding response elicited by desipramine, while significantly potentiating the feeding response to NE. This change in drug responsiveness, which did not occur with lesions in other midbrain locations, was associated with a small to moderate loss of CA fluorescence in the PVN and with evidence for degeneration of CA fibers that course through the dorsal pontine tegmentum and radiate sharply ventrally along the dorsal surface of the superior cerebellar peduncles. In attempting to identify the specific cell group(s) from which these mediating fibers may originate, somewhat more caudal lesions at the level of the pons were examined in animals tested with amitriptyline and NE. Discrete lesions, which damaged the anterior portion of the locus coeruleus (A6) cell group but left intact posterior A6 and ventral (subcoeruleus) cell bodies as well as fibers radiating dorsal to the superior cerebellar peduncles, have so far been found to have little impact on the animals' responsiveness to PVN adrenergic stimulation. Results obtained from current studies examining the effects of more caudal hindbrain lesions will be discussed.

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710 THE EFFECT OF EXOGENOUS INSULIN ON FOOD INTAKE IN THE RABBITS. PART I: THE ROLE OF THE DOSE, DURATION OF ACTION AND THE ROUTE OF ENTRY. V. Havlicek and M. Rezek, Dept. of Physiology, University of Manitoba, Winnipeg, Manitoba, R3E OW3, Canada.

Subcutaneous (SC) administration of 1, 5 and 10 U of Protamine Zinc Insulin (PZI) in free-feeding unrestrained rabbits produced a significant and prolonged elevation of diurnal, nocturnal and total daily food and water intakes. Eating was initiated before drinking and the latencies of both responses were considerably shortened; the maximal consumptions occurred between 3 and 8 hr post-insulin. Feeding and drinking changes were paralleled by a gradually developing and prolonged hypoglycemia which was sustained despite the continuous elevation of food intake; the hypoglycemia was also maximal between 3 and 8 hr postinsulin. The dose of 5 U of PZI was most effective in stimulating food and water intake while 10 U of PZI produced greater hypoglycemia associated with manifestations of behavioral depression as well as the alteration of EEG wave pattern and sleep-waking cycle. Repeated daily administration of 5 U of PZI were equally effective in stimulating food intake as the original administration and resulted in a cumulation of daily caloric excesses which was subsequently reflected in a progressive gain of body weight. The administration of 1, 5 and 10 U of regular insulin via the intraperitoneal (IP) route resulted in a temporary stimulation of food and water intakes which was characterized by an earlier onset, maximal significant increase occurring between 1 and 3 hr postinsulin and a complete caloric compensation by the end of 24 hr. The latencies of both feeding and drinking were significantly shortened but now drinking was initiated before eating. The changes in blood glucose were characterized by an early rapid fall, maximal hypoglycemia between 1 and 3 hr and subsequent faster recovery with baseline levels achieved at 24 hr postinsul in. The dose of 5 U was again most effective in stimulating food and water intake while 10 U was clearly excessive as indicated by lower intakes, marked increase in the shallow slow wave sleep (drowsiness) and abnormalities of the EEG wave pattern. The results of this part of experiment suggest that the expression, duration and intensity of insulin-induced hyperphagia is dependent on the route of insulin entry as well as the dose and duration of insulin action.

711 HYPOTENSION AND THIRST IN RATS AFTER ISOPROTERENOL OR PHENTOLAMINE TREATMENT. Jean A. Hosutt* and Edward M. Stricker. (SPON: David J. Kupfer). Psychobiology Program, Department of Psychology, University of Pittsburgh, Pittsburgh, PA 15260.

Blood pressure and water intake were monitored simultaneously in rats following the administration of hypotensive agents. Arterial pressure was recorded from chronic indwelling aortal catheters for 60 min after injection of isoproterenol (33, 100, or 330 ug/kg, sc) or phentolamine (5 or 10 mg/kg, sc). Most of the drinking to these agents occurred when blood pressure was within the range of 60-90 mm Hg, corresponding to a drop of 25-50 mm Hg. Animals whose blood pressure fell within this range generally drank 3.0 - 9.0 ml. This was true regardless of whether they were intact or nephrectomized. Animals whose blood pressure fell above or below this range usually drank little or nothing in response to these agents. For those animals that did drink, there was no clear relation between blood pressure and water intake, although above 60 mm Hg there was a tendency for larger drops in blood pressure to be associated with higher intakes. In view of the relatively minor effect of β-adrenergic stimulation on venous pressure (Abboud et al., Am. J. Physiol., 209: 383, 1965), the present results are consistent with the suggestion that arterial hypotension alone is sufficient to produce drinking. Removal of the source of renin by nephrectomy did not attenuate drinking during moderate hypotension. Severe hypotension, however, may be so debilitating as to preclude a behavioral response in both intact and nephrectomized animals.

712 LIVING FROM HAND TO MOUTH: EFFECTS OF TRIGEMINAL DEAFFERENTATION UPON FEEDING BEHAVIOR PATTERNS IN RATS. Mark Jacquin* and H.P. Zeigler. Dept. of Psychol., Hunter College, CUNY, New York, N.Y. 10021.

To evaluate the generality of disruptive effects upon food intake seen after trigeminal orosensory deafferentation, rats were trained to obtain food by reaching one paw into the food magazine and scooping out mash. The procedure involves an appetitive response which is easily monitored and linked more directly to eating than an operant lever pressing response, but its sensorimotor control does not directly involve trigeminal mechanisms. Prior to deafferentation rats using this "scooping" response show diurnal feeding patterns similar to those recorded with conventional procedures. Varying degrees of bilateral trigeminal orosensory deafferentation were produced by section of anterior and posterior superior dental, sphenopalatine, inferior alveolar and lingual nerves. Following complete deafferentation "scoopers" exhibited periods of aphagia and hypophagia. Their reduced food intake is mediated by a striking decline in the frequency and duration of "scooping" bouts during the immediate postoperative period. Following partial deafferentations aphagias and hypophagias are reduced or absent. In all deafferented animals, the recovery of feeding is accompanied by a striking reduction in the consummatory efficiency of the "scooping" response as measured both by food spillage and by an increase in the ratio of time spent feeding (bout frequency X bout duration) to food ingested. The reduced efficiency of the deafferented rats reflects a disruption in the organization of the "scooping" response sequences involving either an impairment in the continuity of the hand to mouth sequences or a failure to initiate mouth opening and tongue extension on those occasions when the mash is brought to the mouth. Deafferentation effects upon "scooping" were compared with their effects upon a food-reinforced bar-pressing response and upon species-typical consummatory responses. The results suggest that trigeminal orosensory inputs contribute to both the sensorimotor and motivational control of a variety of response classes related to hunger in the rat.

713 HYPOTHALAMIC OBESITY IN RHESUS MONKEYS: GLUCOREGULATORY AND NEUROCHEMICAL CORRELATES. Joseph W. Kemnitz, Robert W. Goy, and Gary W. Kraemer, Primate Research Center, University of Wisconsin, Madison, WI 53706

Bilateral hypothalamic lesions were produced in three young adult male Macaca mulatta. Two sham-lesioned males served as controls. Histological analysis revealed that the lesions were located in the rostromedial lateral hypothalamus. Analyses of regional catecholamine concentrations indicated that norepinephrine (NE) was depleted in the lesioned animals to 46%, 42%, and 34% of the control mean in dorsal/orbital cortex and to 75%, 10%, and 22% of the control mean in caudate/corpus striatum.

During the year of observation following surgery the lesioned animals became markedly obese. The maximal weights of the lesioned animals were 9.25 kg (104%), 8.75 (93%), and 5.26 (51%) greater than their presurgical weights, while the corresponding values for controls were 1.40 kg (15%) and 2.18 (18%). At autopsy large masses of fat were noted subcutaneously, intraperitoneally, and pericardially; there was fatty infiltration of virtually every organ.

The obesity was largely dependent upon hyperphagia. The lesioned animals were overeating during the time of supranormal weight gain and they lost weight when food was restricted to control levels.

One of the lesioned animals exhibited elevated postprandial serum glucose levels (268 mg/dl vs. 70.5 ± 4.5 for controls) as well as elevated postabsorbtive glucose levels (116 mg/dl vs. 69.0 ± 0.0 for controls) and impaired glucose clearance during an intravenous glucose tolerance test after the obesity was established. There was also a smaller decrease in glucose levels for this animal during an insulin challenge test in this phase of the experiment (8 mg/dl vs. 30.0 ± 11.0 mg/dl for controls and 29.5 ± 9.5 mg/dl for the other lesioned animals). Postprandial and postabsorbtive glucose values were more normal (84 mg/dl and 106 mg/dl, respectively) after a 3-week period of food restriction, but were elevated again after the animal was returned to ad lib feeding (347 mg/dl and 132 mg/dl). The other two lesioned animals and the controls exhibited apparently normal glucoregulation throughout the experiment.

The results are consistent with previous reports of obesity following interruption of ascending NE systems in rodents and primates. They further suggest that glucoregulatory deficits are not an invariable consequence of such lesions and that such deficits, when they occurred, may be described as insulin impedance secondary to obesity.

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714 *ABDOMINAL VAGOTOMY DISRUPTS DRINKING ELICITED BY PREGASTRIC FOOD-CONTINGENT STIMULATION IN RATS.* *F. Scott Kraly. Dept. Psychology, Colgate University, Hamilton, NY 13346*

Drinking in rats occurs in close temporal association with eating. Rats with complete bilateral subdiaphragmatic vagotomy (Vgx-C) with hepatic branch intact fail to drink normally after eating a meal of solid or liquid food; vagotomized rats drink later and less than normal rats and Vgx-C rats' drinking is not related to the amount of food eaten (Kraly, Smith & Carty, 1978). While these findings implicate the abdominal vagus as a neurological substrate for food-related drinking in the rat, the nature of the vagally-mediated signals that stimulate food-related drinking has remained unknown. The results reported below show (1) that food-contingent stimulation of pregastric sites (i.e., mouth and esophagus) is a sufficient stimulus for drinking in normal rats and (2) that Vgx-C disrupts drinking behavior elicited by pregastric food-contingent stimulation. These findings were obtained from Vgx-C (n=6) and normal (n=8) rats sham feeding and drinking (with open stainless-steel gastric fistulas) after 24-hr food deprivation.

Normal rats initiated sham feeding quickly upon being offered liquid food (GIBCO 116EC) and began drinking with a median latency of 16.8 min after having begun eating. They sham drank a mean of 1.8 ± 0.4 ml/100 g BW in the first 30 min of the test and exhibited a water:food consumption ratio of .37 ± .08.

Rats with anatomically-verified complete Vgx-C also initiated sham feeding quickly and sham fed amounts that were not significantly different ($p > .10$) from amounts sham fed by normal rats. Vgx-C rats drank with an abnormally long median latency (23.5 min; $p = .00$, normal vs. Vgx-C), however, and they sham drank significantly less ($p < .01$) water than normal rats in temporal association with sham feeding (0.2 ± 0.1 ml/100 g BW) while exhibiting a smaller ($p < .05$) than normal water:food ratio (.10 ± .06). Analysis of covariance showed that sham-feeding-related drinking was significantly ($p < .025$) reduced by Vgx-C separate from any effects of Vgx-C upon sham feeding behavior. These effects of Vgx-C upon latency to drink (p's from .008 to < .06) and amount of water sham drunk (all p's < .05) persisted for six repeated sham-feeding tests. These findings were not due to differential collection of ingesta through the gastric fistulas (all p's > .10). Thus, preabsorptive pregastric food-contingent stimulation is sufficient to elicit drinking in normal rats and the abdominal vagus has a role in mediating this effect.

715 FUNCTIONAL AND ANATOMICAL STUDIES OF NORADRENERGIC SYSTEM OF THE PARAVENTRICULAR HYPOTHALAMUS THAT CONTROLS FEEDING BEHAVIOR. Sarah F. Leibowitz. Rockefeller University, New York, NY 10021.

The paraventricular nucleus (PVN) and periventricular region of the hypothalamus are densely innervated by noradrenergic and adrenergic neurons. This area has been shown to be uniquely sensitive to the feeding stimulatory effect of exogenous norepinephrine (NE) and epinephrine observed in the rat. A series of tests focused on increasing our understanding of the significance of this phenomenon have yielded the following results.

1) When antidepressant drugs (such as protriptyline and amitriptyline) are injected into the PVN, increased feeding can similarly be seen. This response apparently results from drug-induced release of endogenous NE, as it is selectively blocked by α-adrenergic antagonists, and it fails to occur in animals with a local and selective inhibition of NE synthesis.

2) Electrolytic lesions in the area of the PVN and periventricular hypothalamus attenuate or abolish the feeding response to both NE and the antidepressant drugs.

3) Discrete damage to the PVN also causes hyperphagia leading to obesity, in male as well as female rats maintained on standard lab chow or high fat diet.

4) The increased eating observed with PVN injection of NE is attenuated or abolished by surgical (subdiaphragmatic) removal of the vagus nerve. Cholinergic vagal efferents to the viscera may be involved in this effect, since peripheral injections of the anticholinergic drugs atropine and scopolamine, at very low doses, similarly antagonize the eating evoked by NE.

5) Caudal hypothalamic and midbrain lesions or knife cuts, which presumably sever PVN efferents to the medullary vagal complex, have yet to be found to significantly interfere with the NE eating response.

6) Tests using a self-selection feeding paradigm, where dietary protein, carbohydrate, and fat are systematically manipulated in solid and liquid food, yield suggestive evidence that NE-injected rats, in comparison with mildly food-deprived rats, manifest an increased preference specifically for carbohydrate and, in particular, sugar.

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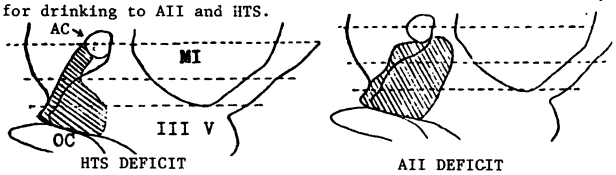
716 CRITICAL TISSUES WITHIN THE PERIVENTRICULAR REGION OF THE ANTERO-VENTRAL THIRD VENTRICLE (AV3V) ASSOCIATED WITH SPECIFIC THIRST DEFICITS. H. Lind*, R. W. Lind, E. E. Shrager*, S. L. Bealer, and A. K. Johnson (SPON: I. Gormezano). Dept. of Psychology, University of Iowa, Iowa City, IA 52242.

Lesions of the AV3V produce acute adipsia and upon "recovery", a chronic refractoriness in drinking to angiotensin II (AII) and hypertonic saline (HTS). Smaller lesions within the AV3V may produce deficits to only one of the dipsogens. This finding suggests that different areas within the AV3V are crucial for drinking to AII and HTS.

Thirty rats were tested prelesion and 2 weeks following AV3V ablations for drinking responses to s.c. injections of AII (1.56 µg/kg) and HTS (1 ml 12% NaCl/100 g). Six rats were refractory to AII and 12 to HTS while seven rats responded normally to AII and 12 drank normally to HTS.

At the completion of testing, serial sections (40 µ) were cut through the area of the lesion. Both sagittal reconstructions (see fig. below) and common overlap analyses at three horizontal levels (dotted lines) were employed to identify common regions of destruction. These procedures identified critical areas for AII and HTS thirst which are distinct from each other. Complete destruction of the nuclei preopticus periventricularis and suprachiasmaticus (SCN) (note: not the SCN of the anterior hypothalamus) and the organum vasculosum was seen in all animals which evidenced attenuated responses to either dipsogen. However, these three structures were also ablated in the majority of lesioned animals that drank normally. Extensive damage to the preoptic nucleus medianus was present in both the AII- and HTS-deficit groups. However, animals refractory to HTS suffered greater damage to this nucleus. Lesions in animals with deficits to AII were more caudal. The critical areas for both of the dipsogens were within .4 mm of the midline. Two animals that responded normally to each of the challenges could not be distinguished on histological grounds from deficit animals. These findings support the hypothesis that different but closely associated areas within the dorsal AV3V contain neural substrates necessary for drinking to AII and HTS.



AC=anterior commissure OC=optic chiasm IIIV=third ventricle MI=massa intermedia

717 ANTEROVENTRAL THIRD VENTRICULAR REGION (AV3V) LESIONS: THIRST DEFICITS TO BETA-ADRENERGIC RECEPTOR AGONIST STIMULATION. R. W. Lind and A. K. Johnson. Department of Psychology and Cardiovascular Center, University of Iowa, Iowa City, IA 52242.

Recent work from this laboratory has demonstrated that lesions of the periventricular tissue surrounding the anteroventral third ventricle (AV3V) produce acute adipsia without a compensatory antidiuresis. Animals that recover ad libitum water intake to normal levels remain hypernatremic and hyperreninemic. They also evidence attenuated drinking responses to central and peripheral injections of angiotensin II (AII) and hypertonic saline (HTS).

The beta-adrenergic agonist, isoproterenol (ISOP), is a potent dipsogen (Lehr, Mallow, & Krukowski, JPET, 158:150-163, 1967). Water intake following administration of this compound has been hypothesized to be mediated via the activation of the renin-angiotensin system (Houpt & Epstein, Physiol. Behav., 7:897-902, 1971). However, this interpretation has recently been challenged (Stricker, JCPP, 91:1220-1231, 1977).

The present experiment investigated the effects of the AV3V lesion on the drinking response to ISOP. Rats were pretested with subcutaneous injections of AII (1.56 µg/kg), HTS (1 ml of 12% NaCl/100 g), and ISOP (100 µg/kg). Following recovery from sham lesioning or ablation of the AV3V, lesioned (n=10) and sham-lesioned (n=9) animals were tested with AII and HTS at the prelesion doses, and 10, 30, 100, 200, and 300 µg/kg of ISOP. Water intakes were measured for 2 hrs following AII and HTS injections and for 3 hrs following ISOP treatment. An analysis of variance yielded a significant difference ($p<.025$) between the drinking responses of lesioned and sham-lesioned rats after ISOP injection. The experimental subjects manifested both an elevation in threshold and a decrease in their maximal response to the beta-adrenergic receptor agonist. The mean responses across doses in AV3V lesioned animals were between 36 and 56% of those in control animals. Responses to the AII dose and 30 µg/kg ISOP (the ED50 in controls) were significantly correlated ($r=.93$, $p<.001$) in lesioned rats. Postlesion drinking responses to HTS did not correlate significantly with water intake following the 30 µg/kg injection of ISOP ($r=.372$, $p>.1$) or AII ($r=.304$, $p>.1$). This lack of relationship corroborates the finding by Lind, Lind, Shrager, Bealer, and Johnson (Neurosci. Abs., 5: 1979) that postlesion response deficits to AII and HTS are associated with destruction of different loci within the AV3V. The high correlation between the residual drinking to AII and ISOP following AV3V lesions supports the interpretation that responses to both of these dipsogens are mediated by common neural substrates.

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718 EARLY METABOLIC EFFECTS OF VENTROMEDIAL HYPOTHALAMIC LESIONS. Robert G. MacKenzie*, Lee L. Bernardis and Jack K. Goldman* (SPON: M.B. Kristal). Dept. Med., Sch. Med., SUNY and VA Med. Ctr., Buffalo, NY 14215.

Male weanling rats (75-100 g), under sodium hexabarbital anesthesia, were lesioned (1.5 mA anodal d.c. for 10 s) at ventromedial hypothalamic (VMH) coordinates (AP 5.0, ML 0.5, DV 8.2). Controls were lesioned 1 mm beneath the surface of cortex. The rats were injected IV with 1.75 mCi ^3H_2O either immediately following lesion, 2½ hr or 5½ hr post-op and then killed 1 hr post-injection. Food and H_2O were unavailable during the experiments. ^3H_2O served as a tracer for lipogenesis and glucogenesis. Lipid and glycogen content of liver and carcass as well as plasma glucose concentration (PGC) were determined along with the specific activity (SA) of these substances and the SA of plasma H_2O.

The results demonstrate that within 1 hr post-VMH lesion, PGC is increased 25% ($p<.01$) and glucogenesis by 51% ($p<.05$) while lipogenesis is decreased 17% ($p<.02$). Between 2½-3½ hrs post-VMH lesion PGC approaches control values (+15%, NS) as incorporation of tracer into liver (+70%, $p<.05$) and carcass (+71%, $p<.05$) glycogen and liver lipids (+40%, $p<.05$) is accelerated. At 5-6 hrs post-VMH lesion, PGC continues the tendency to be elevated but does not differ significantly from control levels. Glucogenesis is increased (23.5%, $p<.02$) as are carcass and liver lipogenesis (42.5%, $p<.01$ and 52%, $p<.05$ respectively). Our hypothesis is the VMH lesions cause an immediate and transient sympathetic discharge promoting glycogenolysis and suppressing lipogenesis. This is followed by increased uptake of plasma glucose by tissues until uptake begins to lower PGC. At this stage gluconeogenesis is increased to maintain PGC.

These data indicate that complex metabolic disturbances occur very early after VMH lesions. It remains to be determined whether these processes seen in the weanling rat also occur in the mature VMH-lesioned rat and whether they underlie the hyperphagia exhibited by the latter preparation.

719 MODULATION OF THE CENTRAL EFFECTS OF ANGIOTENSIN II (AII) BY SODIUM. Johannes F. E. Mann*, Ernesto L. Schiffrin*, A. Kim Johnson, Roger Boucher*, Jacques Genest*. Clinical Research Institute of Montreal, Montreal, P.Q. H2W 1R7, Canada.

We investigated whether sodium influences the actions of AII on its receptor in the brain in vivo and in vitro. Rats were maintained on a normal (controls) or low sodium diet for 2 weeks and received demineralized water ad lib. For intracerebroventricular (i.v.t.) tests chronic cannulas were implanted into the lateral cerebral ventricle. (I) Drinking was induced in the rats by i.v.t. injections of AII (400 pmol), carbachol (400 pmol) and hyperosmolar NaCl (1.5%) in a volume of 4 µl. In rats on a low sodium diet water intake (as compared to controls) was reduced following AII (4.8 vs 13.1 ml/30 min, p<.001, n:18), it was enhanced by administration of 1.5% NaCl (2.0 vs 0.7 ml/30 min, p< .01, n:20) and there was no difference following carbachol (9.7 vs 9.2 ml/30 min, n:19). (II) The pressor effects of AII and carbachol were tested in conscious, unrestrained rats with chronic arterial catheters to monitor blood pressure (BP). Drugs were applied i.v.t. in a volume of 3 µl. BP increases following AII were significantly reduced in sodium deprived rats (n:8) as compared to controls (n:8) at doses of 10 pmol (8.1 vs 11.3 mm Hg), 100 pmol (13.3 vs 16.3 mm Hg), 1000 pmol (14.6 vs 19.4 mm Hg), and 3000 pmol (17.3 vs 25.0 mm Hg). Carbachol elicited BP increases were only diminished at a dose of 1000 pmol (21.9 vs 28.4 mm Hg) but not so at 10 and 100 pmol respectively when sodium deficient rats were compared with controls. (III) ^{125}I-AII binding to brain homogenates in vitro in rats on a low sodium diet was consistently reduced by 30-50% as compared to controls (3 experiments). (IV) Addition of 75 mM NaCl to the incubation medium in vitro enhanced ^{125}I-AII binding to brain homogenates by 30-40% (5 experiments). Unspecific binding (i.e. not displaceable by an excess of cold AII) was not modified by sodium. These results show that the dipsogenic and central pressor effects of AII are reduced in sodium deficient rats. This may be due in part to changes in the binding properties of AII to its brain receptor. AII has been implicated in the physiological regulation of water intake. It is evident from the present experiments that the potency of AII to elicit drinking depends on the state of sodium balance. The same applies to its effect on central mechanisms of BP regulation.

720 BETA-ENDORPHIN: HORMONE FOR THE CONSERVATION OF BODILY RESOURCES AND ENERGY IN ANTICIPATION OF FAMINE. David L. Margules. Dept. Psychol., Temple Univ., Phila., Pa. 19122.

Almost all of the diverse actions of beta-endorphin(bE) are consistant with the theory that this hormone produces a widespread series of adaptive reactions that conserve fuel&water & reduce energy expenditures, thus prolonging survival during famine. Many forms of excretion cease & retention occurs of H_2O, ions, fuels,& CO_2. Energy expending activities cease & lethargy, passivity & skeletal muscle relaxation occur. An attenuation occurs of the arousal capacity of the stimuli for the activation of the sympathetic nervous system including pain, cold, asphyxiation, oxygen lack and the emotions of fear and rage.bE lowers energy usage by the reduction of thyrotropin release, the lowering of set point for body temperature & the elevation of set point for CO_2 tension in the blood. Drops in body temperature,respiration rate, cardiovascular output, sympathetic tone, and thyroid hormone release, reduce the energy production and oxygen consumption in almost every tissue in the body. bE acts in conjunction with insulin & glucagon, whose release it stimulates, to conserve,extract & store nutrient, H_2O, & ions in the body. Sphincters for defecation, urination, and bile release are tightly contracted. Propulsive peristalsis ceases & the gastrointestinal contents are stirred & mixed in place. bE stimulates the release of anti-diuretic hormone which acts on the kidney to conserve H_2O. bE also acts to inhibit the release of the gonadotrophic hormones, thus reducing sexual urges. Starving organisms can not afford the energy necessary to pursue sexual activities. The actions of bE are supported by ACTH release, which stimulates the adrenal cortex to release the mineralocorticoids and glucocorticoid hormones. These hormones further conserve Na^+ and carbohydrates and reduce the rate of their utilization. bE stimulates feeding and drinking and encourages the development of a pre-famine obesity. Obese organisms can survive periods of famine up to ten times longer in duration than lean littermates. The widespread and integrated series of bE mediated changes in many organ systems suggests that this hormone & its associated receptors may be important new modulators of autonomic nervous system activity. Hibernation is an extreme example of the adaptive power of the bE system in coping with seasonal food short age. Part of preparation for hibernation involves storing calories either on the body as adipose tissue or in the nest as hoarded food. Moreover, many of the physiological changes during hiber nation are consistant in direction with the above bE mediated influences. In support of this theory, naloxone (5mg/kg,sc), an opioid antagonist, increases markedly the respiratory and cardiac rate of hibernating Turkish hamsters, and causes premature arousal from hibernation. Endogenous naloxone-like peptides may exist for arousal from hibernation by antagonism of the bE system.

721 A TEST OF THE VASOMOTOR HYPOTHESIS FOR DRINKING TO ANGIOTENSIN II. Richard R. Miselis and Steven J. Fluharty*. Animal Biol., Sch. Vet. Med., Inst. Neurol. Sci., and Psychol., Univ. of Pennsylvania, Philadelphia, PA 19104.

The vasomotor hypothesis states that angiotensin II (AII) is dipsogenic because of special sensitivity within the brain to AII's vasoconstrictive action. AII presumably vasoconstricts the vascular bed of the receptive site to AII. This is detected by mechanoreceptors which drive drinking behavior. The hypothesis is based on the inhibitory effects to AII drinking caused by central administration of substances which are vasodilators (Nicolaidis and Fitzsimons, C. R. Acad. Sci. 281, 1975; Kenney and Epstein, JCPP, 92, 1978). In this study we measure the effects of AII on blood pressure and local cerebral blood flow to infer what happens to resistence (i.e. vasoconstriction). Sprague Dawley male rats were used for subjects. The autoradiographic technique developed by Reivich et al., J. Appl. Physiol., 27, 1969 and Sakurada et al., Am. J. Physiol. 234, 1978 was used to measure local cerebral blood flow quantitatively. This method uses an inert freely diffusable ^{14}C isotope of iodoantipyrine as a tracer for blood flow. Rats were prepared under ether anesthesia with two femoral arterial and two venous catheters. Following surgery they were restrained by taping the trunk to a brick and allowed to recover from the anesthesia for 3 to 5 hours. Two rats given a control infusion of isotonic saline were compared to 3 rats given 32 ng AII/rat/min. The infusion of the tracer for the measurement of cerebral blood flow commenced 4 minutes after the onset of the saline or AII infusion and continued for one minute. The rats were decapitated, the brains rapidly removed and quickly frozen. AII raised blood pressure an average of 18 mm of Hg. Cerebral blood flows in control rats (saline) ranged from 74.8 to 108.1 ml blood/100g tissue/min. for the structures observed. Cerebral blood flows measured after AII were increased for the same structures. However, the increases were heterogeneous ranging from 19 to 101%. The increases for the following areas were: organum vasculosum of the lamina terminalis- 78%, medial preoptic area - 41%, nucleus medianus - 67%, supraoptic nuclei - 41% and the subfornical organ (SFO) - 19%. If the reduced increase observed in the SFO represents more resistance to a passive increase in blood flow due to the pressure rise, then the results can tentatively be interpreted as support for the vasomotor hypothesis. However, the magnitude of the changes suggest metabolically stimulated increases in blood flow, as well.

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722 CHANGES IN THE EFFECTS OF ESTROGEN ON DIURNAL FEEDING PATTERNS FOLLOWING HYPOTHALAMIC KNIFE CUTS. D.M. Nance and C.P. Phelps, Department of Anatomy, College of Med., Univ. South Florida, Tampa, Florida 33612.

In addition to anorexia and weight loss, estrogen priming of ovariectomized female rats results in a marked alteration in diurnal feeding patterns when animals are maintained on a 12:12 light:dark schedule. The changes in meal patterns include: decreases in a.) the number of meals (NM) during light, b.)intermeal interval (IMI) during dark and c.) average meal size (MS) and meal duration (MD) for both photoperiods; but increases in a.) the NM eaten during dark and b.) the IMI during light (Nance & Gorski, Brain Res. Bull. 3:549, 1978). These estrogen induced changes in meal patterns represent an exaggeration in the diurnal distribution of feeding behavior and include a dark phase increase in "nibbling" behavior (many small meals of short duration separated by short intervals) and a further reduction of already low light phase feeding. The net effect of estrogen on MS and MD is an attenuation of diurnal changes in these variables. The neural pathway(s) mediating part of all of these effects of estrogen on feeding behavior have not been established. In the present experiments, the effects of a single injection of 6µg estradiol benzoate (EB) on food intake (FI) and meal patterns were examined in spayed female rats which had received frontal (FC), frontal lateral (FLC) or sham (S) hypothalamic knife cuts (1.5mm radius) in the retrochiasmatic region. FC and FLC were similar except that FLC extended 2.5mm posteriorly along the lateral edge of the ventromedial nucleus (VMN). In rats that had a FC passing through the suprachiasmatic nucleus (SCN) there was a reduced diurnal distribution of feeding behavior before EB and in contrast to S rats, no change in NM and IMI after steroid treatment. In animals with FC posterior to SCN, but anterior to VMN, diurnal variations in meal patterns were present before EB and were altered in the same directions as S rats after EB (increased NM and decreased IMI in dark; decreased NM and increased IMI in light). However, relative to S rats, FLC attenuated diurnal variations in all meal parameters prior to EB, and after EB, NM and IMI of FLC rats were unaffected by EB treatment. Thus, either a FC passing through the SCN or a FLC produced an attenuation in diurnal feeding behavior and prevented EB effects on NM and IMI. These data suggest an interaction between SCN neurons and anterolateral neural connections of the VMN in the mediation of estrogen-sensitive diurnal feeding behavior. Supported by NIH HD11345.

723 EFFECTS OF TESTOSTERONE ON BODY WEIGHT AND FOOD INTAKE: SITES AND MECHANISMS OF ACTION. Antonio A. Nunez*, Linda I. Siegel*, Janet M. Gray* and George N. Wade*. (SPON: J. Meyer). Univ. Mass., Amherst, MA 01003.

Treatment of castrated male rats with low doses of testosterone propionate (TP; .2 mg/day) increases food intake and body weight gain, but long-term (30 days) treatment with a higher dose of TP (1 mg/day) reduces body weight gain and carcass fat content. Concurrent treatment with androsta-1,4,6-triene-3,17-dione (ATD), which blocks the aromatization of androgens to estrogens, prevents the weight-reducing effects of high doses of TP. Long-term treatment with 1 mg TP/day also depletes cytoplasmic estrogen receptors and reduces lipoprotein lipase activity in epididymal fat pads. Both of these effects are blocked by concurrent treatment with ATD and are observed after treatment with estradiol.

Bilateral implants of TP in the ventromedial hypothalamus (VMH) also reduce the food intake of castrated male rats. Similar implants in the preoptic area or VMH implants of 5∝-dihydrotestosterone propionate do not reduce food intake. The effects of VMH implants of TP are comparable to those observed after diencephalic implants of estradiol in both sexes (JCPP, 72: 328-336, 1970; Bull. Psychon. Soc., 3:273-274, 1974).

The results of these experiments suggest that estrogenic metabolites of TP may act on different sites to reduce food intake and body weight. In addition to a direct effect on the hypothalamus, estrogens may reduce body weight gain by inducing changes in adipose tissue metabolism, including lipoprotein lipase activity. These mechanisms may underlie the reductions in body weight, food intake, and carcass fat content seen in gonadally-intact, sexually-active male rats.

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724 ANORECTIC AND NEUROCHEMICAL EFFECTS OF D-AMPHETAMINE IN GENETICALLY OBESE (obob) MICE. Roma Olsauskas and Gary A. Oltmans. Dept. Pharmacol., Chicago Med. Sch., Chicago, IL 60612.

Genetically obese mice (obob mutation) have significantly elevated food intakes and hypothalamic norepinephrine (NE) levels compared to those of lean controls (Oltmans et al., Pharmacol. Biochem. Behav. 5:617, 1976). Food intake and brain catecholamine (CA) levels were studied in obob and lean mice following treatment with d-amphetamine (AMP). Female obob and lean mice (5-6 mos old) were acclimated to a 6 hr food access schedule. Drug injections (3 mg/kg AMP or 0.9% NaCl) were administered ip 30 min prior to food access; food intake was measured 1, 3, and 6 hrs thereafter. After 6-11 days of testing, mice were decapitated either 1.5 or 5 hrs following AMP injection, and the hypothalamus (HT), telencephalon (TEL), and brainstem (BST) were analyzed for CA content.

Food intake during the initial 3 hrs of food access did not differ significantly between saline-treated obob and lean mice (1.49±.34g vs 1.31±.23g). During the second 3 hr period, obob mice ate significantly more food than lean mice (1.46±.44g vs 0.94±.28g). While AMP treatment significantly reduced food intake during the first 3 hr period in both obob (70% of control) and lean mice (74% of control), it did not affect the food intake of either obob (114% of control) or lean mice (106% of control) during the second 3 hr period. Consequently,during this period AMP-treated obob ate significantly more than AMP-treated lean mice (1.55±.25g vs 1.07±.26g).

Saline-treated obob mice had significantly higher HT NE levels (+14%) than saline-treated lean mice. At 1.5 hrs after AMP injection (during the period of anorexia), HT NE levels were significantly reduced in both obob (-16%) and lean mice (-11%). At 5 hrs after AMP injection (when mice had resumed a normal feeding pattern), HT NE levels in both obob and lean mice were not significantly different from those of control mice. HT dopamine (DA) levels in lean mice were not significantly changed at either 1.5 or 5 hrs. HT DA levels in obob mice were initially significantly decreased (-23%) and then significantly increased (+37%) at 5 hrs after the injection. Amphetamine-induced CA changes in other brain areas (TEL and BST) varied between lean and obob mice and, in general, indicated that the CA systems in obob mice respond differently to AMP treatment than those in lean mice.

The results indicate that AMP has a comparable anorectic effect in lean and obob mice in the immediate post-injection period. This anorectic action is paralleled by comparable reductions in HT NE levels in both groups. Subsequent to this period of anorexia normal feeding is restored and obob mice again eat significantly more than lean mice. This period of normal feeding coincides with normal HT NE levels in the AMP-treated obob and lean mice.

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725 MORPHOLOGICAL EFFECTS OF INTRAHYPOTHALAMIC KAINIC ACID. Gary M. Peterson and Robert Y. Moore. Dept. Neurosciences, UCSD Sch. Med., La Jolla, CA 92093.

Bilteral injections of kainic acid (KA), a neurotoxic analogue of glutamate, into the lateral hypothalamic area (LH) of rats duplicates the aphagia and adipsia produced by electrolytic lesions in the same region (Stricker et al., Brain Res., 158: 470, 1978). Because KA preferentially destroys cell bodies and spares axons of passage (Coyle et al., J. Comp. Neurol., 180: 301, 1978), the resulting feeding and drinking deficit has been interpreted as evidence that cells in the LH may have a role in the control of ingestive behaviors (Stricker et al., 1978). Since KA may affect a wide area of tissue but does not affect all cell groups equally, a detailed histological analysis of those cell groups actually destroyed is necessary.

The effects of LH injections of KA on hypothalamic and thalamic nuclei and on extrinsic fibers passing through the LH were examined by light microscopy. Brains from three groups were examined: 1) bilateral LH injections resulting in aphagia and adipsia, 2) bilateral LH injections which did not result in these deficits,and 3) unilateral LH injections. Brains from the two bilateral groups were from Stricker et al. (1978) and received approximately twice the volume and concentration of KA as the unilateral group (1.14 µg/.57µl and 0.3µg/0.3µl, respectively).

All cell groups in the medial hypothalamus were intact. The LH showed moderate to heavy cell loss. Several subthalamic nuclei, the zona incerta, subthalamic nucleus and medial portion of the reticular thalamic nucleus, were consistently affected by the KA and commonly showed more complete cell loss than the LH. The substantia nigra was not affected. The unilaterally injected brains showed the same pattern of cell loss except that the cellular degeneration did not spread as far rostrally as with the bilateral injections. The bilaterally injected group which did not show ingestive deficits had needle tracks which passed entirely through the brain tissue and no cellular loss. Examination of brain sections stained with the Bodian method showed no destruction of fibers of passage. The concentration of dopamine (as determined by radioenzymatic assay) in forebrain areas receiving fibers which pass through the LH was not significantly affected by the injections.

The anatomical substrate of the LH syndrome has not been well elucidated even though the syndrome was described more than 20 years ago. Whereas intrahypothalamic KA injections produce deficits in feeding and drinking identical to those produced by electrolytic lesions, the present morphological study suggests that damage to nuclear regions in the lateral hypothalamus and subthalamus is important to production of the behavioral syndrome. (Supported by USPHS Grant MH-32410.)

726 EVIDENCE FOR THE PRIORITY OF A RENAL RELATED MECHANISM MEDIATING THIRST INDUCED BY ISOPROTERENOL TREATMENT. R. Rettig and A. K. Johnson. Dept. Psychology and Cardiovascular Center, Univ. of Iowa, Iowa City, IA 52242.

Isoproterenol (ISOP), a beta-adrenergic receptor agonist, induces drinking in normal rats but fails to do so in nephrectomized animals. The renin-angiotensin system (RAS) has been considered to be responsible for the mediation of thirst after isoproterenol treatment (Houpt & Epstein, Physiol. Behav., 7:897, 1971). The hypothesis that ISOP induced drinking is completely dependent on the RAS, however, has been questioned. The greater reduction in arterial blood pressure (BP) in nephrectomized as compared to intact rats after ISOP injection has been suggested to produce debilitation incompatible with drinking. It has been concluded that the RAS merely plays a permissive role by allowing the animal to maintain a sufficiently high BP, while the main stimulus for the water intake is hypotension, mediated by arterial baroreceptors (Stricker, Fed. Proc., 37:2704, 1978).

Our studies were designed to investigate the relative importance of arterial baroreceptor vs. renal mechanisms. In Experiment 1 male albino rats were implanted with catheters in the right femoral artery for BP measurement and in the right femoral vein for i.v. infusion.

Two experimental groups and 2 control groups were formed. Rats in group 1 and 2 (n=7/group) were subjected to bilateral nephrectomy, while rats in group 3 and 4 (n=8, n=6) received ureteric ligations. Three to 4 hrs after surgery a drinking test was conducted. ISOP (40 µg/ml) was infused (.2 ml/hr) by means of a syringe pump in groups 1 and 3. Groups 2 and 4 served as controls and received isotonic saline at the same rate. Prior to the start of the infusions there were no differences in BP between groups 1 and 3. The ISOP infusion lowered the BP 20.2 ± 1.54 mmHg in group 1 and 8.3 ± .75 mmHg in group 3. There was no change in BP in groups 2 and 4. After 1 hr the water intakes were: Group 1, 1.3 ± .7 ml; group 2, 1.3 ± .8 ml; group 3, 4.2 ± .9 ml; group 4, .7 ± .3 ml. The water intake in the group receiving ISOP and ureteric ligation (group 3) was significantly greater than that of group 1 ($p<.05$), group 2 ($p<.05$), and group 4 ($p<.01$). In Experiment 2 a total sinuaortic baroreceptor denervation was performed in 7 male albino rats. Five animals received a sham denervation. After 30 days of recovery 33 µg/kg ISOP was injected subcutaneously in both groups. No significant differences in water intake were observed within a 3-hr period after injection. Therefore, no evidence could be found for an involvement of the arterial baroreceptors in ISOP-induced dipsogenesis. From the results of these experiments we conclude that a major factor mediating thirst after ISOP treatment is associated with a renal mechanism, probably the RAS.

727 [^{14}C] DEOXYGLUCOSE MAPPING OF BRAIN AREAS ACTIVATED BY HYPOTHALAMIC STIMULATION ELICITING GNAWING, EATING, AND DRINKING IN RATS. Warren W. Roberts. Dept. Psychol., Univ. of Minnesota, Minneapolis, MN 55455.

Rats having 1 or 2 ipsilateral hypothalamic electrodes that elicited gnawing, eating, and drinking received intermittent electrical stimulation for 45 min following i.v. injection of [^{14}C] deoxyglucose, and their brains were processed according to the method of Sokoloff for determining regional differences in glucose metabolism. To maximize detectability of elicited activity, baseline metabolism was reduced by light barbiturate anesthesia. The stimulation produced predominantly ipsilateral increases in glucose consumption in an extended sequence of structures extending anteriorly from the lateral hypothalamus into the diagonal band of Broca and the septal area, and posteriorly into the central grey, zona incerta, ventral tegmental area of Tsai (VTA), medial raphe, a portion of the tegmentum dorsal to the brachium conjunctivum (BC), parabrachial nuclei, and locus coeruleus. Many areas were similarly affected in control rats by hypothalamic stimulation that did not elicit the three behaviors. The largest differences between experimental and control rats were in the lateral VTA, supra-BC tegmentum, and dorsal and ventral parabrachial nuclei. These and possibly a few other marginal structures appear to be the most likely candidates for the destinations and/or pathways of hypothalamic efferents producing gnawing, eating, and drinking.

728 REDUCTION OF VOLUNTARY ALCOHOL CONSUMPTION BY MALE GOLDEN HAMSTERS AFTER MELATONIN ADMINISTRATION. P. K. Rudeen* and S. K. Symmes*. (Spon: C. P. McGraw). Department of Anatomy, Bowman Gray School of Medicine, Winston-Salem, N.C. 27103

Male golden hamsters preferentially consume alcohol solutions (< 10%,w/v) when given a free-choice between water and the alcohol solution. The pineal gland has been implicated as influencing the predilection for the ethanol solution. Male golden hamsters were allowed to consume a 5% ethanol solution for 72 days. Light-deprived, sham-pinealectomized hamsters drank more ethanol solution (38 ml/day) than did light-deprived pinealectomized hamsters (31 ml/day; $p < 0.001$). Sham-pinealectomized hamsters maintained in a long photoperiod consumed more alcohol solution (35 ml/day) than did their pinealectomized counterparts (30 ml/day). Serum alcohol levels were higher in sham-operated animals and lower in pinealectomized animals. In subsequent experiments, melatonin, a pineal hormone, was administered either daily for 11 weeks as a subcutaneous injection (25 µg/animal) or weekly as a subcutaneous beeswax implant (1 mg melatonin/24 mg beeswax) for 5 weeks to hamsters allowed a free-choice between water or a 10% ethanol solution. Food, water and alcohol consumption was measured on a daily basis. Animals treated daily with melatonin consumed slightly less ethanol (16.5 ml/day) than did animals not given melatonin (18.0 ml/day). However, in light-deprived animals given chronic implants of melatonin, alcohol consumption was reduced (27.3 ml/day) when compared to alcohol consumption by light-deprived hamsters not receiving melatonin (34.4 ml/day). Total fluid consumption was also reduced by melatonin treatment (30.7 ml/day) and was greater in hamsters which did not receive the melatonin implants (40.7 ml/day). Melatonin treatment resulted in reducing daily total fluid intake as well as ethanol consumption in light deprived hamsters. The results indicate that the pineal gland may influence fluid consumption in the hamster, but the data suggest that the pineal gland does not alter the propensity to consume alcohol.

(Supported by a grant from the North Carolina Alcoholism Research Authority.)

729 PREOPERATIVE Na^+ EXPERIENCE AND LATERAL HYPOTHALAMIC LESIONS Jay Schulkin* and Jeff Ruger*. Dept. of Nat. Sci., SUNY-Purchase, Purchase, N.Y.; Dept. of Phil., Univ. of Penn., Philadelphia, PA. 19104. (SPON: Leo M. Hurvich).

Lateral hypothalamic lesions disrupt the rat's ability to respond to a Na^+ deficit by ingesting saline. What we found, however, was that animals given pre-operative experience of Na^+ need and saline ingestion did not show this deficit.

Eighteen lateral hypothalamic lesioned animals were used in this study. The Na^+ appetite was induced by injecting the animals subcutaneously with 10 mg of furosemide (Lasix, Hoechst-Roussel) and 5 mg of deoxycorticosterone acetate (Percorten, CIBA). Nine of the animals underwent this treatment prior to the lesion and 9 did not. Both groups underwent this treatment post-operatively. The animals were given a 3% saline solution and deionized water. The 9 injected animals had access to the saline prior to the lesion and the other 9 did not. After an initial post-operative aphagia and adipsia the animals were once again maintained on normal rat chow. (The coordinates with bregma and lambda at the same horizontal plane were 2.7mm posterior to bregma, 1.5 mm lateral to the longitudinal suture and 9.1 below the skull surface at bregma. Anodal dc was administered with .5 mm exposed at the tip for .5 mA for 20 sec.)

The table shows the mean increase of Na^+ and HoH for the 2 days following the treatment from the 2 days baseline prior to the treatment. T test revealed a significant difference between the two groups for the Na^+ scores ($p = < .01$) but not for the HoH scores.

	No Experience	Experience
Na	1.57	8.89
HoH	14.11	12.56

This research, and earlier studies, are an attempt to demonstrate the relevant stimuli that an animal requires prior to the brain lesion in reducing the behavioral deficit. "The Na^+ appetite paradigm" is a screening system for determining which stimuli are most relevant to which anatomical locus in reducing this behavioral deficit.

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730 EFFECTS OF ATROPINE AND VAGOTOMY ON APPETITE IN HYPOTHALAMIC HYPERPHAGIC RATS. Anthony Sclafani, Paul Aravich*, Martin Landman*, and Steven Xenakis*. Dept. Psychology, Brooklyn College, Brooklyn, N.Y. 11210.

Bilateral parasagittal knife cuts in the medial hypothalamus of adult female rats produced overeating and obesity. The Cut rats also drank more of a 20% sucrose solution than did sham controls during 30 min/day tests. Pretreating the rats with atropine methylnitrate (5 or 10 mg/kg) did not reduce their sucrose intake, and the Cut rats continued to drink more sucrose than did the controls. Atropine reduced 24 hr chow intake, although the Cut rats still ate more than did control subjects. The results suggest that cholinergically-mediated visceral responses are not essential for knife cut induced sucrose overconsumption. In a second experiment subdiaphragmatic vagotomy (Vag) or sham vagotomy (Sham) were performed in knife cut (Cut) and control (Con) female rats. Vagotomy reversed the hyperphagia and obesity of the Cut rats, and also blocked their overconsumption of 20% sucrose solution during 1 hr and 24 hr/day tests. The atropine results of Exp 1 suggest that the vagotomy effect on sucrose consumption may be due to the disruption of vagal afferent activity, or atropine-resistent vagal efferent activity. In a third experiment the subjects of Exp 2 were given ad libitum access to palatable foods (high fat diet, sweetened milk, chocolate-chip cookies) in addition to chow for 40 days. At the start of the experiment the Cut-Vag and Con-Vag groups weighed less than did the Con-Sham group, which weighed considerably less than did the Cut-Sham group. All rats gained weight on the palatable foods, but the Cut-Vag group gained the most (260 g), while the Con-Vag group gained the least (85 g). The Cut-Sham group gained more than did the Con-Sham group (195 vs 145 g). Interestingly, both the Cut-Vag and Con-Vag groups consumed significantly less of the sweetened milk diet than did the Cut-Sham and Con-Sham groups, which is consistent with the suppressive effect of vagotomy on sucrose solution intake observed in Exp 2. When returned to the chow only diet all groups lost weight, but the Cut-Vag group lost the most (190 g/40 days). These results demonstrate that while vagotomy reverses the overeating of chow and sucrose diets induced by hypothalamic knife cuts, it does not block the hyperphagia and enhanced weight gain displayed by knife cut rats offered an assortment of palatable foods.

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731 DRINKING TO CAVAL LIGATION FOLLOWING ABLATION OF PERIVENTRICULAR TISSUE SURROUNDING THE ANTEROVENTRAL THIRD VENTRICLE (AV3V) IS SPECIFICALLY CORRELATED WITH THE POSTLESION RESPONSE TO ANGIOTENSIN II. E. E. Shrager* and A. K. Johnson (SPON: J. A. Harvey). Dept. of Psychology, University of Iowa, Iowa City, IA 52242.

Lesions of AV3V periventricular tissue produce acute, primary adipsia; animals which subsequently regain ad lib water intake demonstrate deficits in the drinking responses to exogenously administered angiotensin II (AII) and hypertonic saline (HYP). These animals also sustain persistent elevations in serum sodium and osmolality (Buggy & Johnson, Am. J. Physiol., 5:177, 1977). In a previous report from this laboratory it was shown that AV3V lesions attenuated or abolished the drinking response to caval ligation. This response failure did not appear to reflect an inability to release renin in response to the surgical manipulation; in fact, lesioned rats demonstrated chronic elevations in basal plasma renin concentration (PRC)(Shrager & Johnson, Neurosci. Abs., 4:180, 1978).

The present studies further explored the contributions of the AV3V region to the control of drinking in response to both exogenously administered and endogenously generated, peripheral AII. Rats were tested to a range of subcutaneously (s.c.) administered doses of AII (0.5,1.0,2.0,4.0,6.0 mg/kg) and of HYP (1-16 cc/kg of 10% NaCl w/w) prior to placement of anodal lesions in the AV3V or sham lesioning procedures. The dose-response curve for AII had an ED50 at 1.0 mg/kg and a maximal response of 7.2 ml (in a 1-hr test) at the 4.0 mg/kg dose. One month postsurgery, dose-response testing was repeated. Half of the lesioned and sham lesioned rats then underwent ligation of the inferior vena cava and were observed for their water intakes over the next 4 hr. The remaining animals were bilaterally nephrectomized and 24 hr later, the water intake to 2.0 mg/kg AII was tested. The drinking response over the 4 hr postligation was positively and significantly correlated with the postlesion response to AII ($r = .686$, $p<.01$). Thus, rats which failed to drink to s.c. AII postlesion also failed to respond to caval ligation. The postlesion response to HYP was not significantly correlated with either the response to AII or to that following caval ligation. The chronic elevation in PRC cannot account for the failure to drink to s.c. AII postlesion as nephrectomy did not reverse the deficit.

These data support the hypothesis that caval ligation acts to induce drinking via those systems mediating the response to s.c. AII, and further, that the lesion produced disruptions of drinking to both the endogenous and exogenous challenges are independent of accompanying alterations of the peripheral renin angiotensin system. Histological analysis for an area of critical damage within the AV3V region, which produced AII response deficits, implicated a portion of the median preoptic nucleus.

732 EFFECTS OF LIMBIC LESIONS ON CIRCADIAN DRINKING RHYTHMS IN THE RAT. Cheryl L. Sisk and Friedrich K. Stephan. Dept. Psychology, Florida State University, Tallahassee, FL 32306.

Large bilateral lesions of the amygdala and ablation of the dorsal hippocampus, including the fornix, significantly enhanced nocturnal drinking and resulted in a tendency toward accelerated re-entrainment following a 12-hr phase shift of the light-dark (LD) cycle. None of the rats were in permanent estrus or anestrus. Knife cuts of the fornix resulted in highly irregular transients after the phase shift of the LD cycle and significantly increased the number of days required to re-entrain drinking rhythms. Since drinking rhythms appeared to free run normally in constant light, the impairment in phase shifting is probably not a deficit in the circadian regulation of drinking. Furthermore, since ablation of the fornix did not produce this deficit, it appears that some other structure damaged by the knife cuts is responsible. All rats in the fornix and hippocampal lesion groups displayed a change in the pattern of drinking bouts for the duration of the experiment but average daily water intake was not affected. It was concluded that the major extrahypothalamic limbic structures may play a modulatory role in the drinking of rats but are not essential to the generation or entrainment of the circadian rhythm in water intake. (Supported by NSF Grant BNS 78 2497 and NIH Grant MH 11218.)

733 FEEDING IN RESPONSE TO SYSTEMIC AND INTRAVENTRICULAR 5-THIOGLUCOSE. Peter G. Slusser* and Robert C. Ritter (SPON: M.E. DeSantis). College of Veterinary Medicine, Wash. State Univ., Pullman, WA 99164

Systemic or intracerebroventricular injection of 2-deoxy-D-glucose (2DG) causes sympothoadrenally mediated hyperglycemia and increased food intake in most mammals. Both of these physiological responses to 2DG are thought to be mediated in part or in total by brain glucoreceptors which detect decreased glucose utilization, glucoprivation. Unfortunately, the feeding and hyperglycemic response to glucoprivation have not been studied in animals treated with other potent antimetabolic glucose analogues. Accordingly, we have been studying the effects of several hitherto unexamined glucose analogues upon food intake and hyperglycemia in rats. We have devoted particular attention to 5-thio-D-glucose (5TG), a glucose analogue in which the glucopyranose ring oxygen has been substituted with sulfur.

5TG is a potent inhibitor of phosphoglucomutase and glucose-6-phosphate dehydrogenase, two enzymes which are not strongly inhibited by 2DG. We have found that intracardiac infusion of 5TG produced marked dose dependent increases in food intake and blood glucose concentrations. In fact, at 0.30 and 0.61 mmols/kg 5TG produced significant elevations of food intake ($N=10$, $p<0.01$) above control values (1.5 ± 0.3 g and 2.6 ± 0.3 g respectively) while equimolar 2DG doses produced no statistically significant changes from control intake ($N=13$, $p>0.1$). Food intake in response to 5TG increased linearly with dose up to 1.22 mmols/kg. However, 2DG induced intake was not dose dependent. At 1.22 mmols/kg, 5TG increased food intake by 5.5 ± 0.7 g and 2DG increased intake by 6.2 ± 0.9 g. These were the maximal responses for both analogues. 5TG also produced 3.0 to 4.6 times greater plasma glucose elevations than did 2DG and appeared to be a more potent inhibitor of insulin release in the presence of food than is 2DG. Work in progress is investigating the comparative potencies of 5TG and 2DG for elicitation of feeding when infused intracerebroventricularly.

The known biochemical actions of 5TG differ markedly from those of 2DG. Therefore, the greater efficacy of 5TG for elicitation of feeding suggests that the metabolic events inhibited by 5TG deserve closer scrutiny with regard to control of food intake.

734 SELECTIVE GASTRIC VAGOTOMY DECREASES THE SATIETY EFFECT OF CHOLECYSTOKININ IN RATS. G.P. Smith, C. Jerome*, R. Eterno* and B. Cushin*. Dept. Psychiatry, Cornell Univ. Med. College and E.W. Bourne Behavioral Research Lab., The New York Hospital, White Plains, N.Y. 10605.

Bilateral abdominal vagotomy markedly decreases or abolishes the satiety effect of the C-terminal octapeptide of cholecystokinin (CCK-8) in rats (Lorenz and Goldman, 1978; Smith and Cushin, 1978). Resection of the hepatic branch is not necessary for this effect (Smith and Cushin, 1978). In an attempt to localize the specific abdominal vagal terminal field that mediates the satiety effect of CCK-8, we performed selective resections of the coeliac (n=8) or gastric (n=9) branches of the abdominal vagus. After recovery from surgery, rats were adapted to overnight (17 hr) food deprivation and offered their maintenance diet of sweet milk. When intakes stabilized, rats were injected i.p. with CCK-8 (1-16 mcg/kg) or with saline 15 min before food was offered. Gastric vagotomy decreased the satiety effect of CCK-8, but coeliac vagotomy had no effect. The dose for 50% inhibition of food intake in gastric vagotomy rats was about 16 mcg/kg compared to about 2 mcg/kg in sham operated rats. The results suggest that (1) the stomach is the major site of action for the satiety effect of CCK 8; and (2) vagal afferents relay the satiety signal to the brain.

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735 ANTICIPATION OF 24 HR FEEDING SCHEDULES IN RATS WITH LESIONS OF THE SUPRACHIASMATIC NUCLEI AND HYPOPHYSECTOMY. Jennifer M. Swann* and Friedrich K. Stephan. Dept. of Psychology, Florida State University, Tallahassee, FL 32306.

We have shown previously that rats with lesions of the suprachiasmatic nuclei (SCN) anticipate restricted access to food by an increase in wheel running as readily as intact rats and presented evidence that this behavior shows some of the basic properties of a circadian rhythm, despite the absence of circadian rhythms in activity and drinking in ad lib. conditions. Anticipatory activity was not prevented by adrenalectomy (Stephan, Swann and Sisk, Behavioral and Neural Biology, 1979, in press). To elucidate the possible role of the pituitary gland in anticipatory activity, hypophysectomized rats and controls were maintained in constant light with ad lib. access to food and water for 10 days, followed by a restricted feeding schedule (one or two daily access periods) at 24 hr intervals for 20 consecutive days. Under ad lib. conditions, both groups showed free running activity rhythms with a period of approximately 25 hr. Under restricted access conditions, the dominant period of activity became exactly 24 hr in both groups, although a weaker activity component with a period longer than 24 hr persisted in some animals. However, while controls showed distinct anticipatory activity within 2-5 days after initiation of the restricted feeding schedule, hypophysectomized rats required 10 days or longer before anticipatory activity appeared. Furthermore, the amount of anticipatory activity was significantly reduced compared to controls. Preliminary data on hypophysectomized rats with SCN lesions indicate that these rats are still capable of anticipating restricted feeding schedules. While some rats no longer showed anticipatory wheel running, simultaneously collected event records of drinking indicated the presence of anticipatory licking. These results show that restricted access to food at 24 hr intervals synchronizes activity of hypophysectomized rats and that anticipatory activity is reduced, but not prevented, by hypophysectomy. Furthermore, SCN lesions in hypophysectomized rats may abolish anticipatory wheel running but not anticipatory drinking. Thus, the locus of control for anticipatory behavior remains to be determined.

737 INSULIN AND SATIETY IN OBESE AND NORMAL-WEIGHT RATS. Dennis A. VanderWeele, Edward Haraczkiewicz* and Theodore B. Van Itallie*. Dept. Neurol., Sch. Med., NYU, New York, NY, 10016 and Obesity Res. Cent., Dept. Med., Columbia Univ., New York, NY, 10025.

The role of insulin was evaluated in normal-weight, male rats using the osmotic minipump to elevate endogenous insulin levels. Normal-weight animals reduced food consumption by lowering meal sizes without any change in meal frequency when 1.92 U regular insulin was delivered via minipump. Meal sizes averaged 21% smaller with 1.92 U insulin compared to isotonic saline delivered via the pump. Body weight gain was significantly decreased in animals receiving 1.92 U but not in subjects receiving 0.96, 4.0, and 6.0 U insulin per 24 hr, when compared to animals receiving saline. Thus, normal-weight animals, with induced elevation of insulin levels, may increase satiability and limit weight gain by clearing the blood of nutrients more efficiently.

The above findings indicate a sustained modest elevation of insulin is associated with reduced food intake. Obese animals with attendant hyperinsulinemia have been shown to reduce food intake when the manipulation inducing the obesity is terminated and, therefore, it was hypothesized that impairment of insulin release would attenuate the hypophagic response. To test this proposal, insulin release was severely impaired with injections of streptozotocin at 45 mg/kg body weight, based on the weight of the lightest subject. Obese, diabetic animals consumed 17% less food daily than nonobese, diabetics; obese, nondiabetics consumed 16% less food daily than nonobese, nondiabetics; and mild diabetics which were also obese (<2% glycosuric) consumed 21% less food daily than did nonobese, nondiabetics. These differences are all significant at the 0.05 level. A dietary snack-food paradigm had been used to induce obesity in the rats, but food intake comparisons post-streptozotocin were made with regular Purina chow only. Thus, the hypophagic behavior of dietary obese rats shifted to less palatable diets does not appear to depend upon obesity-induced insulin excess. Insulin-enhanced satiety in normal-weight animals may not be inconsistent with the obese animal effects due to increased insulin resistance which would develop in the obese animals.

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736 INHIBITION OF ADH SECRETION AND SATIETY FOLLOWING WATER INGESTION IN WATER-DEPRIVED DOGS. T.N. Thrasher*, J.F. Nistal Herrera*, L.C. Keil*, C.J. Brown* and D.J. Ramsay. Dept. of Physiol., UCSF, San Francisco, CA 94143 and Ames Res. Cent. Moffett Field, CA 94035.

It is well known that some species, such as dogs, sheep and goats, are able to repair a water deficit due to deprivation within the first few min. of access to water. Since, in dogs, drinking is complete long before ingested water alters plasma tonicity or volume, the mechanism which matches water intake to deficit must rely on other afferent information. Stimuli which elicit thirst usually also elicit ADH secretion. Therefore, we questioned if ADH secretion was reduced before restoration of plasma tonicity in water-deprived dogs. A preliminary experiment on four dogs, dehydrated for 24-hours, indicated that plasma ADH fell before a detectable change in plasma sodium or osmolality occurred. The two most likely stimuli which could account for this phenomenon are drinking-induced oro-pharyngeal stimulation or gastric distention. In order to test between these two stimuli, a second population of dogs was prepared with permanent gastric fistulae, thus allowing either immediate removal of ingested water, or loading of water directly into the stomach. Blood was sampled before dehydration, after 24-hours of dehydration, and at 3, 6, 9, 12, 15, 30 and 60 min. after allowing access to water. Three experiments were carried out: (1) oral rehydration with water available to satiation; (2) oral rehydration coupled with simultaneous removal, via the fistula, of the ingested water; and (3) gastric loading of water, via the fistula, the amount being determined from (1) above. Dogs allowed to drink ad lib were satiated within six min. and showed a significant reduction in plasma ADH before a detectable change in plasma sodium or osmolality occurred. When offered water 60-min. later, it was ignored suggesting complete satiation. Dogs which drank but lost the water via the fistula also were satiated within six min. Plasma ADH was significantly reduced at 3, 6, 9 and 12 min. after drinking but rapidly increased to pre-drinking levels. No changes in plasma sodium or osmolality were detected at any time. When offered water 60 min. later, they drank approximately the same volume that was consumed in (1) above. Gastric administration of a water load equivalent to that drunk in the first experiment produced a simultaneous reduction in plasma ADH, sodium and osmolality. When offered water 60 min. later, they drank 20% of the volume consumed in (1) above. Since plasma sodium and osmolality had returned to predehydration levels, this drinking suggests that oro-pharyngeal stimulation is required for complete satiety. These data indicate that oro-pharyngeal influences, but not gastric distension, acts to suppress ADH secretion in water-deprived dogs. Supported by NIH Grant AM 06704.

738 ONTOGENY OF CHOLECYSTOKININ-INDUCED SATIETY IN RATS. C.H. Wang* and S. Hsiao*. (SPON: M. Wetzl) Dept Psych, U Arizona, Tucson

Three hundred and fifty-eight neonatal rats selected from 44 litters of different ages were used in four experiments to investigate the development of behavioral response to a satiety hormone cholecystokinin (CCK). The CCK-induced satiety was first seen in 9 day-old rat pups followed by a disappearance of the effect at day 10 through 12 and then permanently reappeared at day 13. This phenomenon was replicated at the critical ages of day 9 through day 13. Thyroxine injections during the first postnatal week facilitated the onset of CCK-induced satiety whereas hypothyroidism induced by treating the nursing mothers with n-Propylthiouracial (PTU) during their late pregnancy retarded the onset of CCK-induced satiety. These alterations of behavioral response by manipulating the thyroid hormones were parallel to the alterations of morphological growth rate such as teeth eruption, ears and eyes openings.

739 CIRCADIAN DRINKING RHYTHMS AND MODULATION BY EXOGENOUS LIGHT. Andrea L. Wesley. Dept. Psy., Univ. Sou. Miss., Hattiesburg, Ms 39401.

Physiological and behavioral studies have shown that the light-dark cycle exerts a pronounced exogenous influence upon endogenous eating and drinking rhythms. The exogenous stimulus of light was used to investigate its effects upon the rhythmic pattern of water intake as well as the total daily consumption. Low, medium, and high intensity light levels (1 log difference) were used in a series of three experiments to study the interaction of exogenous and endogenous mechanisms in three strains of rats, Wistar (WS), Long-Evans (LE), and Charles River (CH). In experiment 1 with constant light of the three intensity levels, WS and CH rats reduced daily water intake significantly ($p < .01$). LE rats, however, did not exhibit a significant decrease in water intake, even at high light intensities. Nocturnality of drinking (%dark/total daily intake) was reduced in both CH ($p < .01$) and WS ($p < .02$) after exposure to a light-dark cycle with low intensity light periods in experiment 2. With high intensity light periods nocturnality increased in these two strains ($p < .01$). Again, LE rats did not exhibit a significant modification of drinking in response to light intensity manipulations in the light periods. In experiment 3 the superior accessory (SAOT) and primary optic (POT) tracts were lesioned to evaluate the possible influence of the accessory optic system; this lesion resulted in disinhibition of the inferior accessory optic system (IAOT). When compared with non-lesioned controls for each respective strain, LE rats reduced daily water intake at medium ($p < .001$) and high ($p < .05$) intensity levels of constant light. These reductions were comparable to those exhibited by WS and CH rats ($p < .02$). The results suggest that in the LE rats, the SAOT is able to compensate for the effects of light intensity on water, preserving the homeostasis of fluid balance as well as nocturnal drinking patterns. In summary these experiments demonstrate the interaction of exogenous factors with the rhythmic controls of drinking in the rat.

740 AV3V DESTRUCTION IN INFANT RATS AND THE DEVELOPMENT OF DRINKING BEHAVIORS AND BODY WATER REGULATION. Robert L. Wyatt* and C. Robert Almli. (SPON: C. Spirito). Dept. Psychol., Ohio Univ. Athens, Ohio 45701.

Infant albino rats (males and females) sustained electrolytic lesions of the periventricular tissue surrounding the anteroventral third ventricle (AV3V) at 10 days of age. Following weaning at 25 days of age, and throughout development, drinking behaviors were measured daily. Also measured were drinking behaviors following specific dipsogenic treatments, as well as various blood and urine variables.

Daily ad lib water intakes of AV3V rats were elevated over control intakes from the first measurement period and thereafter. Female AV3V rats were reliably hyperdipsic by 25-30 days of age, and males were reliably hyperdipsic by 45-50 days of age. The daily ad lib hyperdipsia was maintained through 200-250 days of age for both sexes, and reached 216% of control intakes. When the rats were tested for drinking behaviors following specific manipulations, the AV3V rats (of both sexes) were hyperdipsic in response to hypovolemia produced by polyethylene glycol injections, they were hyperdipsic during 24 hour food deprivation, and they were hyperdipsic following 24 hour water deprivation. More noteworthy, because of the contrast with rats sustaining AV3V lesions as adults, the rats sustaining AV3V lesions at 10 days of age were also hyperdipsic in response to cellular dehydration produced by injection of hypertonic NaCl solution. Latency to initiate drinking following hypertonic NaCl solution injection did not differ between AV3V and control rats. Water intakes of AV3V and control rats did not differ following injection of isoproterenol, and AV3V rats displayed a normal sodium appetite following formalin injection.

Under ad lib conditions, urine output:water intake ratios of AV3V rats were elevated over controls, and their urine was more dilute than controls. However, the AV3V rats were hyperdipsic, and even with an elevated urine output, these rats were retaining more body water than controls. This water retention was associated with serum hyponatremia for AV3V rats under ad lib conditions. These results suggested a possible disruption of water savings mechanisms; thus, the rats were water deprived for 24 hours. The AV3V rats displayed ability to reduce urine volume (AV3V = 64-74%, control = 26-51%) and to increase urine concentration (AV3V = 200-300%, control = 100-200%) during 24 hour water deprivation. Thus, the hyperdipsia of the present AV3V rats seemed primary, and the etiology of the hyperdipsia is under study. Supported by: NICHHD (HD-08504) and OURC (OU-520).

741 NEUROPHYSIOLOGICAL CORRELATES OF GUSTATORY AND VISCERAL SENSATION OF ALCOHOL AND OTHER NUTRIENTS IN THE RAT. Warren G. Young* and J. Anthony Deutsch* (SPON: R.B. Livingston). Dept. Neurosciences and Psychology, UCSD, San Diego, CA 92093.

The nucleus of the solitary tract receives gustatory and visceral afferents arising from the facial (VII), glossopharyngeal (IX), and vagus (X) nerves. Extracellular neuronal recordings have been carried out in this nucleus only as a function of sapid, thermal, and tactile stimulation of the tongue.

Gustatory information is essential for the neural mechanism of taste aversions and for modulating hunger-satiety levels. We know that rats have an extraordinarily well-developed mechanism of bait shyness. Our experiments have consistently shown that naive rats will avoid alcohol ingestion. However, forced intubation will yield a chronically dependent rat.

We are investigating this disparity by recording extracellular single unit activity from the nucleus of the solitary tract. Correlations are being made between the naive and the chronic rat and their respective neuronal response patterns. Furthermore, there is an integration of gustatory neural coding with visceral neural coding arising from vagal afferents synapsing on the nucleus. We have found evidence substantiating differential gastric sensibility (J.A. Deutsch, W.G. Young, and T.J. Kalogeris, Science 201, 165 (1978)). We are continuing investigations into this total quality coding of alcohol and dietary nutrients by recording unit activity correlated with gustatory and visceral parametric manipulations. Full results will be presented at the meeting. (This work is being supported by grant AA 07129-04 to J.A. Deutsch).

742 WATER INTAKE AFTER VOLUME DEPLETION IN SHEEP: EFFECTS OF CRUSHING THE LEFT ATRIAL APPENDAGE. Mark B. Zimmerman*, Edward M. Stricker and Edward H. Blaine*. Merck Institute for Therapeutic Research, West Point, PA 19486, and Dept. of Psychology, Univ. of Pittsburgh, Pittsburgh, PA 15260.

Reduction of the intravascular fluid volume has been shown to induce water ingestion in a variety of mammalian species. The present experiments provide the first direct evidence that cardiovascular baroreceptors play an important role in mediating this response. In one group of adult female sheep (I; 7 experiments on 5 animals), removal of 2460-3208 ml of protein-free, isoosmotic fluid was accomplished using a Gambro dialyzer and an extracorporeal blood pumping system. Filtration occurred over a 5-hr period in conscious food- and fluid-deprived animals, followed immediately by access to food and water for 2 hr. In a second group of sheep (II; 5 experiments on 4 animals), the appendage of the left atrium of the heart was crushed 15-20 days prior to filtration. A similar procedure has been shown to abolish the increases in urine flow and heart rate that normally occur following distention of the atrial appendages in dogs (Kappagoda et al., J. Physiol. 227: 233, 1972). The results are tabulated below (mean ± SEM; B = value before filtration, A = value after filtration):

		ABP mm Hg	PP g%	PRA ng/ml/hr	P_{Na} mEq/L	GFR ml/min	RPF ml/min	△INTAKE ml/2 hr
I	B	90±2	6.0±0.2	1.2±0.2	142±1	62±3	454±44	
	A	59±6	7.8±0.3	27.6±6.4	142±1	9±4	91±43	1238±265
II	B	83±2	7.0±0.3	3.0±0.3	145±1	64±7	535±74	
	A	57±4	9.0±0.4	49.6±7.5	147±1	7±3	106±29	163±106

In both groups of animals, the filtration procedure raised plasma protein concentration (PP) and plasma renin activity (PRA) while it reduced arterial blood pressure (ABP), renal plasma flow (RPF), and glomerular filtration rate (GFR); plasma sodium concentrations (P_{Na}) remained unchanged. The major finding of this study is that hypovolemic intact sheep ingested considerably more water than usual, whereas sheep with crushed appendages did not. (△INTAKE was the volume of water consumed after filtration minus the mean water intake averaged from comparable testing periods of the previous 5 days.) Control experiments showed that neither ad libitum water intake nor the drinking response to intracarotid infusion of 4 M NaCl solution was affected by the crushing. These data suggest that volume receptors located in the area of the left atrium may play a role in mediating thirst during hypovolemia.

HYPOTHALAMUS

743 INDIVIDUAL BEHAVIORAL RESPONSES TO HYPOTHALAMIC STIMULATION PERSIST DESPITE DESTRUCTION OF TISSUE SURROUNDING ELECTRODE TIP. Susan E. Bachus* and Elliot S. Valenstein. Dept. Psychobiology, Univ. Michigan, Ann Arbor, Michigan 48109.

Striking individual differences in the behavior evoked by electrical stimulation of the lateral hypothalamus (ESLH) have been noted in animals, even with indistinguishable electrode placements and identical testing procedures (Cox & Valenstein, Brain Behav. Evol. 2:359, 1969). It has been argued that individual differences in response to ESLH may be due to variability in localization of critical neural substrates, but responses have been reported not to change after incremental movements of the electrode (Wise, Physiol. Behav. 6:569, 1971). In the latter experiment, however, the possible roles of current spread or stimulation experience were not considered. We investigated whether the same behavior would be elicited by stimulation of different substrates in individual rats by retesting after producing lesions around the tips of the electrodes, in rats with either extensive or minimal stimulation experience prior to the lesions.

Sprague-Dawley and Long Evans rats were implanted with bipolar electrodes, and screened for elicitation of drinking by ESLH. Some S's received daily tests until current thresholds for eliciting drinking had stabilized. Other S's were given "minimal stimulation" experience (as few as 2 stimulus trains) to establish the presence of elicited-drinking. Electrolytic lesions were then administered through the stimulating electrodes. One group of S's received a series of progressively larger lesions, each succeeding extensive daily testing. In another group, single stage large lesions were produced in S's which had minimal stimulation experience. In all cases, drinking was elicited by ESLH after extensive lesions, usually at greatly elevated thresholds. Histological examination revealed that drinking was evoked after lesions in which damage spread over 3 mm along an anterior-posterior axis through the medial forebrain bundle, with a maximum diameter of over 1 mm.

When the low stimulating current levels (as low as 1.0uA, 60 Hz sine wave) that were initially sufficient to evoke drinking are considered along with the large area of destruction around the electrode tips, we infer that the neuronal elements that were directly activated by the stimulation during screening were completely destroyed, or at least, the circuitry radically altered; yet the initial response persisted. These results suggest that individual differences in response to ESLH cannot be attributed solely to variation in the neuroanatomical locus of stimulation. Moreover, this persistence does not reflect changes induced by a long regimen of stimulation or experience. We conclude that some animals are predisposed to drink in response to stimulation within a relatively large hypothalamic area.

744 A NEUROPHYSIOLOGICAL DETERMINATION OF LATERAL HYPOTHALAMIC AND LATERAL PREOPTIC AREA INTERCONNECTIONS. F. C. Barone, M. J. Wayner, W. H. Tsai* and F. E. Barash*. Brain Research Lab, Syracuse Univ, Syracuse, NY 13210.

Single unit recordings were made from central neurons in anesthetized male hooded rats. In some animals the effects of lateral hypothalamic stimulation on lateral preoptic area-medial forebrain bundle (LPA-MFB) unit activity were determined. In other animals the effects of LPA stimulation on lateral hypothalamic-medial forebrain bundle (LH-MFB) neural activity were determined. Recordings were made ipsalateral to the stimulating electrode. A stable baseline discharge frequency was established and single pulses, 0.5 msec in duration, and/or trains of pulses were applied to the stimulation site through a concentric bipolar electrode. Both frequency, usually 0 to 20 Hz, and voltage, 0 to 10 volts, were varied in a random manner. The reliability of effects was established by repeated testing. The results of single pulse stimulation on neural activity were examined on a storage oscilloscope and photographed. The results of trains of pulses on neural activity were illustrated graphically by means of a strip chart recorder. Frequency and voltage response relationships were established. Both excitatory and inhibitory effects were observed. Analysis of single pulse stimulation indicated that the effects were mono and polysynaptic. The effects occurred with relatively short latencies and low voltages indicating that interconnections between the LPA and LH exist. Data obtained from repetitive stimulation confirmed the results of single pulse effects. These results together with neuroanatomical data unequivocally demonstrate a significant degree of interconnectivity along the extent of the LPA-LH-MFB neuropil.

745 AFFERENT PROJECTIONS TO THE MEDIAL PREOPTIC AREA AND MEDIAL HYPOTHALAMIC REGIONS OF THE RAT. Mitchell L. Berk* and Judith A. Finkelstein. Dept. Anat., N.E. Ohio Univs. Col. Med., Rootstown, Ohio 44272.

Many neuronal pathways to the medial preoptic area (MPOA) and hypothalamus of the rat have been determined by the use of a variety of neuroanatomical techniques. However, in order to obtain a complete overview of the afferent projection to this region, the horseradish peroxidase (HRP) technique was utilized. Iontophoretic injections of HRP were placed in the MPOA-anterior hypothalamic area and the regions of the paraventricular, dorsomedial (DMH) and ventromedial hypothalamic (VMH) nuclei using standard stereotaxic procedures. Survival times were usually 24 hrs. Sections were processed by the tetramethylbenzidine (TMB) procedure which produces a blue reaction product.

The following description of labeled cells pertains to all injection sites, unless stated otherwise. In the telencephalon, HRP granules were localized ipsilaterally in the perikarya of the ventral subiculum, medial amygdala, and the lateral septal nucleus. The basolateral nucleus of the amygdala was labeled only with VMH injections. Labeled cells in the bed nucleus of the stria terminalis were found in VMH and DMH injections. At midbrain levels, labeled cells were located in the ventromedial part of the periaqueductal gray and the dorsal and median raphe. Injections in the VMH region resulted in intensely stained cells bilaterally (ipsilateral> contralateral) in the peripeduncular nucleus. With all other injection sites only ipsilateral peripeduncular cells were labeled; the intensity of the staining was less than with the VMH region injections.

At pontine levels, labeled cells were located bilaterally (ipsilateral> contralateral) in the lateral parabrachial nucleus and the locus coeruleus. The dorsolateral tegmental nucleus, as well as cells medial and ventral to the dorsal tegmental nucleus, were labeled. Also, DMH-VMH injections resulted in labeled perikarya in the dorsal and ventral tegmental nuclei.

In the medulla, labeled perikarya were located bilaterally (ipsilateral> contralateral) in the nucleus of the solitary tract and lateral reticular nucleus (possibly the A2 and A1 catecholaminergic cell groups respectively). Labeled cells of the lateral reticular nucleus were more intensely stained on the ipsilateral than on the contralateral side. Labeled medullary reticular cells were found bilaterally in the area bordering the medial longitudinal fasciculus, lying ventral to nucleus prepositus hypoglossi. These results indicate that a multiplicity of areas send their input to cells in the medial hypothalamus where they may modulate homeostatic mechanisms. Supported by NIH Grant 5 R01 NS14344 and NSF Grant BNS 77-19302.

746 HYPERPHAGIA AND OBESITY FOLLOWING LESIONS OF THE MEDIAL HYPOTHALAMUS IN MAN. Gastone G. Celesia, Carol R. Archer* and Hyung D. Chung*. Veterans Administration Medical Center and St. Louis Univeristy, St. Louis, MO 63104.

Food intake is regulated by the hypothalamus. Bilateral destruction of the ventromedial nucleus (VM) in animals induces abrupt onset of voracious eating while bilateral destruction of the lateral hypothalamus at the level of VM causes cessation of eating. Similarly in man, lesions of the lateral hypothalamus causes anorexia and emaciation; in contrast the effects of VM lesions are more controversial.

A 28-year-old man developed progressive left hemiparesis followed three weeks later by face and trunk cutaneous vasodilatation and hyperphagia. He began eating five to six meals per day and constantly felt hungry, he gained thirty-five pounds in sixty days. Computerized tomography with coronal and sagittal reconstruction revealed a neoplastic lesion involving the right lenticular nucleus, the right internal capsule, crossing the midline via the hypothalamus and reaching the contralateral lenticular nucleus. Endocrine studies including HTSH, growth hormone, prolactin and cortisol serum levels were normal. Hyperphagia and consequent obesity was caused by bilateral destruction of VM, cutaneous vasodilatation was related to involvement of preoptic area.

It is suggested that in man a medial hypothalamic syndrome is characterized by hyperphagia, obesity and cutaneous vasodilatation. Additional signs may be hyperthermia and diabetes insipidus.

747 ULTRASTRUCTURE OF NEURONS OF THE VENTROMEDIAL NUCLEUS OF OVARIECTOMIZED AND ESTROGEN-TREATED FEMALE RATS. Rochelle S. Cohen* and Donald W. Pfaff, The University of Illinois at the Medical Center, Chicago, Il. and The Rockefeller University, N.Y., N.Y.

Cells in the ventrolateral area of the ventromedial nucleus in the hypothalamus have a high affinity for estradiol. We are studying the cellular architecture of these cells to see what differences exist between such cells and other neurons, and to look for estrogen effects.

Estradiol benzoate was injected daily subcutaneously in one group of ovariectomized rats. The control group was injected subcutaneously with the vehicle, sesame oil. After 20 days, exposure to estrogen, the experimental rats showed a strong lordosis reflex response and at this time the rats were sacrificed by anesthetizing with nembutal and perfused with fixative according to standard electron microscopy procedures (Peters, 1970). Three regions of the brain were dissected from each rat: the ventrolateral and dorsomedial subdivisions of the ventromedial nucleus of the hypothalamus and, as a control, the ventrobasal thalamus. These areas were embedded and thin-sectioned for viewing with the electron microscope.

The overall morphology of the ventromedial nucleus consists mainly of neurons and a few interspersed glial cells. The neurons contain large oval nuclei, the nuclear membrane of which is often invaginated; the nucleoplasm is finely granular with a densely granular nucleolus. The assemblage of organelles present include rough endoplasmic reticulum (RER), a Golgi complex with coated and uncoated vesicles attached or in the vicinity, small (~125nm) dense-cored vesicles, lysosome-like bodies, dense, granular, non-membrane bound structures referred to as inclusion bodies, multivesicular bodies, and occasionally, myelin figures are present.

Two trends seen as differences in the ventrolateral subdivisions of the two groups include a greater stacking of RER and a greater number of dense-cored vesicles in estrogen-treated rats compared to the control group. King et al. (1973) also found changes in the organization of the RER and dense-cored vesicles, among other changes, in arcuate neurons of the rat during the estrus cycle.

748 AUTORADIOGRAPHIC ANALYSIS OF HYPOTHALAMIC AREA CONTROLLING CARDIOVASCULAR RESPONSE TO EMOTION. J. DeVito, O. Smith, J. Stein and K. Walsh*, Reg. Primate Res. Ctr., Univ. Wash., Seattle, WA 98195

The cardiovascular (CV) responses to an acute emotional situation in unanesthetized chair restrained baboons include elevations in heart rate, blood pressure, and terminal aortic flow and a complex biphasic reduction in renal flow. The same CV responses can be produced by stimulating an area in the hypothalamus. Furthermore, bilateral ablation of the hypothalamic area eliminates CV responses to the emotional behavior while responses to exercise, free feed and sleep remain unaltered. Efferent projections of the hypothalamic site were traced by means of autoradiography.

Prior to the isotope injection baboons were fitted with recording devices for heart rate, blood pressure and renal flow. They were anesthetized and stereotaxic coordinates determined by ventriculography. An electrode was lowered through a guide cannula into the hypothalamus until stimulation produced the appropriate CV responses. The electrode was then removed, a ul syringe needle was inserted through the cannula to the predetermined site, and 0.15-0.25 ul of a 20-uCi/ul mixture of ^{3}H-isotopes was slowly injected. After a 3-day survival, the brain was processed for autoradiography.

From the injection site, fibers ascended to the nucleus of the stria terminalis and the lateral septal nucleus. There was widespread label in the preoptic area and fibers spread laterally from this region over the optic tract (OT) to the substantia innominata and amygdala. A band of fibers remained above the OT until passing medially into the midbrain. The rostral hypothalamus was widely labeled and fibers ascended at this level into the midline thalamic nuclei. Further caudally there were small projections to densocellularis part of dorsomedial nucleus and to pretectum ventral to habenular nuclei. In the posterior hypothalamus, only the medial and lateral mamillary nuclei were free of label. As fibers passed out of the hypothalamus, they coursed in central grey (CG), zona incerta (ZI) and dorsal to substantia nigra (SN). In midbrain, fibers from ZI were joined by the band descending above OT and passed medially to a dense terminal area in the dorsal tegmentum (DT) medial to medial lemniscus. The fibers paralleling SN appeared to terminate in central tegmentum. Fibers in midbrain CG spread laterally to join medially coursing fibers in DT and appeared to terminate lateral to CG. At this level in the midbrain, an area in lateral CG also gave the appearance of terminal rosettes. There were light projections to the pedunculopontine and lateral parabrachial nuclei. Moderately heavy label was present in dorsal and lateral CG throughout the midbrain.

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749 SUPERIOR COLLICULUS AND RETINAL PROJECTIONS TO THE HYPOTHALAMUS James H. Fallon and Robert Y. Moore. Dept. Anatomy, UCI and Dept. Neurosci., UCSD; Irvine, and La Jolla, CA

Recent Golgi and electron microscopic evidence has revealed that dendrites of hypothalamic origin extend into the optic and supraoptic tracts (Riley et al., '79). In a series of anatomical studies with anterograde tracing methods, we now report that these dendrites receive both superior collicular and retinal inputs in the rabbit. Injections of (^{3}H) proline were made into the intermediate or deep layers of the superior colliculus and silver grains could be traced over the supraoptic and optic tracts to the midline. The number of silver grains diminished at the midline with the remaining crossing fibers projecting to the contralateral thalamus and tegmentum. However, the fate of the "disappearing" superior collicular axons in the hypothalamic region of the optic and supraoptic tracts could not be ascertained with certainty. In order to determine if the fibers terminate within the optic and supraoptic tracts, we injected horseradish peroxidase (HRP) into the superior colliculus of three rabbits and demonstrated, electron microscopically, the termination of superior colliculus axons containing HRP reaction product onto dendrites extending into these tracts. In order to determine the cell bodies of origin of these dendrites, (^{3}H) adenosine was injected into the superior colliculus in four rabbits. Anterograde transneuronal transport of (^{3}H) adenosine resulted in autoradiographic labeling of cell bodies in the diffuse supraoptic nucleus, retrochiasmatic area and ventral sectors of the lateral and anterior hypothalamic areas. This projection is predominantly ipsilateral. In separate experiments, the pattern of anterograde labeling of retinal axons in the hypothalamus was studied. With (^{3}H) proline injections into the vitreous of the eye of three rabbits, projections to the hypothalamus included the suprachiasmatic nucleus and a few scattered labeled axons dorsal to the lateral wing of the supraoptic nucleus. With (^{3}H) adenosine injections into the vitreous of the eye of three rabbits, cellular labeling was found not only in the suprachiasmatic nucleus but also in the contralateral lateral hypothalamic area, retrochiasmatic area and supraoptic nucleus. In spite of the potential problems with axonal "leakage" of adenosine or its metabolites, these results provide some evidence that in the rabbit, as in the rat (Riley et al., '79), the retina projects to several hypothalamic nuclei outside the well-known retino-suprachiasmatic projection system. These results also show that separate and overlapping visual and multimodal inputs from the superior colliculus, and visual input from the retina may directly influence cells in a broad expanse of this ventral tier of the hypothalamus. Interestingly, many of these projections are to dendrites located within the sensory lemniscus.

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750 RECOVERY OF FUNCTION FOLLOWING TWO-STAGE POSTERIOR HYPOTHALAMIC LESIONS IN RATS. Michael Gruenthal* and Stanley Finger. Dept. Psychol., Wash. Univ., St. Louis, MO 63130.

The "serial lesion effect" (Finger et al., Brain Res., 1973) refers to the observation that under some conditions bilateral brain lesions made in 2 discrete stages are less incapacitating than those produced in a single operation. While this phenomenon has been intensively studied with learning and perceptual tasks following cortical damage, less research has been conducted on staged lesions of the brainstem, and these results have not always been consistent. We therefore investigated the effects of 1- and 2-stage posterior hypothalamic lesions on swimming ability (Robinson et al., Canad. J. Psychol., 1974) and open-field activity to determine if 2-stage lesions would result in greater recovery.

42 male rats received 1-stage or serial (30 days between sides) lesions of the posterior hypothalamus and were tested 1, 3 and 5 weeks after surgery. Subjects with 2-stage lesions swam significantly better than animals with 1-stage lesions across all testing periods. With respect to open-field activity, however, subjects with serial lesions did not demonstrate significantly enhanced recovery until the 3rd postoperative week. These data suggest that serial lesions of the posterior hypothalamus can enhance recovery although the time-course for such recovery may vary with the specific deficit being evaluated.

751 SEROTONERGIC INNERVATION OF THE LATERAL HYPOTHALAMUS: EVIDENCE FROM SYNAPTOSOMAL UPTAKE STUDIES. J. Heym* and W. E. Gladfelter. Dept. of Physiology and Biophysics, West Virginia University Medical Center, Morgantown, WV 26506.

In rats the lateral hypothalamus (LH) has been shown to contain relatively high concentrations of serotonin (5HT) and tryptophan hydroxylase, the rate limiting enzyme for serotonin biosynthesis. Anatomical findings have suggested that ascending 5HT fibers turn off from the medial forebrain bundle and synapse within the LH. The present study has explored further the possibility of a serotonergic terminal field within the LH. ^{3}H-5HT uptake was examined in crude synaptosomal preparations of rat lateral hypothalami. Aliquots (0.2 ml) of the synaptosomal suspension were preincubated at 37°C for 5 minutes in a modified Krebs-Ringer phosphate buffer (pH = 7.4) after which radiolabelled 5HT was added and the incubation was continued for an additional 4 or 5 minutes. When the concentration of ^{3}H-5HT present in the incubation media was varied from 2 X 10^{-8} M to 5 X 10^{-6} M, transport of 5HT by LH synaptosomes occurred by two distinct processes, one which was linearly related to 5HT concentration and the other which was saturable at 5HT concentrations greater than 10^{-7} M. Kinetic constants for the high affinity uptake of 5HT were calculated from a Lineweaver-Burk plot of the saturable component of total uptake at 37°C. The derived constants were: affinity constant (Km) = 4.03 X 10^{-8} M and maximum uptake velocity (Vmax) = 21.6 picomoles/mg protein/5 minutes, which are consistent with values reported for other brain regions.

The relationship between log concentration and % of 5HT uptake inhibition for three differentially selective uptake blocking drugs revealed that fluoxetine, a specific inhibitor of uptake by 5HT neurons, was the most potent inhibitor of 5HT uptake by LH synaptosomes. Fluoxetine was 23 times more potent than desipramine, a selective uptake blocker for noradrenergic neurons, and 437 times more potent than benztropine, a selective uptake blocker for dopaminergic neurons.

Disruption of ascending serotonergic projections at points caudal to the LH significantly reduced ^{3}H-5HT uptake by crude synaptosomal preparations of LH. Electrolytic lesions of either the median raphe nucleus or the dorsal raphe nucleus decreased this uptake by 45% and 63% respectively. Bilateral intracerebral infusions of 5, 7 dihydroxytryptamine into ascending serotonergic pathways also reduced this uptake by as much as 78%.

Collectively, these data support the contention that serotonergic terminals are present within the LH. The majority of these terminals are provided by ascending projections from neurons situated in the midbrain raphe nuclei.

752 COMMISSURAL FIBERS OF THE SUPRACHIASMATIC NUCLEUS. Stephen K. Itaya. Dept. Anat., Univ. Iowa, Iowa City IA 52242.

The paired suprachiasmatic nuclei (SCN) are thought to regulate timing for a variety of biological rhythms, e.g., feeding, drinking, and sleeping, making the study of the SCN both intriguing and important. If the neuroanatomical connections of the SCN were understood, in terms of intrinsic wiring and extrinsic inputs and outputs, a foundation would be available for physiological and biochemical studies. At the present time, little is known about how information is processed within the SCN. To gain further insight of SCN anatomy, the gold-toning Golgi method (Fairen et al., J. Neurocytol. 6:311, 1977) was used on adult rat brains. Neurons located along the ventromedial SCN border were observed which have unmyelinated projections in two main directions: medially, through the infundibular tract to the contralateral SCN, and laterally, as short fibers within the SCN of origin. Terminals appear to be situated within the two SCN, thus suggesting that one function of these neurons is to provide a communicating link between the two SCN. By electron microscopy, there are no features which distinguish these neurons from others in the SCN. From previous work, all SCN neurons have small cell bodies (approximately 8-10 microns diam.), often with a small rim of cytoplasm surrounding an indented nucleus; organelles commonly observed include large dense core vesicles (90-140 nm), ribosomes, lysosomes, mitochondria, some granular ER, and cilia.

The SCN are thus interconnected by commissural fibers from typical SCN neurons. Further studies are underway to ascertain the synaptic connections of these and other possible interneurons in the SCN.

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753 CONNECTIONS OF MEDIAL PREOPTIC NEURONS WITH MEDIAN EMINENCE AND AMYGDALA : AN ELECTROPHYSIOLOGICAL STUDY IN THE RAT. B.S.Layton* and L.P. Renaud, Division of Neurology, Montreal General Hospital and McGill University, Montreal, Canada, H3G 1A4

Neurons in the amygdala have direct reciprocal connections with neurons in the mediobasal hypothalamus; these connections are particularly prominent on neurons within the ventromedial nucleus (VMH). Furthermore, some of these VMH neurons have been identified as tuberoinfundibular cells, based on electrophysiological evidence of their projection to the median eminence (J. Physiol.260: 237-252,1976). The amygdala is also known to project to the medial preoptic area (MPOA), a region also shown to contain neurons with median eminence projections. The aim of the present study was two-fold : to further examine the connections between MPOA neurons and the median eminence, and to compare the responses of both MPOA and VMH neurons to electrical stimulation in the amygdala. Experiments were performed on pentobarbital anaesthetized male Sprague-Dawley rats implanted with bipolar stimulation electrodes in various nuclei of the amygdala. Following a transpharyngeal exposure of the hypothalamus, a concentric bipolar electrode was positioned on the surface of the median eminence to activate the terminals of tuberoinfundibular neurons. Extracellular records obtained from more than 400 MPOA neurons during 1 Hz single stimulation of the median eminence indicated evidence of antidromic activation i.e. tuberoinfundibular neurons (25% of cells), orthodromic excitation (13% of cells) or inhibition (2% of cells); the remaining cells were unresponsive. Relatively few spontaneously active MPOA tuberoinfundibular neurons displayed excitability patterns that suggest a possible recurrent inhibitory pathway. Single amygdala stimuli evoked a predominantly negative field potential within MPOA; this potential was seldom as prominent as that evoked within the VMH of the same animal. Recordings from 265 cells indicated evidence of antidromic activation (2% of cells), orthodromic excitation (45% of cells) or inhibition (20% of cells); the remaining neurons were unresponsive. Studies on MPOA tuberoinfundibular neurons indicated that only 22% of tuberoinfundibular neurons displayed evidence of orthodromic activation (18%) or inhibition (4%) from amygdala. On the other hand, comparative studies indicated that the majority (90%) of VMH tuberoinfundibular neurons displayed orthodromic excitation (80% of cells) or inhibition (10% of cells) following amygdala stimulation. These data suggest that the amygdala influences adenohypophyseal function predominantly through tuberoinfundibular neurons in VMH rather than MPOA. We have yet to determine the nature of other MPOA neurons that receive the majority of amygdalo-preoptic actions. Supported by the Canadian MRC.

754 THE TUBEROINFUNDIBULAR SYSTEM OF THE RAT AS DEMONSTRATED BY HORSERADISH PEROXIDASE RETROGRADE TRANSPORT FOLLOWING MICROIONTOPHORETIC INJECTION. R.M. Lechan*, S. Jacobson, J. Nestler*, S. Reichlin, Endocrine Division, Dept. of Medicine, Tufts New England Medical Center Hospital and Dept. of Anatomy, Tufts University School of Medicine, Boston, MA 02111

In order to identify the neuronal perikarya of the tuberoinfundibular tract, retrograde transport of horseradish eroxidase (HRP) was determined following minute microiontophoretic injections into the median eminence of the rat. Under stereotactic control, 20% aqueous HRP was applied directly to the median eminence of fifty-eight 250-300gm, female, CD, albino rats. Using currents of 2.5-3.5 microampers for ten minute intervals, and glass pipettes with tip diameter of fifteen microns, satisfactory local injections of HRP, limited to the median eminence with little or no diffusion into the basal arcuate nucleus, was achieved in six animals. Visualization of HRP-reaction product was accomplished by fixation in Karnovsky's aldehyde solution by cardiac perfusion and incubation of forty micron, coronal, serial sections with tetramethylbenzadine (TMB).

HRP was distributed throughout the median eminence even after strictly localized lateral injections and perikarya were rather symetrically labled indicating diffusion through the substance of the median eminence. HRP-containing beaded neuronal processes resembling axons, were seen to take a ventromedial arching path from the median eminence into the arcuate nucleus, to course for long distances in a subependemal location in the periventricular region, and to lie within the hypothalamic-hypophyseal tract. Based on distribution HRP-containing perykarya, we conclude that the following nuclear regions project to the median eminence: arcuate nucleus (predominantly dorsal and rostral portions); periventricular nucleus, in a diffuse perykaryal system as far anterior as the preoptic region; and the medial division of the paraventricular nucleus. Lesser numbers of cells arise from the medial preoptic, supraoptic, and lateral divisions of the paraventricular nuclei and rare cells from the ventramedial nucleus. The following regions do not appear to project to the median eminence and therefore are presumably not the source of the tuberoinfundibular neuron system: dorsomdeial, suprachiasmatic, anterior, mamillary and the vast majority of the ventromedial nucleus. Failure to find labeling in the septum and in the region of the organum vasculosum of the lamina terminalis (OVLT), supports the view that the neuronal system of this region and that of the median eminence are relatively independent.

755 AN HRP STUDY OF THE AFFERENT CONNECTIONS OF THE VENTROMEDIAL HYPOTHALAMIC NUCLEUS (VMN) IN THE HAMSTER. C.W. Malsbury, V.S. Harris*, D. Weisberg* and J. Daood*. Depts. Psychiatry & Psychology & the Neuropsychobiology Training Program, Western Psychiatric Institute & Clinic, Univ. Pgh. Sch. Med., Pittsburgh, PA 15261.

Horseradish peroxidase (HRP) was applied iontophoretically to sites in the medio-basal hypothalamus in female golden hamsters. After approximately 24 hrs, animals were sacrificed and the brains processed according to the histochemical procedure described by Mesulam (1978) using tetramethyl benzidene as a chromogen.

When injections were centered in the VMN, labelled neurons were found in five extrahypothalamic cell groups: 1) bed nucleus of the stria terminalis (restricted parts), 2) the rostral pole of the medial amygdaloid nucleus, 3) the amygdalo-hippocampal area (area B3), 4) the peripeduncular nucleus of the midbrain, and 5) the nucleus of the lateral lemniscus (NLL). When injections were restricted to the VMN, septal neurons were not labelled. A relatively small number of labelled cells were also seen in the rostral pole of the central amygdaloid nucleus, the ventral subiculum, and the dorsal and median raphé. Within the hypothalamus and preoptic area labelled neurons were found in: the most medio-ventral part of the preoptic area in the region of the lamina terminalis; the medial preoptic area at the level of the anterior commissure; the medial anterior hypothalamus; and the supraoptic, paraventricular and suprachiasmatic nuclei.

VMN lesions and sagittal-plane hypothalamic knife cuts disrupt sexual receptivity (Malsbury & Daood, 1978). We proposed that sagittal cuts produce this effect by cutting efferent connections of the VMN which travel in the supraoptic commissures (SOC). However we now present evidence that such cuts may also interrupt afferent connections of the VMN. Following a knife cut which severed the SOC, HRP was injected into the region of the ipsilateral NLL. When the tissue was reacted and examined, a pile-up of reaction product was seen lateral to the cut in axons of the SOC as they entered the hypothalamus. This appears to be a result of anterograde transport of HRP within axons of VMN afferent neurons whose perikarya are located in the NLL. Thus cutting the SOC may disrupt sexual receptivity by cutting VMN efferent and/or afferent connections.

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756 SUPRACHIASMATIC NUCLEUS AFFERENTS IN THE RAT: AN HRP-RETROGRADE TRANSPORT STUDY. R.Y. Moore, E.R. Marchand and J.N. Riley. Dept. Neurosciences, Univ. Calif., La Jolla, CA. 92093.

Localized injections of horseradish peroxidase (HRP) were placed in the suprachiasmatic nucleus (SCN) of rats by iontophoresis. Sections of aldehyde-fixed brain were prepared by the benzidine dihydrochloride method for demonstration of HRP and examined microscopically. Neurons showing retrograde labelling with HRP were observed in brainstem and diencephalon following injections completely localized to the SCN.

Labelling of brainstem neurons was observed in the nucleus centralis superior and in the lateral part of the dorsal tegmental nucleus. Labelled neurons were observed only occasionally in raphe dorsalis. In the hypothalamus numerous neurons were labelled in the medial preoptic area, the anterior hypothalamic area (particularly in the region adjacent to the SCN), the ventromedial nucleus and the periventricular hypothalamic nucleus from its rostral to caudal extent. Scattered arcuate nucleus neurons were labelled. In the thalamus labelled neurons were present in the paraventricular nucleus, nucleus reunions and the ventral nucleus of the lateral geniculate body.

The material also provided evidence for a projection from one SCN to that on the contralateral side. Even in cases of very small injections totally confined to the limits of the SCN, labelled neurons were evident in the contralateral SCN and anterograde labelling of axons was evident with the axons crossing the midline beneath the third ventricle and entering the contralateral SCN.

These observations confirm previous reports of projections to the SCN. They further extend information on the innervation of the SCN by demonstrating previously undescribed projections from brainstem, thalamus and hypothalamus and a projection interconnecting the nuclei on each side. Supported by USPHS Grant NS-12267.

757 ON THE SOURCE OF A NEW PATHWAY FOR VISUAL INPUTS TO THE RAT HYPOTHALAMUS. Daniel A. Pasquier and Juan H. Tramezzani*. Instituto de Neurobiología, Serrano 665, 1414 Buenos Aires, Argentina.

Horseradish peroxidase (HRP) axonal transport was used for tracing afferent fibers to the nuclei of the mediobasal hypothalamus. Iontophoretic delivery of HRP or injections of 0.02-0.03 microliters of a 30 % HRP saline solution through microsyringe were made. Highly specific labeling of the target nuclei was obtained and, from this material, the afferents to the retrochiasmatic area (RCA) are reported herein.

Following HRP deposit in the RCA consistent neuronal labeling was found in the ventromedial hypothalamic nuclei, the lateral part of the substantia nigra and the parabigeminal nucleus along the lateral midbrain-pontine tegmentum. This pattern appeared different from that shown after HRP labeling of other areas of the mediobasal hypothalamus.

HRP studies were also done of the afferents to the superior colliculus and lateral geniculate nucleus for two reasons: a) to see whether the parabigeminal nucleus also projects to the visual centers in the rat, and b) because many of the fibers from the parabigeminal nucleus going to the superior colliculus and lateral geniculate nucleus pass by the supraoptic commissures. HRP injections in the superior colliculus gave rise to neuronal labeling in the parabigeminal nucleus, most of which appeared contralateral and primarily in its ventral part. However, even large HRP injections in the lateral geniculate nucleus failed to show labeled neurons in the parabigeminal nucleus.

These results are the first demonstration of the afferents to the rat retrochiasmatic area and they suggest that those fibers originating in the parabigeminal nucleus might carry visual information towards the hypothalamus.

758 COMBINED EFFECTS OF FRONTAL HYPOTHALAMIC CUTS AND PREOPTIC (POA) STIMULATION ON ANTERIOR PITUITARY HORMONE RELEASE. C.P. Phelps and J.A. Colombo, Dept. of Anatomy, College of Medicine, Univ. of South Florida, Tampa, Florida, 33612.

The present set of experiments continues our investigation of the general problem of segregation of functions within the POA-hypothalamic complex related to the control of LH, prolactin (Prol.) and TSH release by the anterior pituitary. Recently we have examined the effects of chronic (90d) frontal retrochiasmatic cuts (FC) on LH, Prol. and TSH release after electrical stimulation of the POA. We found that when compared with sham surgery (S) values, that POA stimulation 90d after FC resulted in a plasma LH increase (ΔLH) of 166±27ng/ml vs. 233±59ng/ml for S rats 30 min poststimulation and a plasma Prol. increase (ΔProl) of 68±24ng/ml (FC) vs. 182±29ng/ml (S) at the same poststimulation interval. Increases in plasma TSH (ΔTSH) in the same animals, however, were enhanced by FC at 30 min (275±79ng/ml) when compared with those of S (ΔTSH-57±12ng/ml). In subsequent experiments, adult male rats were subjected to retrochiasmatic hypothalamic FC or S placement with a retractable Halasz knife of 1.5mm radius. One and one-half weeks later, under pentobarbital anesthesia, sequential 30 min blood samples were withdrawn before and after bilateral electrical stimulation of the POA. Stimulation was effected through bipolar stainless steel electrodes using monophasic 0.5 msec width pulses, 200μA, in 30 sec on/off trains for 30 min at a pulse rate of 50 Hz. LH, Prol. and TSH were measured by RIA. In rats that were subsequently found to have histologically complete FC, LH levels were approximately doubled by POA stimulation (0 time-50±8ng/ml; 30 min-112±27ng/ml) and this ΔLH was similar to those of S animals (0 time-63±27ng/ml; 30 min-139±38ng/ml) after POA stimulation. The ΔProl. in the same (FC and S) plasma samples showed equal 3-fold increases. Comparisons of ΔTSH in the latter plasma samples revealed no significant change from 0 time baseline levels in S rats (153±4ng/ml) across the 90 min sampling period, however, FC rats showed an approximate doubling at 60 min poststimulation (232±67ng/ml) when compared with their 0 time TSH concentrations (124±14ng/ml). The results of these experiments indicate that effects of frontal hypothalamic cuts on POA electrically stimulated release of LH and Prol. are not demonstrable between 8 and 12 days after surgery. Pituitary release of TSH after POA stimulation is, however, potentiated by FC done 8-12 days earlier. Thus, the differential effects of FC on POA-stimulated anterior pituitary hormone release appears to vary with the time interval between POA stimulation and FC. Investigation of a possible mechanism(s) for these effects are currently under way. Supported by NIH HD11345.

759 OPIATES SELECTIVELY DEPRESS SPONTANEOUS ACTIVITY IN THE HYPOTHALAMIC SLICE PREPARATION. Quentin J. Pittman, James D. Hatton* and Floyd E. Bloom. A.V. Davis Ctr. for Behav. Neurobiol., Salk Inst., La Jolla, CA 92037.

Opiates and opiate peptides administered intracranially and systemically modify neurohypophysial secretion. To investigate a possible mechanism for this effect we have applied opiates by perfusion to hypothalamic slices in vitro while monitoring extracellular unit activity in the paraventricular nucleus (PVN). This nucleus contains cell bodies of vasopressin- oxytocin- and other peptide-containing (e.g., enkephalin) neurons which project to the neurohypophysis.

Brains were quickly removed from 200 g, male Sprague-Dawley rats with or without prior transcardial perfusion of the animal with oxygenated Hank's BSS (4°C) and coronal sections (500 μ) of the diencephalon were cut on a tissue chopper. The slices were placed in a chamber where they were perfused over both surfaces at 1.5 ml/min with a heated (35°C), oxygenated medium (composition in mM: NaCl, 138; $NaHCO_3$ 4.2; $Na_2HPO_4 \cdot 7H_2O$, 0.3; KCl, 8.03; KH_2PO_4 0.4; $CaCl_2$ 0.75; $MgCl_2$ $6H_2O$ 0.5; $MgSO_4 \cdot 7H_2O$ 0.4, glucose 11; phenol red 0.03). Recordings were pursued in the anatomical area of the PVN localized with the aid of a dissecting microscope. 71 probable PVN neurons in 13 slice preparations displayed spontaneous activity (1-5 Hz) which was influenced by Ca^{++} and K^+ levels in the perfusate. 7 neurons displayed bursting activity and another 4 neurons displayed phasic activity similar to that associated with vasopressin neurons. Low intensity electrical stimulation delivered from bipolar metal electrodes placed on the neurohypophysial tract elicited antidromic action potentials in 6 PVN neurons.

Responses to opiate application were investigated in 27 PVN neurons. On 15 of these neurons, including 4 phasic cells, neither the level of spontaneous activity nor the pattern of activity were affected by addition of morphine, naloxone or enkephalin to the perfusate (all at 10^{-6}M). On 12 tonically firing neurons, 10^{-6}M morphine caused a decrease in spontaneous activity which, on 7 neurons, was reversed by naloxone (10^{-6}M). These results demonstrate a direct depressant action of opiates on certain cells in the PVN; however, opiate effects on neurohypophysial secretion in vivo may be indirect and dependent on preservation of appropriate neural circuitry.

760 SUPRAOPTIC PHASIC NEUROSECRETORY NEURONS : RESPONSE TO STIMULATION OF AMYGDALA AND DORSAL HIPPOCAMPUS, AND SENSITIVITY TO MICROIONTOPHORESIS OF AMINO ACIDS AND NORADRENALINE. L.P.Renaud and E.Arnauld*, Division of Neurology, Montreal General Hospital and McGill University, Montreal, Canada, H3G 1A4

It was recently demonstrated by electrophysiological techniques that supraoptic (and paraventricular) neurosecretory (NSO) neurons could be functionally differentiated as oxytocin or vasopressin neurons dependent upon the presence of 'continuous' or 'phasic' activity patterns respectively (Proc.Roy.Soc.Lond.B, 196: 367-384, 1977). The present communication presents our preliminary observations on the responses of 'phasic' NSO neurons (a) during stimulation in the ipsilateral amygdala and dorsal hippocampus and (b) during microiontophoresis of amino acids (glutamate, aspartate, GABA) and norepinephrine. Experiments were performed on pentobarbital anaesthetized male Sprague-Dawley rats previously implanted with bipolar stimulation electrodes in the ipsilateral amygdala and dorsal hippocampus. The hypothalamus was exposed by a ventral approach and a bipolar electrode was inserted into the posterior pituitary in order to identify NSO neurons by antidromic activation. For synaptic studies, evidence of afferent connections were derived from short latency stimulus induced excitability changes determined from poststimulus histograms. Data from 22 phasic NSO neurons indicated that fewer than 10% of cells displayed short latency inhibitory responses following amygdala or dorsal hippocampus stimulation. Microiontophoretic applications of glutamate and aspartate both enhanced the activity of phasic neurons; NSO neurons tended to tolerate aspartate much better than glutamate. These agents appeared capable of initiating and prolonging bursts of activity. GABA depressed most NSO phasic neurons; when GABA was applied in the middle of a burst, phasic cells tended to resume activity following termination of the application. During norepinephrine microiontophoresis most phasic cells tended to increase their excitability : this was usually manifested as a gradual increase in the intraburst firing frequency, but on occasion norepinephrine appeared to initiate a burst of activity or to prolong an ongoing burst of activity. Norepinephrine was seen to depress the activity of a few phasic neurons. On the premise that vasopressin release is correlated with action potential frequency, these results suggest that it's release may be diminished by GABA and promoted by aspartate, glutamate and (?) norepinephrine. Pathways that may be involved in vasopressin release are under investigation.

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761 AFFERENT PROJECTIONS TO THE SUPRAOPTIC NUCLEUS OF THE RAT R.C. Rogers, K. Talbot, D. Novin and L.L. Butcher. Brain Res. Inst. & Dept. Psychology, UCLA, Los Angeles, CA 90024.

The afferent projections to the supraoptic nuclei (SON) have been studied with a variety of techniques in several species. Thus far, no anatomical evidence exists for relatively direct projections from the viscero-gustatory relay nuclei (solitary nucleus, parabrachial nucleus and ventrobasal thalamus) and the SON though clearly, viscero-gustatory receptor inputs alter ADH release. Recently investigators have explained viscero-gustatory activation of ADH release on the basis of pathways leading from the solitary nucleus to the paraventricular nucleus, which also releases ADH (Ricardo & Koh; Brain Res., 153:1, 1978).

We sought to demonstrate the afferent connections of the SON by iontophoretically injecting minute quantities of horseradish peroxidase (HRP) into the SON and by processing the tissue with the new and sensitive de Olmos modification of the HRP histochemical method (Exp. Brain Res., 29:541, 1978). Using this technique, we find that both the solitary and parabrachial nuclei (but not the ventrobasal thalamus) project to the SON. Projections from other brainstem and limbic structures to the SON were also demonstrated. They included the lateral septum; nucleus of the diagonal band; paratenial nucleus; periventricular, medial and lateral preoptic nuclei; the bed nucleus of the stria terminalis; subfornical organ; the anterior, lateral, perifornical, circular, premammillary, posterior and supramammillary hypothalamic nuclei; the medial amygdala; subiculum; CA1 and CA3 fields of the hippocampus; central grey; dorsal tegmental nuclei; ventral tegmental area; locus coeruleus; dorsal raphe nucleus; nucleus centralis superior; raphe pontis; nucleus reticularis tegmenti pontis; and the nucleus reticularis parvocellularis.

Placements of HRP into the immediately overlying anterolateral hypothalamus revealed a different afferent projection pattern; it was essentially the reciprocal of efferents to this area described by Saper et al. (J. comp. Neurol., 183:689, 1979).

These results indicate that the lower order viscero-gustatory nuclei project directly to the SON as do many other brainstem and limbic structures involved with autonomic, homeostatic processes.

In addition to these results, some fortuitous HRP injections revealed the efferent projections of the SON alone. Axons leaving the SON ascend vertically for about 1 mm into the anterior hypothalamus before turning sharply in the opposite direction. Upon contact with the medial accessory SON, the fibers turn ventromedially toward the infundibulum.

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762 UPTAKE OF HORSERADISH PEROXIDASE (HRP) FROM FRONTAL HYPOTHALAMIC KNIFE CUTS IN RATS. S. Saporta and C. P. Phelps, Department of Anatomy, College of Medicine, University of South Florida, Tampa, Florida 33612.

Frontal retrochiasmatic cuts made in the rat hypothalamus with a Halasz knife result in anterograde fiber degeneration in the periventricular and ventromedial nuclei of the hypothalamus, the optic chiasm, supraoptic commissures, optic tracts, medial forebrain bundle, stria medullaris, stria terminalis, the fimbra and fornix (C. P. Phelps and D. M. Nance, Endo. 102:164A, 1978). In order to better describe which neurons are interrupted in the course of hypothalamic surgery in rats, we have designed a retractable Halasz type knife which can deliver HRP during hypothalamic knife cuts. The knife retracted within a cannula was lowered 5.5 mm and then extruded in the median sagittal plane. A frontal cut (FC) (radius 1.5 mm) was made, unilaterally, by rotating the blade 90° during which time 15 - 25 μl of 15% HRP was delivered. Sham surgery consisted of merely extruding the blade in the midline and delivering 15 μl of HRP. Animals were sacrificed 4, 18 and 23 hrs after surgery and the brains processed for HRP. Damaged neurons, filled with homogeneous HRP reaction product, were visible along the course of the cut at all survival times. With the longer survival times, HRP positive neurons were identified in the lateral habenular nuclei; dorsal medial, reticular, parataenial, and intralaminar nuclei of the thalamus; and in the locus coeruleus, periaqueductal grey and midline raphe nuclei. Within the hypothalamus HRP positive somata were found in the paraventricular, dorsal medial, periventricular, ventromedial, arcuate and supraoptic nuclei. In addition, all but the last structure had HRP filled fibers running through their neuropil. These results indicate that a wide variety of hypothalamic afferents are interrupted after FC, as well as a number of efferents, and that the structures involved are not necessarily reciprocally connected. Supported by NIH HD11345.

763 NEURAL PATHWAYS NECESSARY FOR A BEHAVIORAL RESPONSE TO ANDROGEN. Charles W. Scouten*, Linda Burrell* & Craig F. Cegavske. Dept. of Psychology, State University of New York, Binghamton,NY 13901

Knife cuts were placed in the sagittal plane between the medial forebrain bundle and the medial preoptic-anterior hypothalamic region in male rats. Copulatory behavior, body weight and deposition of urine on a threaded rod were observed in weekly test sessions conducted for the next 20 weeks. Urine deposition is an androgen supported behavior, and declines to about 50% of normal following castration (Price, Hormones and Behavior 6: 393-397, 1975).

In males in which the cuts separated at least the anterior 50% of the anterior hypothalamus and all of the medial preoptic area from the medial forebrain bundle, no copulation was observed even after 20 weeks postsurgery. Subcutaneous implants of silastic capsules of testosterone effectively restored copulatory behavior to castrates, but not to the knife cut males. These animals also deposited 50% less urine on the threaded rods (thus resembling castrated males) than did sham operated males. The testosterone implants did not produce any increase in urine marking in these knife cut males, although intact (sham operated) or castrated males sharply increased urine deposition after implant. Cuts placed anteriorly or posteriorly so as to spare as little as 25% of the area lateral to the medial preoptic area or anterior anterior hypothalamus allowed a nearly normal increase in urine deposition following androgen implant. The cuts appear to selectively block the influence of androgen on urine marking. Since the cuts caused only a decline to the level of castrates, no influence other than that of androgen was removed, and the medial preoptic area is not an integrative area for urine marking.

In contrast, the cuts did not block an androgen induced weight increase.

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764 EFFERENTS FROM THE MEDIAL ANTERIOR HYPOTHALAMIC AREA IN THE GUINEA PIG: AN AUTORADIOGRAPHIC STUDY. Ching Liang Shen and Conwell H. Anderson. Dept. Anat., U. Illinois Med. Ctr., Chicago, IL 60612.

Efferent projections from the medial anterior hypothalamus (MAHA) were studied using tritiated amino acid autoradiography in guinea pigs. 48-56 hours after injecting tritiated proline the animals were perfused with 10% formalin and processed through a paraffin technique for autoradiography. Axonal projections from MAHA projected not only to local areas, such as suprachiasmatic n, retrochiasmatic area, paraventricular n and bed nucleus of stria terminalis, but also to other structures in the brain. Labelled axons and diffuse grains could be seen in POA and OVLT, and in the diagonal band of Broca and its horizontal and vertical nuclei. Some of these labelled fibers passed through the diagonal band of Broca and ascended to the septum. Medial, lateral and triangular septal nuclei also received projections which ascended directly from the medial anterior hypothalamus through POA. Dorsally, through the stria terminalis the labelled fibers could be followed to the amygdala. Through the stria medullaris, anterior hypothalamic axons reached the lateral habenula and the central gray of the midbrain. Following the periventricular fiber system, the axonal projections from MAHA terminated in the periventricular and other midline nuclei of the thalamus, and also distributed to the central gray of the midbrain. Laterally, it projected to the lateral hypothalamus, supraoptic n, substantia innominata and the anterior and medial amygdaloid nuclei. Lateral hypothalamus including medial forebrain bundle (MFB) were labelled heavily. The medial part of MFB was labelled heavier than the lateral part. Posteriorly, MAHA projected directly into the medial basal hypothalamus including ventromedial, dorsomedial and arcuate nuclei. The arcuate nucleus was only slightly labelled. The median eminence was labelled in both internal and external zones. Some axons also distributed to the ventral and dorsal premammillary and supramammillary nuclei, and to the posterior hypothalamus. MAHA fibers travelled via the capsule of the mammillary nucleus and reached the ventral tegmental area of Tsai or ascended caudodorsally merging with the periventricular fiber system and distributed to the central gray of the midbrain. Diffuse central gray label could be followed to the rostral pons. A few labelled fibers in the MFB projected to the tegmental area of the midbrain and pons. Contralateral medial POA, MAHA, paraventricular n, and posterior hypothalamus were labelled, and the heaviest contralateral label was in the retrochiasmatic area and ventral and dorsal premammillary nuclei. (Supported in part by NICHHD grant HD 5865)

765 LATERAL HYPOTHALAMIC STIMULATION IN HEMIFOREBRAIN ABLATED RATS: REWARD EFFECTS. James R. Stellar, Judy Illes*, and Linda E. Mills*. Dept. Psych. & Soc. Rel., Harvard Univ., Cambridge, MA 02138.

Electrical stimulation of the medial forebrain bundle (MFB) at the level of the lateral hypothalamus (LH) will support vigorous operant self-stimulation behavior in rats and is therefore judged to be rewarding. These rewarding effects can be quantitatively characterized (Gallistel, J. Comp. & Physio. Psych. 92, 1978), but due to the widespread connections of MFB to brainstem and forebrain, anatomical inferences are difficult. In a dramatic report, Huston and Borbely (Physio. & Behav. 12, 1974) demonstrated that rats with complete forebrain ablations, having the thalamus and hypothalamus as the most rostral structures in the brain, would self-stimulate via tail elevation to LH stimulation. However, the basic behavioral impairments prevented a more refined, quantitative analysis of these reward effects in this preparation.

As a first approximation to the bilateral preparation of Huston and Borbely, adult male rats were given unilateral forebrain ablations by surgical aspiration. After recovery these animals fed and cared for themselves under standard laboratory conditions. They were then implanted with a bilateral array of LH stimulating electrodes, and were trained to lever press for LH stimulation on the forebrain-ablated side. Subsequently, they were trained to run a runway for LH stimulation reward according to the techniques of Gallistel.

Alterations in the current or number of pulses of reward stimulation were shown to lead to systematic variations in running speed. These variations occurred over the first 3-5 trials after the reward was changed. Alterations in the pretrial or priming stimulation level were also shown to lead to systematic variations in running speed but these variations occurred, in full, on the very next trial. These results are in good agreement with previous findings in intact rats (Gallistel, Stellar, & Bubis, J. Comp. & Physio. Psych. 87, 1974) and suggest that connections between the MFB and its ipsilateral forebrain targets are not necessary for what appears to be normal LH self-stimulation reward.

766 AFFERENT PROJECTIONS FROM THE PERIAQUEDUCTAL AND PERIVENTRICULAR GRAY TO THE MIDBRAIN RETICULAR CORE. M. Steriade and A. Parent., Lab. Neurophysiol. and Lab. Neurobiol., Fac. Med., Laval Univ., Québec, Canada.

A descending input from the midline diencephalic areas to the mesencephalic reticular formation has been envisaged to underly a tonic, reinforcing control of the waking brain. In order to substantiate morphologically this hypothesis and to precise the exact source of this descending input, unilateral injections (0.04-0.06 µl) of 50% horseradish peroxidase (HRP) were made and confined to the nucleus cuneiformis and the adjacent central tegmental field of cat. The animals were allowed to survive 48 hours and the sections were treated according to either the diamino-benzidine, or the tetrametyl-benzidine, or the benzidine-dihydrochloride procedure, the latter with a counterstaining by means of neutral red to distinguish the limits of nuclear groups. Positive cells, with HRP granules in the soma and processes, were consistently found ipsilaterally (and very few contralaterally) at 0.2 to 0.8 mm from the wall of the aqueduct and the third ventricle. Thus labeled neurons were seen in the midbrain periqueductal gray (mostly in its medio-dorsal aspect), in the periventricular gray medially to the retroflex bundle (contrasting with complete lack of positive cells in the adjacent parafascicular-centromedian thalamic complex), in the periventricular posterior hypothalamic area, and continuing up to the periventricular anterior hypothalamus. These results demonstrate that, in addition to a previously described subthalamic input to the midbrain reticular formation (Soc. Neurosci. Abstr., vol. 4, p.49, 1978), a midline system extending from the mesencephalon to the anterior hypothalamus provide afferent fibers to the upper brainstem reticular core. The nature of this descending projection is now being investigated electrophysiologically. Supported by MRC grants MT-3689 and MT-5781.

767 IMMUNOHISTOCHEMICAL EVIDENCE FOR BIDIRECTIONAL PATHWAYS BETWEEN THE PARAVENTRICULAR NUCLEUS, LOCUS COERULEUS AND DORSAL VAGAL COMPLEX. L.W. Swanson and B.K. Hartman. Depts. of Anat. & Neurobiol., and Psychiatry, Wash.Univ.Sch.Med., St. Louis, MO 63110.

The results of two groups of experiments suggest that the locus coeruleus (LC) and the adrenergic cell group centered in the dorsal motor nucleus of the vagus and adjacent parts of the nucleus of the solitary tract (the dorsal medullary group[1]) project in part to the paraventricular nucleus of the hypothalamus (PVH) in the rat. First, electrolytic lesions were placed in the dorsal vagal complex, or knife cuts were made just rostral to it in 12 animals. After 1-4 wks, the hypothalamus was processed by Hartman's method for the immunohistochemical localization of dopamine-β-hydroxylase, and damage in the brainstem was assessed in thionin-stained frozen sections. In those experiments with lesions involving the dorsal medullary group, and in animals with deep knife cuts, the density of adrenergic varicosities in the caudal half of the ipsilateral PVH was reduced by about one-third. Second, 10-40 nl of HRP (33%) was injected stereotaxically into the PVH and adjacent regions of 10 rats. After 24-48 h survival times, 30 µm frozen sections of the hypothalamus and brainstem were processed with TMB histochemistry. In each experiment, retrogradely-filled cells were identified in the nucleus of the solitary tract[2] and dorsal motor nucleus of the vagus, and in the LC. This evidence, when considered together with the results of the lesion studies, suggests that adrenergic neurons in the LC and dorsal vagal complex project to the PVH. Previous autoradiographic studies have shown that the LC[3] and nucleus of the solitary tract[2] project to the PVH, although the neurotransmitter involved in the latter pathway could not be identified.

In earlier immunohistochemical studies[4], we showed that oxytocin-stained fibers from the PVH innervate the LC and dorsal vagal complex. These anatomical results are discussed in light of physiological data which support the hypothesis that the PVH, LC and dorsal medullary group together are involved in neural circuitry that regulates peripheral homeostatic mechanisms, as well as similar functions within the CNS itself.

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768 HORSERADISH PEROXIDASE ANALYSIS OF HYPOTHALAMIC AREA CONTROLLING CARDIOVASCULAR RESPONSES TO EMOTION. K. Walsh*, J. DeVito, O. Smith, C. Astley*, Reg. Primate Res. Ctr., Univ. Washington, Seattle, WA 98195.

The cardiovascular (CV) responses to an acute emotional situation in unanesthetized chair-restrained baboons include elevations in blood pressure, heart rate, terminal aortic blood flow and a complex biphasic reduction in renal flow. Electrical stimulation of an area of the hypothalamus produces the same CV responses and when this area is destroyed bilaterally the CV responses accompanying the emotional behavior are eliminated. The neural structures providing afferents to this hypothalamic area were studied with the HRP method in three baboons.

Flow probes were aseptically implanted on the renal artery. Two weeks later the animal was anesthetized with chloralase (90 mg/g) and an arterial cannula was placed in the femoral artery from which arterial pressure and heart rate were recorded. Using a ventriculographic stereotaxic procedure, the appropriate location in the hypothalamus was identified by lowering a stimulating electrode through a guide cannula and recording the CV responses to electrical stimulation (100 Hz, 0.5 ma, 0.2 ms). When the response mimicking the emotional response was obtained the electrode was withdrawn and replaced at that identical location with an injection cannula. A volume of 0.15-0.25 ul of 30-40% HRP (Worthington) was injected into the site. In one animal a control injection was placed in n. ventralis anterior. After 3 days the animals were sacrificed, perfused with 1% paraformaldehyde and 1.5% glutaraldehyde. The next day the brains were cut on a freezing microtome and sections reacted with tetramethyl benzidine (TMB).

The experimental animals showed substantial retrograde labeling of cells in the subiculum, amygdala (medial, cortical and basal nuclei), lateral septal nucleus, organum vasculosum lamina terminalis, subcallosal gyrus, supraoptic nucleus, periventricular system, arcuate nucleus, tuberomammillary nuclei, paraventricularis thalami, interpeduncular and centralis superior, parabrachial nuclei, periacqueductal grey, dorsal raphe and very heavy labeling of the locus ceruleus. Lighter labeling of only a few cells was found in the lateral hypothalamic area, subfornical organ, lateral reticular formation, lateral pre-optic and nucleus solitarius. Contralateral labeling was found in locus ceruleus, periacqueductal gray, lateral parabrachial and solitarius. Of special note was bilateral heavy labeling in and around the dorsal motor nucleus of the vagus and nucleus ambiguus as well as scattered cells forming a connection between these structures. At least some of these cells could be identified as pigmented. Supported by NIH grants RR00166 and HL16910

769 GOLGI STAINING IN THE HYPOTHALAMUS: CELL GROUPS IMPREGNATED FOLLOWING INTRAVENTRICULAR INJECTIONS OF PUROMYCIN. Steven Warach, William E. Armstrong, and Glenn I. Hatton. Neuroscience Program and Psychology Dept., Michigan State Univ., E. Lansing, MI 48824

It has been established (Switzer, Neurosci. Lett. 2:301, 1976) that intracranially administered puromycin induces neural argyrophilia which results in a Golgi staining upon treatment of the tissue with the cupric-silver technique (DeOlmos, Brain Res. 33: 523, 1971). This staining compares favorably to the results of conventional Golgi procedures. Furthermore, the impregnation of neurons is of sufficiently high quality to serve as the basis for distinguishing morphological cell types in at least one nucleus (Armstrong et al., Soc. Neurosci. Abst. 4:329, 1978). Our interest in the hypothalamic neurosecretory nuclei led us to administer puromycin to the hypothalamus via the ventricular system. The widespread hypothalamic impregnation which consistently resulted warrants this report on the extent and quality of the staining. Injections of 5 µl of puromycin dihydrochloride (0.1M or 0.2M) were delivered into the right lateral ventricle near the foramen of Monroe of rats under pentobarbital anesthesia. The animals were allowed to survive from 6 to 48 hr after which the tissue was fixed, gelatin embedded, and processed as 60 µm sections. Survival times of 12 and 18 hr proved best.

The highest concentration of well-impregnated cells were found in the bed nucleus of stria terminalis, the anterior hypothalamic area (especially a ventrolateral group lying dorsomedial to the supraoptic nucleus), and the ventromedial nucleus. Adequate staining was also observed in the lateral preoptic area, the anterior commissural nucleus, the paraventricular nucleus (especially the posterior subnucleus), the dorsomedial nucleus, and the entire rostro-caudal extent of the periventricular zone (usually excluding the 100 µm nearest to the third ventricle). Although often absent or incomplete, staining occasionally occurred in the medial preoptic area, the lateral hypothalamic area, the perifornical region, and the supramammillary and medial mammillary nuclei. Notable exceptions to the puromycin-induced argyrophilia were the suprachiasmatic, supraoptic, and arcuate nuclei.

Lateral regions stained better than medial ones rostrally, while the reverse was true caudally. In general, the concentration and adequacy of staining decreased along a rostro-caudal gradient. The results suggest that too great as well as too little an amount of puromycin made available to specific regions leads to inferior impregnations.

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770 POST-LESION HYPERPHAGIA, HYPERKINESIA AND STOMACH ULCERATION INDUCED BY ANODAL AND CATHODAL HYPOTHALAMIC LESIONS IN RATS. N. I. Wiener* & J. N. Nobrega* (SPON: W. A. Mackay), York Univ., Downsview, Ontario, M3J 1P3, Canada.

Lesions of the medial hypothalamus have been known to produce a number of effects in the early post-operative period, including hyperactivity, voracious eating, and stomach ulcers. It has been suggested that some of these effects may result from stimulation by metal ions deposited by anodal current rather than tissue destruction. However, since anodal current tends to produce larger lesions than cathodal current of the same intensity and duration, the effects of current directionality and lesion size may often be confounded. In the present study the effects of small and large ion-depositing anodal and deposit-free cathodal lesions were tested on four types of responses in the 24 hr post-operative period. Epoxylite-insulated #00 stainless steel electrodes were used to produce bilateral VMH lesions in male Wistar rats. Animals were divided into five groups: Group SA received small anodal lesions (.5 mA/10 sec); Group LA received large anodal lesions (1 mA/20 sec); Group SC received small cathodal lesions (1 mA/20 sec); Group LC received large cathodal lesions (2 mA/40 sec); control rats received anesthetic only. Half of the animals in each group were placed in activity boxes after surgery and had their stomachs examined 24 hr later; the others were given food and saline for 24 hr. ANOVAs were used to test the effects of lesion type and size on activity counts, food and fluid intake at 6 and 24 hr and three measures of stomach pathology. Of the nine response variables a significant effect of type of lesion (current) obtained only for the total amount of activity at 6 hr and saline consumption at 6 and 24 hr. Single comparisons indicated that LA lesions produced higher activity counts than any other in the first 6 hr post-lesion; in the next 18 hr, however, LC rats had higher activity counts, and as a result at 24 hr LA and LC groups had equally high activity counts. Large anode rats drank more than all other groups, again mostly in the first 6 hr after the lesion. The only factor which proved significant for all nine variables was size of lesion. Small-lesion groups behaved indistinguishably from controls on all measures, regardless of type of lesion received. These results indicate that large cathodal lesions can produce as much stomach pathology, eating (but not drinking), and motor activity as anodal lesions of comparable size, although the two types of lesions may produce their effects at different rates.

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771 WHAT VENTROMEDIAL HYPOTHALAMIC LESIONS DO TO BRAIN METABOLIC ACTIVITY AS MEASURED WITH THE ^{14}C-2-DEOXY-D-GLUCOSE METHOD.

John S. Yeomans, Neil I. Wiener* and Jose Nobrega*. Dept. Psych. University of Toronto and York University, Toronto, Ont. M5S 1A1.

Anodal (2mA for 20 sec) or cathodal (2mA for 40 sec) electrolytic lesions were made with monopolar stainless steel electrodes aimed at the ventromedial hypothalamus of rats. Both unilateral and bilateral lesions were performed. Behaviorally these anodal lesions produce hyperactivity within two hrs after the lesion, accompanied by voracious eating or stomach ulcers (Wiener, Nobrega, Ossenkopp, in preparation). Cathodal lesions produce only ulcers.

Three and one-half hrs after the lesion, the animals were injected with 30 µCi of ^{14}C-2-deoxy-D-glucose. The procedures of Sokoloff were used to obtain auto-radiographs of brain sections from these rats.

Anodal lesions produce profound decreases in labelling in a sphere around the lesion site almost 2mm in diameter. Furthermore there is usually an intense increase in labelling in some specific ipsilateral regions away from the lesion site including, for example, zona incerta, lateral hypothalamus, globus pallidus, and substantia nigra, zona reticulata. The cathodal lesions, although longer in duration, produce a much smaller region of decreased labelling around the lesion site. These radiographic results were compared with the anatomical changes in these sites observed with conventional histology.

The dynamic three-dimensional changes in local brain activity produced by lesions over time can be observed with this method. This promises to help us understand the neural basis of the behavioral syndromes produced by lesions.

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INVERTEBRATE NEUROBIOLOGY

772 SOMA SPIKE OF NEUROENDOCRINE BAG CELLS OF APLYSIA CALIFORNICA. Juan Acosta-Urquidi and F. Edward Dudek. Dept. Zool. and Erindale College, Univ. of Toronto, Mississauga, Ont. L5L 1C6.

Repetitive firing of bag cells in Aplysia leads to egg laying in vivo and secretion of a peptidergic egg-laying hormone (ELH) in vitro. Extracellular recordings from bag cell neurites have characterized the propagation of spikes during afterdischarge. Variations in amplitude and/or duration of spikes during repetitive firing and the ionic basis of the action potentials were examined in soma spikes elicited by intracellular current injection. Spikes in artificial sea water (ASW) were either graded with increasing cathodal current pulses, or had a well-defined threshold. The latter typically had faster rise times, larger overshoots and more fully-developed hyperpolarizing afterpotentials. Repetitive stimulation led to frequency potentiation (FP), which was expressed as an increase in overshoot amplitude with a concommitant increase in duration of successive spikes in a train. FP was usually detectable at 0.5 Hz, and fully expressed between 0.8 - 4 Hz. Accommodation occurred rapidly at ≥5 Hz. The increase in spike duration during FP resulted from a progressive enhancement of an inflection on the falling phase. The expression of this inflection was dependent on membrane potential; depolarization enhanced it, hyperpolarization reduced it. A short conditioning train of strong hyperpolarizing pulses caused inactivation of spike electrogenesis, which lasted a few seconds. Experiments with 0-Na^+ ASW (Tris-substituted) and/or 0-Ca^{++} (1mM EGTA) ASW perfusion revealed mixed Na^+/Ca^{++} spikes with variable degrees of Na^+ versus Ca^{++} dominance. Na^+-dominant spikes showed less FP. Similar to 0-Ca^{++} ASW, solutions containing Cd^{++}, Co^{++} and Mn^{++} reversibly abolished the inflection on the falling phase; the spike was severely attenuated at higher concentrations. FP was generally abolished only if the spike was severely attenuated. The inflection was augmented in 3-aminopyridine (4mM), thus increasing spike duration. Ba^{++} (substituted for Ca^{++}) attenuated spikes and in some cases increased the duration. Results with tetrodotoxin (10^{-5}-10^{-4}M) in normal ASW were equivocal. These experiments generally support the hypothesis that soma action potentials of bag cells have both Na^+ and Ca^{++} components. It is proposed that FP results primarily from potassium-inactivation which then unmasks a calcium current. FP may play a significant role in augmenting ELH release.

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773 KINETICS OF THE SLOW CURRENTS DURING NORMAL BURSTING IN APLYSIA CELL R15. William B. Adams and Irwin B. Levitan. Friedrich Miescher-Institut, P.O.Box 273, CH-4002 Basel, Switzerland.

Bursting activity in cell R15 in the abdominal ganglion of Aplysia is mediated by voltage- and ion-dependent conductances having slow kinetics. During a burst the cell is depolarized by a flow of inward current. Using voltage-clamp techniques, we have found a transient increase in the inward current flow following each action potential. The increase amounts to 1 to 2 nA, lasts from 1 to 3 seconds, displays temporal summation when action potentials are sufficiently close together, and tends to maintain the burst. In addition, each action potential elicits a smaller but longer-lasting increase in outward current. The increments in outward current are only a small fraction of a nanoampere, but they persist for tens of seconds and summate throughout the burst. Because of the different kinetics of the two current components, the outward current plays a more important role as the burst progresses. Eventually the net current is outward and the burst is terminated. Thus, the pattern of burst activity appears to depend on the amplitudes and recovery kinetics of these two current components. The normal burst pattern can be altered by dopamine, serotonin, vasopressin and modifiers of cyclic nucleotide metabolism. Attempts to elucidate the effects of these agents on the parameters of the individual current components are in progress.

774 TWO-DIMENSIONAL GEL ELECTROPHORESIS OF NEUROSECRETORY POLYPEPTIDES IN THE CRUSTACEA. R.D. Andrew * (SPON: H. Kwan). Biology Dept., York University, Toronto, Ontario, Canada M3J 1P3.

Two-dimensional polyacrylamide gel electrophoresis is capable of resolving hundreds of proteins from microgram amounts of complex biological material. Proteins are focused according to charge in the first dimension and then, at right angles, according to size in the second dimension. Determining the precise location of neurosecretory cell bodies is prerequisite for studying the synthesis and processing of neurosecretory polypeptides stored in axon terminals comprising the sinus gland of the crustacean eye stalk. Structural data establishes that the crayfish X organ represents 90 - 95% of the cell bodies actively synthesizing neurosecretory vesicles stored in the neurohemal sinus gland. These cell bodies rarely accumulate neurosecretory vesicles as judged by light and electron microscopy suggesting that neurohormone precursors, but not processed products, might be found there. Two-dimensional electrophoresis of sinus gland and X organ homogenates support this hypothesis. In crayfish, lobster and blue crab, stained two-dimensional gels display a number of sinus gland-specific polypeptides whose high concentrations and low molecular weights are consistent with stored neurosecretory material. These neuropeptides are not detected in X organ homogenates or in non-neurosecretory neural tissue with Coomassie Blue staining. By decreasing the porosity of the second dimension, the two-dimensional gel technique has proven useful in determining the molecular weights of a variety of neurosecretory polypeptides (MW 4-20K) stored in the sinus gland. The crayfish and lobster store several polypeptides weighing ~ 7 K daltons. The blue crab stores two 7 K, two 13 K and three 20 K sinus gland polypeptides detected in stained gels.

Following a 4 hr incubation in ^3H-labelled amino acids, predominantly labelled 19 - 21 K molecular weight polypeptides are detected in crayfish X organ homogenates with 2-D gel autoradiography. Concomitantly, three labelled polypeptides (MW 4 - 10 K), which are not stored in enough quantity to be detectable with staining, appear in the sinus gland. Their rapid synthesis but low storage level suggest that they are quickly released. This study is the first to examine neurosecretory precursors (19-21 K) and their putative cleavage products (~ 7 K) in the Crustacea.

775 BAG CELL DEGENERATION FOLLOWING SURGICAL TRANSECTION OF A PLEUROABDOMINAL CONNECTIVE. S. Arch and G. Thayer*. Biological Laboratories, Reed College, Portland, OR 97202.

Two clusters of neuroendocrine cells (the bag cells) associated with the parietovisceral (abdominal) ganglion of Aplysia californica are known to be responsible for the synthesis and secretion of a peptide hormone that causes egg laying. Because the cell clusters are morphologically set apart from the ganglion proper and are structurally, chemically, and electrically homogeneous, they represent an accessible and analytically tractable preparation. Since it has been shown that deafferentation of these neurons results in cessation of spontaneous egg laying (Pinsker and Dudek, 1977, Science 197:490), we were interested to determine if the neurons undergo changes correlated with the elimination of afference. Animals were anesthetized, a small incision was made in the dorsal body wall, and one or the other of the two nerves known to carry bag cell afferents was transected. Most of the animals survived this operation and resumed apparently normal behavior. At various post-operative intervals animals were sacrificed and their parietovisceral ganglia were fixed, embedded, and sectioned for light microscopic analysis. For the first few weeks following transection there was little change evident in the bag cell clusters. However, there was marked hypertrophy of glial and connective tissue in the bag cell organ ipsilateral to the transection. By the sixth post-operative week, extensive degeneration of the ipsilateral bag cells was evident. Surprisingly, the bag cell cluster contralateral to the transection also showed marked degenerative change. Since this cluster was still innervated normally, we had assumed that it would serve as an internal control. The level at which the transections were made (1-2 cm rostral to the cell cluster) were chosen to minimize the likelihood that bag cell neurites would be cut. Studies of axoplasmic transport of radiolabeled egg-laying hormone have shown no indication of hormone movement beyond 1 cm rostral to the cell cluster. Similarly, we have been unable to backfill bag cells with horse-radish peroxidase in preparations having connective nerves longer than about 1 cm. Thus, while neurite transection may have occurred, only a very small proportion of the bag cells could have been involved. It seems unlikely that retrograde degeneration of the entire ipsilateral cluster would ensue as a consequence of the neurite transection that may have occurred. In any case, loss of the contralateral cluster must also be explained. Our present hypothesis is that unilateral transection of the connective nerve has removed afference to the bag cells and that the functional integrity of this afference is crucial to the maintenance of the differentiated state in these neuroendocrine cells.

776 TWO NEUROHORMONES PARTICIPATE IN THE CONTROL OF CIRCADIAN NEURONAL ACTIVITY IN THE CRAYFISH. Hugo Aréchiga, Alberto Huberman*and Irene González* Dept. Fisiol. Ctr. Estud. Avanzados, I.P.N., and Inst. Nac. Nutrición, México, D.F.

It has been postulated that a hormonal channel modulates the excitability of the nervous system of decapod crustaceans along the 24-hour cycle (Aréchiga, H., Fed. Proc. 36:2036, 1977), and a neuropeptide (Neurodepressing Hormone) has been identified in the nervous system and blood of the crayfish, exerting a depressant action on the excitability of an ample variety of neuronal elements (Aréchiga, H., Cabrera-Peralta, C. and Huberman, A., J. Neurobiol., 1979, In Press.) The concentration of this peptide varies along the 24-hour cycle, being highest at day-time.

In experiments measuring the activity of Neurodepressing Hormone (NDH) at different times, it became evident that another substance was present in the nervous system, capable of increasing the spontaneous activity of motoneurons in isolated ganglia. By successive steps of Sephadex G-25 and G-15 chromatography, it has been possible to separate this neuroactive substance, which appears to be a peptide of low molecular weight, eluted in fractions neighbour to those containing NDH in G-15. The greatest amount of this substance is contained in the eyestalk, but lesser quantities are found in the supraoesophageal ganglion and thoracic ganglia. Its excitatory effect does not show noticeable desensitization. The concentration of this peptide in the eyestalk varies along the 24-hour cycle, attaining a high level at night and decreasing during the day. This rhythm persists in constant darkness and is opposite in phase to that of NDH. The rhythm of secretion of both peptides can be phase-shifted by entraining animals to different light:darkness regimes.

From these evidences, it appears that the level of excitability of the crayfish nervous system is modulated in a circadian manner by two peptides with opposite effects.

777 **COMPLEX RECEPTOR NEURONS IN TRITONIA: NEURONAL CORRELATES OF A CHANGE IN BEHAVIORAL RESPONSIVENESS.** **Gerald Audesirk and Teresa Audesirk. Friday Harbor Labs, Univ. of Washington, Friday Harbor, WA 98250.**

When contacted by the tube feet of certain predatory sea stars, the nudibranch Tritonia diomedea performs an escape maneuver composed of an initial swim (Willows, 1967) followed by an "escape run" consisting of rapid crawling locomotion. Although the usual response of a crawling Tritonia to food presentation is to stop locomotion and begin to feed, during the escape run the animal will not feed, and in fact behaves as if it cannot tell that food is being presented.

The CNS of Tritonia contains a population of complex receptor neurons which are both primary mechanoreceptors and secondary mechano- and chemoreceptors receiving sensory input from the oral veil and anterior foot. These receptors normally respond strongly when the oral veil is touched with a sea whip, Tritonia's normal food. After a swim, during the time of the escape run, these receptors are much less responsive (50%) to food stimuli, although their input from the periphery is not qualitatively different.

During the swim, the receptors are synaptically driven to produce bursts of spikes, in phase with ventral flexions. Three hypotheses can be advanced to account for the change in responsiveness of the receptors: (1) aftereffects of swim spiking reduce excitability; (2) aftereffects of swim synaptic activity reduce excitability; (3) synaptic inputs during the escape run inhibit spiking. These hypotheses were tested by hyperpolarizing a receptor neuron during a swim to prevent spiking and by driving an "artificial swim" by current injection. It was found that if spikes were prevented during a swim, nearly all of the responsiveness change was abolished. Second, an almost normal responsiveness change could be induced by an artificial swim of similar frequency and temporal patterning to a natural swim. These excitability changes occurred whether assayed by natural food stimulation of the oral veil or by direct intracellular current injection. Similar changes still occurred when interneuronal firing was reduced by high calcium solutions. These results support the first hypothesis, but minor effects due to the second and third hypotheses cannot be ruled out.

We propose that the responsiveness change is intrinsic to the complex receptors, and is elicited by spiking during a swim. This change in receptor excitability is a possible mechanism by which Tritonia behavior is modified by immediate past experience, contributing to behavioral flexibility.

778 **PROPERTIES OF COMPLEX RECEPTORS IN TRITONIA DIOMEDEA.** **Teresa Audesirk and Gerald Audesirk. Friday Harbor Labs, Univ. of Washington, Friday Harbor, WA 98250.**

The lateral cerebral ganglia of Tritonia diomedea contain a group of approximately 10 neurons ranging in size from 50 to 100 microns. These usually send out three axons, one each in cerebral nerves 2 and 3 innervating the oral veil, and one in pedal nerve 1 to the anterior foot. Ipsilateral cells are electrically coupled to one another. Application of a variety of chemical stimuli to the oral veil produces a burst of synaptically driven spikes in these cells, with food extracts being most effective. Mechanical stimuli delivered to the oral veil and anterior foot also result in synaptic activity and spiking.

When a brain - oral veil preparation is bathed in high Mg^{++}, low Ca^{++} SW to block chemical synaptic activity, the chemoresponsiveness of these cells is eliminated. Responsiveness to mechanical stimuli is retained as blocked afferent spikes, indicating that these are primary mechanoreceptors. Mapping studies reveal that most of these receptors have multiple receptive fields for tactile stimuli in the ipsilateral oral veil and anterior foot. Responses evoked by stimuli delivered to the contralateral oral veil or foot were never observed. When the brain is placed in high Mg^{++}, low Ca^{++} SW and the oral veil is in normal seawater, blocked afferent spikes are recorded in response to food extract as well as mechanical stimuli. This indicates that some peripherally located chemoresponsive cells make chemical synapses on these receptors. When the oral veil is placed in high Mg^{++}, low Ca^{++} SW and the brain is in normal seawater, synaptic activity and spikes are present in response to mechanical stimuli, but no activity is elicited by chemical stimuli. Therefore all chemoreceptive input must pass through peripheral synapses. Some is communicated by way of both peripheral and central synapses to account for the synaptic activity observed in response to food extract when the entire preparation is bathed in normal seawater.

The result of this organization is a neuron responsive to chemical and tactile stimuli, and which responds most energetically when the two are combined. It is hypothesized that Tritonia uses these receptors to determine contact with an object and on which side of the body, and whether or not the object is food.

779 NEURONAL ORGANIZATION OF THE SALIVARY NEUROEFFECTOR SYSTEM OF HELISOMA: REDUNDANCY OF BILATERAL COORDINATING MECHANISMS. Fred Bahls, Stanley B. Kater and Ronald W. Joyner*. Depts. of Physiol. & Biophys. and Zool., Univ. of Iowa, Iowa City, IA 52242.

The salivary neuroeffector system of Helisoma consists of the paired salivary glands and two identified buccal ganglion neurons, 4R and 4L. In contrast to most gastropod neuroeffector systems, both the central and peripheral components of this system are readily accessible to intracellular analyses. Previous work has shown that two different mechanisms can exist to ensure coordinated activation of the salivary glands. First, the two neurons 4 can receive identical synaptic input from higher order buccal ganglion neurons. Second, the two neurons 4 are electrically coupled, with a mean coupling coefficient of .42 (N=28, S.D.= .16). Activity within each salivary gland is coordinated by extensive electrical coupling. However, bilateral coordination of activity between the two salivary glands requires neural control, because there is no communication between the two glands.

We now provide morphological and electrophysiological evidence for a third mechanism for bilateral coordination: innervation of each salivary gland by axons from both neurons 4. Intracellular injection of the fluorescent dye Lucifer Yellow has shown that both neurons 4 can send an axon to both salivary glands. Electrophysiological evidence for dual innervation was obtained in the following manner. When one neuron 4 is stimulated at frequencies $\geq$ 2 Hz., a delay occurs between the spikes in the two neurons 4. Owing to the electrotonic interaction associated with this delay, the waveform of the spike in the stimulated 4 becomes a sensitive monitor of activity in the follower 4. Thus, we monitored the activity of both neurons 4 while recording from only one neuron 4 and a salivary gland cell, and were able to demonstrate in 6 out of 8 preparations in which this stimulus paradigm was used, that at least one of the two salivary glands received innervation from both neurons 4.

Thus, there can be at least three levels of interaction which maintain bilateral synchrony of activity in the salivary glands, indicating a high level of redundancy in this gastropod neuroeffector system. Data from other, less easily studied systems, indicate that redundancy may be a general feature of the organization of gastropod neuroeffector systems.
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780 THE ACTIVE ZONE AT APLYSIA SYNAPSES: ORGANIZATION OF PRESYNAPTIC DENSE PROJECTIONS. C.H. Bailey*, P. Kandel*, and M. Chen*(SPON: J. Koester) Div. of Neurobiol. and Beh., Dept. Physiology, Columbia Univ. College of P & S, New York, N.Y. 10032

We have recently developed criteria for recognizing active zones at Aplysia synapses. To obtain independent evidence for these sites and to study their characteristics we have applied to the Aplysia neuropil two special staining techniques - 1) ethanolic phosphotungstic acid (E-PTA) (Bloom and Aghajanian, 1968)and 2) bismuth-iodine impregnation followed by uranyl acetate and lead staining (BIUL) (Pfenniger et al., 1969).

As is the case with active zones at vertebrate synapses, Aplysia ganglia treated with E-PTA or BIUL reveal a series of presynaptic dense projections arising from, or connected by, a thin band of electron-dense material attached to the cytoplasmic leaflet of the presynaptic membrane. Analysis of sections cut perpendicular to the presynaptic membrane suggest some variability of the shape and size of individual dense profiles. A common configuration is that of a flattened or slightly rounded pyramid (mean height=29nm ± 0.8nm S.E.M., N=87 mean base diameter = 46 nm ± 1.4 nm S.E.M., N=87). Additional configurations include small, irregular swellings with smooth borders and larger more complex bodies with short spine-like extensions. Viewed in sections taken perpendicular to the membrane these projections can appear either isolated, or interconnected at their bases by strands of electron-dense material. The center-to-center spacing of presynaptic projections varies from 45 nm to over 100 nm with a mean value of 69nm ± 2.5 nm S.E.M., N=52. Preliminary analysis of sections cut parallel to the synaptic cleft suggest that at least some of these presynaptic densities are discrete projections (as opposed to bar-type densities, Wood et al., 1977), although the precise geometric nature of the presynaptic region is not yet clear.

Postsynaptic specializations occur as a narrow, uniform electron-dense sheet (mean width = 8nm ± 0.5 nm S.E.M., N=11) attached along the length of the postsynaptic membrane or as an interconnected series of irregular expansions (mean width=17nm ± 1.1nm S.E.M. N=14). The periodicity of these postsynaptic tufts is variable although some appear to occur more frequently between opposing presynaptic projections than directly opposite a dense projection.

These techniques confirm the existence of specialized active zones in Aplysia and should prove useful for the quantitative study of changes in synaptic function with experience or age.

781 NEUROTRANSMITTER SYNTHESIS IN THE VISUAL SYSTEM OF LIMULUS B.A. BATTELLE* (SPON: H.B. Pollard) LVR, National Eye Institute, NIH, Bethesda, MD.

The synthesis of putative neurotransmitters in the ventral and lateral eyes of Limulus was studied using a modification of the procedure of Hildebrand et al. (J. Neurobiol. 2, 231-246, 1971). Results are summarized in the table below. Ventral eye preparations were dissected into a photoreceptor rich (p-fraction) and a nerve fraction (n-fraction) before extraction and electrophoresis.

^{3}H labelled precursor	Neuro-transmitter	Ventral Eye P	Ventral Eye N	Lateral Eye	Lateral Eye Nerve
Choline	Ach	-	+	-	+
Glutamate	GABA	+	++	+	++
Tryptophane	5-HT	-	-	-	-
Tyrosine	NE	-	-	-	-
	DA	-	-	-	-
	Oct	+	+	+	+
Tyramine	Oct	++	+	++	+

Several interesting observations emerge. 1. The types of putative neurotransmitters synthesized, and the distribution of synthetic activity between cell-rich and nerve fractions are similar in the lateral and ventral eye preparations. 2. Although serotonin has a number of important pharmacological effects on cells in the lateral eye, no serotonin synthesis is detected. 3. Incubations with tyramine show clearly a selctive synthesis and accumulation of octopamine by cell-rich fractions of both ventral and lateral eye preparations, thus octopamine becomes of interest as a possible neurotransmitter or neurohormone in these preparations. Further support to this idea comes from observations that both ventral and lateral eyes contain significant levels of endogenous octopamine (6.02 and 32.8 p moles octopamine per mg tissue wet weight respectively) and that the photoreceptor cells of the ventral eye stain with neutral red.

Incubations of ventral and lateral eye preparations with tyramine yield two other major radioactive products in addition to octopamine. The nature of these products is unknown, however one is a mixture of octopamine and tyramine metabolites. There is no detectable accumulation of octopamine metabolite when tyrosine is used as the precursor.

782 THE SALIVARY SYSTEM PROVIDES SENSORY INPUTS TO FEEDING NEURONS IN LIMAX. Barbara Beltz and Alan Gelperin. Dept. Biology, Princeton University, Princeton, New Jersey 08544.

The central nervous system of the terrestrial slug Limax maximus provides a convenient system in which to study the interactions between the salivary and feeding systems. Feeding and salivary neurons were monitored extracellularly via their axons in buccal roots while the salivary duct musculature was stretched by microliter injections of saline into the cannulated salivary duct. These injections expanded the duct less than the spontaneous duct expansion measured in vivo.

The feeding motor program (FMP) can be activated by two types of stretch stimuli. When the duct is held inflated for a minute or more, FMP is often initiated when the stretch is released. A series of short inflations of the duct can also result in FMP activation. FMP is detected by monitoring the phasing of motoneuron activity as recorded from the salivary nerves and buccal roots (Gelperin et al, J. Neurobiol. 9(4):285-300, 1978).

Post-synaptic potentials elicited by duct stretch can be recorded intrasomatically from presumptive feeding neurons. Both hyperpolarizing and depolarizing inputs onto these buccal neurons, caused by duct stretch, have been demonstrated.

The salivary bursters (SBs), which are coactivated with feeding, are bilateral buccal neurons each of which sends a process into its ipsilateral salivary nerve. The activity of these autoactive, bursting neurons is also modulated by duct stretch.

This modulation of SB activity can be caused by a chemically mediated inhibitory input to the SBs, evoked in response to stretch of the ipsilateral salivary duct. The SB rebounds from the inhibition with a burst of higher frequency activity than was recorded before duct stimulation.

There is also evidence that the SBs, which are motoneurons to the salivary duct musculature, are also primary sensory neurons activated by duct stretch (Beltz and Gelperin, Neuroscience Abstracts, Volume IV, 1978).

These reflex phenomena are examples of regulation of centrally generated output by peripheral feedback. SB modulation by duct stretch may represent a mechanism to coordinate the processes of salivary secretion and transport during non-feeding periods. FMP initiation caused by salivary duct stretch indicates that mechanosensory input from the salivary network may also contribute to the activation of feeding activity in vivo. (Supported by NSF grant BNS 76-18792 and NIH training grant 5 T01 MH13445.)

783 SALINITY INDUCED CHANGES IN THE ELECTRICAL PROPERTIES OF MOLLUSCAN NEURONS. Linda S. Beres* and Sidney K. Pierce* (Spon: H. Levitan). Dept. Zoology, Univ. of Maryland, College Park, MD 20742.

Many marine invertebrates are subjected to large fluctuations in environmental salinity. Further, in osmoconforming species, the blood ionic composition undergoes changes which reflect those occurring in the external medium. Since many aspects of neuronal physiology are dependent upon the ionic composition of the extraneuronal medium, exposure to low salinity may have pronounced effects on the properties of the nervous system in these animals. This possibility was examined using conventional intracellular electrophysiological techniques to record from single neurons in isolated visceral ganglia of the clam Mya arenaria, which is an osmoconformer inhabiting salinities as low as 10% of the salt content of normal seawater. Recordings obtained from animals maintained in normal (≈100%) seawater reveal resting membrane potentials of -40 to -80 mV; overshooting action potentials could be elicited by intracellular current injection. Exposure of these neurons to 75% seawater resulted in an immediate hyperpolarization (15 to 30 mV) of the membrane potential. This hyperpolarization was transient, lasting 2 to 10 mins, and was followed by a secondary depolarization of 30 to 60 mV. Further, the changes in membrane potential were accompanied by an increase in apparent input resistance. Despite this increase in input resistance, the excitability of the cells was reduced. Higher current intensities were required to elicit action potentials; in some cases, no action potential could be produced even with a three-fold increase in stimulating current. Action potentials, when present, were reduced in amplitude following the salinity change. Occasionally, spontaneous synaptic potentials were observed in some cells; when they occurred, invariably their frequency was increased by reductions in salinity. Recordings over longer periods of time (2-3 hrs from the salinity change) showed that the membrane potential slowly returns to normal. Preliminary investigations suggest that the excitability also returns. In addition, neurons from animals acclimated for 3-4 weeks to lowered salinity exhibit electrophysiological properties similar to those found in animals maintained in 100% seawater. These results indicate that, following exposure to low salinity, the neurons of Mya arenaria undergo changes in both the membrane properties and excitability. After a period of time, however, the neurons appear to adapt to the change in salinity, as evidenced by a return of the electrical properties to normal levels.

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784 CIRCADIAN RHYTHM IN THE EYE OF APLYSIA RECORDED IN VIVO. G. D. Block, Department of Biology, University of Virginia, Charlottesville, Virginia 22903.

The eye of Aplysia expresses a circadian rhythm (CR) in the frequency of compound action potentials (CAPs) when recorded in vitro (Jacklet, Science 164:562-563, 1969). Aplysia also exhibit a circadian periodicity in locomotor activity. Although extraocular photoreceptors and oscillators are involved in controlling the locomotor rhythm, changes in locomotor patterning following eye removal suggest that the eye also contributes to locomotor control as a circadian oscillator (Strumwasser, Physiologist 16:9-42, 1973; Lickey et al., J. Comp. Physiol. 118:121-143, 1977). Unfortunately, the expression and waveform of an ocular CR in vivo cannot be confidently inferred from in vitro CRs, since the optic nerve contains efferent fibers which, when active, modify the patterning and frequency of CAPs (Eskin, Z. vgl. Physiol. 74:353-371, 1971). Thus to further define the role of the ocular CR in controlling locomotor activity it is necessary to: (1) confirm that a CR in CAP activity is expressed in vivo and (2) evaluate the in vivo phase relationship between the ocular and locomotor CR's.

In situ optic nerve activity was recorded by means of .003" teflon coated Pt-Ir hook electrode. Continuous recordings were made for up to 90 hr from Aplysia maintained in 20 l glass aquariums. CAPs recorded in this manner displayed amplitudes ranging from 20-100 μV and in most cases showed irregular burst patterning, suggesting effective efferent modulation.

The results indicate the presence of an ocular CR in vivo. When Aplysia were maintained in constant darkness CAP frequency peaks (200-400/hr) occurred at projected dawn with near peak levels persisting for 6-10 hr. The overall waveform of the ocular CR was similar to CR's obtained in vitro with the cerebral ganglion left attached. When Aplysia were placed on light cycles (LD 12:12), CAP frequencies remained near peak levels for the entire photoperiod, dropping to low levels at dusk (5-10/hr) and remaining at this baseline until 2-3 hr before dawn. Simultaneous recording of locomotor activity by time-lapse cinematography indicated that locomotor onsets closely followed the predawn increase in CAP activity.

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785 NUMBERS AND SIZES OF CELLS IN MOLLUSCAN GANGLIA. M. B. Boyle*, L. B. Cohen and E. R. Macagno. Dept. Physiology, Yale Univ. Sch. Med., New Haven, CT 06510; Dept. Biol. Sci., Columbia Univ., New York, NY 10027

One criterion that might be used in the selection of a preparation for studying the neuronal basis of behavior is the number and size of neurons in the central ganglia. Ganglia from three gastropod molluscs were dissected out, pinned out in Sylgard dishes, stained in methylene blue overnight, fixed in 4% formaldehyde in sea water, dehydrated in acetone, cleared in methyl salicylate and mounted between cover slips. Cell counts were performed with the aid of a computer-interfaced light microscope (Macagno et al, Ann. Rev. Biophys. Bioeng. 8,1979). Considering the cells as approximate prolate ellipsoids, the smaller diameter of each cell was entered into the computer. Objects smaller than 4μm were not counted or measured. The measured diameters were multiplied by 1.4 to account for shrinkage during preparation and by 1.16 to give the mean diamter of each cell. We think the cell counts do not differ from the actual number by more than a factor of two.

Species	Ganglion	No. of Cells	Mean Diam.(μm)
Navanax inermis	cerebral	600	24
"	pedal	700	34
"	pleural	200	45
"	supraintestinal	160	46
"	visceral	350	52
"	buccal	240	35
Aplysia californica	buccal	800	28
Hermissenda crassicornis	cerebral	600	14
"	pedal	500	23

Some differences were found. The cell bodies in Hermissenda were only half as large as those in Navanax, and the buccal ganglia of Aplysia had about three times as many neurons as the corresponding Navanax ganglia. Although counts were made on only one example of each ganglion, inspection of additional preparations suggested that these differences between species are not due to variation from individual to individual. There seem to be substantial species differences in cell number and size. The total number of neurons in the Navanax central nervous system was about 4500. We plan to measure ganglia from additional species searching for a preparation with relatively few and relatively large neurons.

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786 VASCULARIZATION OF THE ABDOMINAL CENTRAL NERVOUS SYSTEM OF THE CRAYFISH: CONSEQUENCES FOR PHYSIOLOGY. Stephen K. Brown*, Dyane N. Sherwood, and Jeffrey J. Wine. (SPON: Angela C. DiBerardino). Dept. of Psych., Stanford U., Stanford, CA 94305.

The importance of an intact circulatory system to the normal function of the crayfish's nervous system was investigated. This is of interest, for decapod crustaceans lack a blood-brain barrier as it is known in vertebrates while possessing barriers to the diffusion of chemicals into the nervous system from the surrounding open return system (Lane & Abbott, Cell Tiss. Res. 156, 173, 1975). The anatomy of the vascularization of the ventral nerve cord was examined; a technique by which the nerve cord could be perfused via the circulatory system was developed; and neurophysiological experiments were performed to determine whether the perfusion technique provides results different from those obtained with conventional methods.

Injections of ink into the heart revealed extensive vascularization within the ventral nerve cord. Within the ganglia, which received many more major arterial inputs than did the connectives, the greatest vascularization was associated with the nerve cell bodies. The vascularization of the neuropil was also relatively heavy and consisted of numerous fine arterioles which assumed a variety of orientations. The vascularization of the connectives consisted primarily of four or five arterioles of a relatively large diameter running parallel to each other and longitudinally in the central area of each hemiconnective. The roots were the most lightly and variably vascularized.

In order to discover whether arterial solutions have access to central synapses, the arteria descendens was cannulated through the bulbus arteriosis with Silastic tubing, and various solutions were perfused through the system at rates of 50 ml/hr. To test synaptic transmission during perfusion, responses of the lateral giant neurons to both direct and root stimuli were monitored. Root stimuli activate well-known pathways that contain both chemical and electrical synapses and converge onto the lateral giant neurons (Zucker, Kennedy & Selverston, Sci. 173, 645, 1971). Although bathing the nerve cord with high Mg^{+2}/low Ca^{+2}saline for up to one hour did not affect synaptic transmission, arterial perfusion with high Mg^{+2}/low Ca^{+2} saline rapidly blocked chemical but not electrical synapses. These results show that cannulation of the artery provides a means of delivering to the nervous system solutions that do not penetrate the ganglionic neuropil when delivered via the bathing medium.

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787 A COMPARISON OF THE NEURAL CIRCUITS FOR INKING BEHAVIOR AND GILL-WITHDRAWAL IN APLYSIA CALIFORNICA. John H. Byrne. Department of Physiology, School of Medicine, University of Pittsburgh.

As our understanding of the neural control of simple behaviors progresses, it becomes of interest to examine to what extent different behaviors utilize different neural substrates. Defensive inking behavior and gill-withdrawal in Aplysia offer simple test systems in which these questions can be examined. While a good deal is known regarding the neural circuit for gill-withdrawal (Kandel,1979), little is known about the neural circuit for inking other than it is mediated by 3 identified motoneurons (L14A,B,C) in the abdominal ganglion (Carew & Kandel,1977). I have begun to identify the sensory- and inter-neurons which mediate inking behavior in order to compare these two defensive reflexes at the cellular level.

The ink motor cells are activated by strong tactile stimulation of the mantle region. At least part of the sensory input is mediated by previously identified LE and RE cluster sensory neurons (Byrne et al., 1974) as well as newly identified RF cluster sensory neurons. The RE and LE cells innervate the mantle shelf and siphon skin while the RF cells overlap these regions and also innervate the gill. Some sensory neurons make direct monosynaptic connections to ink motoneurons, others have mono- and polysynaptic components, while others have only polysynaptic components. There are at least 2 types of polysynaptic excitatory pathways. Sensory neurons make monosynaptic connections onto interneurons, such as cell R17, which in turn make monosynaptic increased conductance EPSPs to ink and gill motoneurons. Tactile stimulation of the skin also excites a cluster of at least 3 interneurons one of which has been identified as L31. L31 makes a slow decreased conductance EPSP to L14 but makes no connections to gill motoneurons. In addition to the excitatory input to L14 there is a pronounced slow inhibitory input from a cluster of at least 4 cells, one of which has been identified as L32. In contrast to the slow inhibition of ink motoneurons, L32 produces fast EPSPs in gill motoneurons. Tactile stimulation inhibits L32 and at least part of the inhibition is mediated by the previously identified (Castellucci et al., in prep.) interneuron L16. L16 is weakly electrically coupled to L14 but not to gill motoneurons. All of the interneurons, as well as the RF cluster neurons send axons in the pleuroabdominal connectives. It is unknown whether these axons are input routes or outputs to drive other neurons such as the opaline motoneurons (Tritt & Byrne, 1978) in the pleural ganglion.

The two circuits utilize a large number of common sensory- and interneurons and are mediated by both mono-and poly-synaptic pathways. However some neurons (L32) have different synaptic actions, others (L31) are used for one circuit and not the other, while one neuron (L16) is coupled to L14 but not the gill motoneurons. In addition the ink motoneurons are coupled while the gill motoneurons are not (Carew & Kandel, 1977). Thus while some general features of organization of these reflexes are common, specific differences have developed which contribute to differences in the expression of the two behaviors (Carew & Kandel, 1977, Shapiro et al., in press). Supported by NIH grants NS13511 & NS00200.

IDENTIFIED MOTORNEURONS INNERVATE IDENTIFIED PHARYNGEAL MUSCLE BANDS AND REGIONS IN NAVANAX, AN OPISTHOBRANCH MOLLUSC. M.S. Cappell, D.H. Hall*, A.J. Susswein, D.C. Spray and M.V.L. Bennett. Div. Cell. Neurobiol., Dept. Neurosci., Einstein Col. Med., Bx,NY

Previous work has identified two populations of neurons in the buccal ganglia that control pharyngeal musculature: expansion neurons innervate radial fibers, causing pharyngeal expansion and circumferential neurons innervate circumferential fibers, causing pharyngeal constriction. The present work provides additional evidence that both neuronal types make monosynaptic connections onto muscle and shows that identified cells innervate specific regions of the pharynx. Longitudinal fascia divide the pharynx into two dorsolateral thirds and a ventral third. A dorsal and a ventral nerve on each side provide motor innervation to dorsolateral and ventral pharynx respectively with insignificant overlap as determined by EMG recordings. Circumferential musculature is composed of distinct bands which are remarkably constant in number over a 15-fold range of pharyngeal weights for both dorsolateral and ventral regions. (Dorsolateral bands: M=31.6, S.D.=1.2, N=52. Ventral bands: M=24.3, S.D.=0.5, N=15) Subpopulations of the bands differ morphologically and are also quite constant in number. Evidence that particular neurons end monosynaptically on the muscle includes: short and constant latency of EMGs, unchanged latencies of EMGs with elevated divalent ion concentrations and appropriate antidromic latencies with peripheral electrical stimulation. EMGs were recorded from a grid work of up to 40 pin electrodes passed through the pharyngeal wall at specific bands to determine motorneuron (MN) fields. Identified circumferential cells appear to innervate single subpopulations of bands. The anterior sphincter is formed from overlapping bands which comprise a distinct subgroup. Four MNs innervate this sphincter: one on each side activates it bilaterally, another on each side activates it only ipsilaterally. Several additional MNs innervate more posterior bands. Expansion MNs innervate particular areas of radial muscle via specific combinations of the dorsal and ventral pharyngeal nerve. Both giant (G) cells innervate the entire pharynx via all four nerves as indicated by EMG recordings, dye injection, and serial section reconstruction. It is not yet determined whether each G cell innervates every radial fiber. The left ventral medium sized (M) cell innervates the anterior dorsal pharynx from circumferential #25 to 31 via the dorsal pharyngeal nerves. Another smaller left ventral cell innervates the posterior area of the ipsilateral pharynx via ipsilateral dorsal and ventral pharyngeal nerves. Several other cells with various motor fields have been observed but not adequately characterized with respect to identifiability and uniformity. This work is providing a framework for studying the contribution of individual MNs to generation of feeding behavior. Supported (in part) by NIH grant 5T 32GM7288.

HORMONAL MODULATION OF EGG-LAYING BEHAVIORS IN APLYSIA BRASILIANA. Jerald Cobbs and Harold Pinsker. Marine Biomedical Institute, UTMB, Galveston, TX 77550.

Egg laying in Aplysia is a sequence of fixed acts which follows natural firing of the neuroendocrine bag cells or injection of bag cell hormone (BCH). BCH has been implicated in the release of mature eggs from the ovotestis and in the prolonged modulation of electrical activity of identified central neurons. However, it is unknown whether behaviors associated with egg laying are released directly by BCH or are secondarily triggered reflexes. Furthermore, the sequence of behavioral events that surround a natural bag cell discharge are unknown.

We simultaneously monitored bag cell activity via chronically implanted cuff electrodes on the pleurovisceral connectives and ongoing behavior via time-lapse video recording. A sequence of behavioral events could precede a spontaneous bag cell discharge by as much as 10-15 mins. One component of egg-laying behavior, head undulations (a rhythmic dorso-ventral flexion of the head) began to increase in frequency on the average 5 mins before a bag cell discharge. There was also a predischarge tendency toward decreased locomotion and the mounting of a vertical surface where eggs are subsequently laid. Head weaving (a side-to-side movement of the head used in the dispersion of eggs about the substrate) began 15 min after discharge onset. The appearance of eggs and tamping behavior followed discharge onset by 32 min.

To determine the role of egg movement in releasing the sequence of behaviors following BCH injection, the oviduct was ligated at one of several points along the passageway from ovotestis to periphery. It was found that movement of eggs through the oviduct was required for a complete sequence of egg-laying behaviors. Two components of egg-laying behavior, head undulation and head weaving, were dependent on egg movements in hormone injected animals. A transient inhibition of feeding seemed to be directly initiated by BCH, however egg movement was required to maintain this inhibition. We are attempting to mimic egg movement by cannulating the small hermaphroditic duct near the ovotestis in hormone and non-hormone treated animals. Removal of both abdominal and genital ganglia does not appear to change either the quantity or quality of egg-laying behavior following hormone injection.

These findings suggest BCH triggers a chained reflex. The initial event is release of eggs from the ovotestis whereas egg movement along the oviduct triggers and maintains subsequent behaviors.

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789 THE NEUROPEPTIDE, EGG LAYING HORMONE OF APLYSIA: PURIFICATION, AMINO ACID SEQUENCE AND ANTIBODIES. A. Y. Chiu*, M. Hunkapiller* and F. Strumwasser (SPON: M. M. Nass). Division of Biology, California Institute of Technology, Pasadena, CA 91125.

When the bag cell (BC) neurons of A. californica are electrically stimulated to produce an afterdischarge, they release a number of peptides one of which causes egg laying and related behavior. We have purified this egg laying hormone (ELH) by a two step procedure to yield a homogeneous product with biological activity. Direct microsequence analysis of ELH revealed a single amino acid sequence of 36 residues with a calculated m.w. of 4385 and a calculated pI of 9.7. These results are compatible with previous reports on the size of ELH from SDS gels and gel filtration studies and on the apparent pI of the molecule on isoelectric focusing gels. The amino acid composition of ELH after acid hydrolysis shows good agreement with the sequence data and we report that the amino acid sequence of ELH is: N-Ile-Ser-Ile-Asn-Gln-Asp-Leu-Lys-Ala-Ile-Thr-Asp-Met-Leu-Leu-Thr-Glu-Gln-Ile-Arg-Glu-Arg-Glu-Arg-Tyr-Leu-Ala-Asp-Leu-Arg-Gln-Arg-Leu-Leu-Glu-Lys-OH.

In the purification procedure, the supernatant of a homogenate of BC clusters is subjected to cation exchange chromatography on Sephadex SP C25 followed by gel filtration on Bio-Gel P-6. This results in a 100-fold enrichment of ELH from BC homogenates and a 36% recovery of purified, radiolabeled marker ELH. We calculate, therefore, that a sexually mature animal contains 19-40 µg of ELH in a pair of BC clusters.

The egg laying behavior triggered by ELH includes suppression of feeding and locomotion, head weaving movements, and extrusion and deposition of the egg strand. It is known that neuronal targets respond to the in vitro application of ELH and it is likely that there are also nonneuronal targets. Testing of specific ELH fragments is in progress to locate the active site(s) on the molecule (work in collaboration with R. E. Miller of Monsanto Co.). We have also generated antibodies specifically directed against ELH coupled to Thyroglobulin. This will enable us to localize, by immunohistochemistry, sites of hormone storage within cell bodies and processes of the BC clusters and abdominal ganglion. [This work was supported by a graduate fellowship from the L. A. Hanson Foundation and a Li Ming Scholarship to A.Y.C. and NIH grant NS 15183 to F.S.]

791 DEVELOPMENT OF INDIRECT FLIGHT MUSCULATURE IN A FLIGHTLESS MUTANT OF DROSOPHILA MELANOGASTER. Walter J. Costello. Dept. of Biology, Yale Univ., New Haven, Conn. 06520

Genetic mutations affecting the development of neuromuscular systems can be used as tools to elucidate the parameters important in the development of such systems. A mutation which disrupts the normal development of indirect flight muscle in Drosophila is currently being investigated. Flight in Drosophila is powered by two opposing sets of muscle, the dorsal longitudinal muscles (DLM) and the dorso-ventral muscles (DVM). Both sets are fibrillar type with no known histochemical differences. A mutant allele stripe (Sr) is highly specific in its expression, affecting only the DLM. An adult fly homozygous for stripe contains both sets of flight muscle. However, the size of the DLM is greatly reduced, especially at the anterior insertion; motor neuron connections are present but the organism cannot fly (1973, Levine & Wyman, PNAS 70: 1050).

If a fly homozygous for stripe is crossed with one heterozygous for a deletion at the stripe allele locus (Df(3)Sr), some of the F_1 will possess genotypes with only one copy of the allele: Sr/Df(3)Sr. In most cases these flies contain no DLMs whatsoever (1977,Coggshall,Neurosci. Abstr. 3:173). The adjacent DVMs are always present. In those few cases (~10%) in which some muscle tissue is present where the DLM should be, the tissue is clumped into one mass, extends less than one-half the length of the thorax, and can be asymmetrical (i.e. only one side contains muscle tissue, the other lacking it entirely).

The development of the muscle in Sr/Df(3)Sr flies during pupation has been investigated. In normal and Sr/Sr pupae, adult myocytes congregate around three larval longitudinal muscles during early pupation (<20 hrs). By the 24th hr, each larval muscle is being split in two by the myocytes producing the progenitors of the six DLMs on each side (cf 1956,Shatoury,J. embryol. exp. morph. 4:228). In Sr/Df(3)Sr pupae, these early processes are exactly alike.

By the 40th hr, in normal and Sr/Sr pupae the DLM progenitors have been completely split apart to become separate fibers, though sarcomeres are not yet formed. However, in Sr/Df(3)Sr pupae of the same age the DLM tissue on each side has become clumped; the splitting begun earlier remains incomplete. The muscle is highly vacuolated and during subsequent hours degenerates. The development of innervation in the DLM tissue in normal and Sr/Df(3)Sr pupae is presently being investigated. (Supported by grants from MDA and NIH)

792 LABORATORY REARING REDUCES BEHAVIORAL VARIABILITY IN HERMISSENDA. T. Crow and J. Harrigan*. Sec. on Neur. Systems, Lab. of Biophys. NINCDS, NIH, MBL, Woods Hole, MA 02543.

Laboratory-reared animals may show reduced variability in behavioral and electrophysiological studies. Recently, laboratory specimens of the nudibranch mollusk Hermissenda crassicornis were cultured from fertilized eggs of field-collected animals. We now describe sources of variability that are reduced by using laboratory-reared Hermissenda in our behavioral studies.

Hermissenda exhibit photopositive behavior when maintained on a 6.5 hour light: 17.5 hour dark or 12 hour light: 12 hour dark schedule and tested during the light phase of the schedule. Response to light was assessed by measuring latencies of individual Hermissenda to enter an illuminated area. After entering the light field Hermissenda remain in the illuminated area a significantly greater percentage of time during the observation periods as compared with animals tested in darkness ($p<.01$). Response latencies to move into the light area are faster when animals are tested in the light as compared with the same animals or different groups tested in the dark. Response latencies of animals from the F1 and F2 generations (n=15) and F3 generation (n=17) were compared with two samples of field-collected animals. We found that the variability in the response to light of laboratory reared Hermissenda was significantly less than that of field-collected animals maintained under identical laboratory conditions ($p<.01$). The response latencies of the laboratory-reared animals entering the light field were significantly shorter than those of field-collected animals maintained under the same laboratory conditions three weeks before testing ($p<.005$). The latencies for the first movement toward the light were positively correlated with the response latencies to enter the light field ($r=.97$).

We next examined the response to light of laboratory-reared animals following three training days of light paired with rotation (n=15) and random light and rotation (n=12). As was previously shown for field-collected Hermissenda, response latencies to enter light are significantly longer following training for groups receiving light paired with rotation as compared with random controls ($p<.05$). Animals from the F1 and F2 generations had a smaller range of response latencies to enter light following training for both paired and random groups as compared with the greater range for animals from the F3 generation and field-collected animals. A post-training dissection of our F1 and F2 generations revealed that all animals had statocysts that contained one statoconium. Dissection of animals from the F3 generation revealed variation in the number of statoconia per statocyst. This morphological difference may help elucidate the role of the statocyst in the behavioral modification and the reduced behavioral variability of laboratory-reared Hermissenda following training.

793 BEHAVIORAL AND PHYSIOLOGICAL LOCALIZATION OF WIND STIMULI IN FIRST INSTAR COCKROACHES. Daniel Dagan* and Susan Volman*. (SPON: J. A. Paton). Dept. of Behav. Biol., Technion, Haifa 32000, Israel, and Section of Neurobiol. and Behav., Cornell, Ithaca, N.Y. 14853.

Adult cockroaches (Periplaneta americana) are known to respond to wind puffs by turning away from the source of wind and running (Camhi and Tom, J. Comp. Physiol. 128, 193-201, 1978). The wind excites filiform receptor hairs located on two abdominal appendages called cerci. In the adult, each cercus has about 220 hairs arranged in 15 columns of like directional response. There is a single receptor cell at the base of each hair. In any column all the receptors depolarize most strongly when the hairs are deflected in their common "best" direction, and hyperpolarize for deflections in the opposite direction (Dagan and Camhi, J. Comp. Physiol., in press).

First instar nymphal cockroaches have only two hairs on each cercus. Extracellular recordings with tungsten electrodes at the base of a cercus were used to determine the directionality of the response to wind for these hairs. Mechanical deflection of each hair shaft indicated which of the two was being recorded in a given experiment. For the two hairs of each cercus, the most strongly depolarizing directions of deflection were roughly orthogonal to each other. The combined responses of all four hairs are sufficient to discriminate many different directions of wind.

The directionality of behavioral responses to wind was tested in free ranging 1st instar nymphs. They were stimulated with a wind puff of standard intensity (280 mm/sec); the responses were filmed at 64 frames per second and analyzed frame by frame. As with the adults, the turns were highly significantly correlated with the angle of wind — despite the fact they have only 4 as compared to 440 hairs. After cercal sensory input was eliminated by covering or removing all four filiform hairs, the animals were unresponsive to standard wind puffs. In a semi-restrained animal, a single hair was adequate to detect wind and elicit a running response. When one or more hairs were removed from free ranging nymphs, the directionality of the behavior was predictable from the known physiological properties of the remaining hairs.

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794 I-V CHANGES IN RESPONSE TO CYCLIC NUCLEOTIDE AGENTS IN APLYSIA NEURONS. Peter F. Drake* and Steven N. Treistman, Department of Biology, Bryn Mawr College, Bryn Mawr, PA 19010.

The bursting cells R15 in Aplysia and F-1 in Helix are subject to synaptic and hormonal alteration which persists for long periods and may be mediated by cyclic nucleotides (Treistman and Levitan, Nature,1976; PNAS,1976). We have shown (Brain Research, 1979) that agents which affect cyclic nucleotide levels (e.g., phosphodiesterase inhibitors, cAMP derivatives, and adenylate cyclase activators) elicit response in many Aplysia neurons other than R15, and these responses include the induction of bursting in silent neurons. Current clamp analysis of the I-V characteristics in one such cell, the metacerebral cell (MCC), which bursts after incubation with the phosphodiesterase inhibitor IBMX, shows the induction of anomalous rectification and hysteresis in response to triangular ramp current injection.

Steady state I-V plots under voltage clamp indicate that the MCC usually exhibits a negative slope resistance region (NSR) which increases with bath perfusion of IBMX and benzylthio-cAMP derivative. Similar increases in NSR occur in the bursting left upper quadrant cells treated with cyclic nucleotide agents. When slow triangular ramp techniques were used in voltage clamped MCCs, where voltage is measured on the horizontal axis and current on the vertical scale, IBMX and the cAMP derivative increase the hysteresis and induce or enhance a downward deflection in the curve at a level more hyperpolarized than holding (resting) potential. In addition, the apparent fast transient inward current in the MCC is shown to decrease markedly after perfusion with IBMX. We are presently investigating whether this is an actual decrease in the inward current, or is the result of a concurrent increased fast outward current.

The importance of Ca^{++} in the burst cycle of R15 has recently received attention (Gorman and Thomas, J. Phys.,1978; Eckert, Science,1978) and we have examined the possible involvement of a transmembrane Ca^{++} current and/or an intracellular shift of Ca^{++} from intracellular stores in response to IBMX. Two techniques have been used to inhibit the IBMX-induced bursting: 1) intracellular injection of EGTA and 2) extracellular perfusion with a solution of 30 mM cobalt hypertonically added to stock ASW. These results imply an induced transmembrane Ca^{++} current, and intracellular accumulation of Ca^{++}. Application of cobalt-ASW extracellularly and of EGTA intracellularly both result in a reduction of anomalous rectification present in MCC. Subsequent extracellular perfusion with IBMX and the cAMP derivative restore and enhance the anomalous rectification.

Intracellular injection of cyclic nucleotide agents under voltage clamp as well as clamp results of injected EGTA solutions will also be discussed. (Work supported by NSF grant # BNS 77-01548.)

795 PHASE RESPONSE CURVES AND ENTRAINMENT OF THE INTERNEURON II OSCILLATOR IN INTACT BEHAVING APLYSIA. I. Eberly, M. Lasseter* and H. Pinsker. Marine Biomedical Institute, Depts. Physiol. & Biophys. and Psychiat. & Behav. Sci., UTMB, Galveston, TX 77550.

Spontaneous contractions of the gill, siphon and parapodia occur at frequent but often irregular intervals in Aplysia californica. These contractions are stereotyped and quite conspicuous in the unstimulated animal. The behavior is mediated by a network of interneurons in the abdominal ganglion called Interneuron II (INT II), composed of pacemaker and premotor neurons that function as an endogenous neuronal oscillator (Byrne and Koester, 1978). A similar patterned behavior can also be triggered by a tactile stimulus to the siphon (Kanz et al., 1979), in addition to the monosynaptic component of the reflex (Kupfermann et al., 1974).

Spontaneous INT II activity produces a specific pattern of either excitation or inhibition in motoneurons innervating the mantle organs. This pattern can be monitored in the siphon nerve by extracellular cuff electrodes implanted in freely-behaving animals. INT II activity triggered by external stimulation can also be followed using chronic recordings.

We measured phase-response curves by presenting siphon stimuli at various parts of the cycle. Stimuli presented late in the cycle can phase advance the INT II oscillator by triggering a short-latency INT II burst. However, if presented early (up to 15 sec after a spontaneous burst), the stimulus does not trigger an INT II burst. We also examined entrainment of the INT II oscillator by varying the interstimulus interval (ISI). The probability of triggering INT II bursts is dependent on ISI within a particular range which is close to the free run period. Entrainment tends to break down at lower ISIs.

The behavioral significance of the INT II burst can be demonstrated by video analysis of gill contractions in restrained animals. Under these conditions even very weak stimuli often trigger an INT II burst. When present, the INT II burst significantly increases the amplitude of gill contractions. Thus the INT II burst contributes a stereotyped amplitude component to reflex withdrawal which tends to produce a maximum contraction. The oscillatory and phase response characteristics of the INT II network therefore predict a variety of behavioral outcomes to a stimulus series depending on ISI and free run period. (This research was supported by NSF grants BNS 76-18480 and 77-25584 to H.P.).

796 ANOMALOUS CONNECTIONS BETWEEN IDENTIFIED NEURONS FOLLOWING MICROLESION IN THE LARVAL PRAWN MACROBRACHIUM ROSENBERGII. David R. Friedlander* and Cyrus Levinthal. Dept. of Biological Sciences, Columbia University, New York, NY 10027.

The specificity in the formation of synapses between identified neurons was investigated by following the anatomical effects of killing the cell body of a giant motorneuron (GMN) in the developing giant fiber system responsible for the escape response in Decapod crustaceans. Analysis was done by observation of complete serial thick (1 μm) and thin (0.1 μm) sections of 80 ganglia, fixed from 1 to 56 days after being irradiated in the first larval stage. The giant fiber system in the adult prawn consists of two pairs of bilaterally symmetric giant interneurons (GIs) and the GMNs, a pair of fused neurons, whose central process splits in two axons that leave the CNS through the motor roots, separately innervating left and right flexor muscles. Each GI synapses exclusively with the ipsilateral GMN axon in the motor root region by means of a short colateral. (Holmes, W., Philos. Trans. B 231:293-311, 1942; Macagno, E. and Friedlander, D.R., Soc. Neurosci. Abst. 2:174, 1976). The adult GMN syncytium is the result of the fusion of individual larval neurons, with axons contralateral to their somata. Connections between the GIs and the GMNs are already established at the time of lesion, the first day after hatching.

Microlesions are produced by focusing a beam of light which has a significant percentage of its energy in the ultraviolet, on a region of the ganglion containing the GMN, immediately above the thin ventral carapace. Within a day, the GMN soma cannot be recognized. Signs of axonal degeneration have been observed two days post-lesion; complete disappearance of the axon takes about 10 days. This indicates that the remarkable ability of some invertebrate neurons to survive without a soma (Hoy, R.R., J. Exp. Zool. 172:219-232, 1969) is not present in early larval stages of the prawn.

The most common result of the removal of the GMN axon is the resorption of the colaterals of the GIs deprived of their target GMN axon. More interestingly, in 30% of the ganglia analyzed 21 or more days after irradiation, one or more anomalous connections were found, usually involving a GI deprived of its normal target, with the contralateral GMN which remains intact. This synaptic plasticity supports the notion of a hierarchical order in the rules governing the formation of neuronal connections, where neuron type ranks higher than laterality.

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797 SYNAPTOGENESIS IN THE FLY'S VISUAL SYSTEM. A. Fröhlich* and I.A. Meinertzhagen, Life Sciences Centre, Dalhousie University, Halifax, N.S. Canada.

The formation and selection of synapses during development is the last step in a chain of cellular control mechanisms by which neurons interconnect with great specificity. To study the nature of the rules which control this step we have examined anatomically populations of synapses in the first optic neuropile, or lamina, of the fly Musca in the adult and in various immature stages. Synapses have been scored from limited series of up to 50 consecutive micrographs prepared from material fixed and processed conventionally for electron microscopy. Synapses are segregated in classes according to the identity of the presynaptic element and for each representative the identity of up to four postsynaptic processes established. Studying populations of synapses from serial sections provides not only a direct count of synapse frequency but also some measure of the variability with which different postsynaptic elements associate at a given synapse class, addressing the question of the specificity with which synapse formation occurs. Analysis of different developmental ages provides evidence on how this specificity is attained and may be correlated with the acquisition of adult synaptic ultrastructure.

We have found that synapses form with great specificity. In the adult, the first-order synapse is an elongate tetrad with dyadic symmetry. > 97% of all examples are consistent with L1 and L2 occupying the lateral dyadic positions as previously described (Burkhardt and Braitenberg: Cell and Tiss. Res. 173, 287) while there is variability in the occupants of the polar dyadic positions. The orientation of synapse long axis is also nonrandom. The formation of these synapses occurs relatively late in development (50-80% of pupal life) after the neural elements are represented in their final positions, but before the surrounding glial cell processes have ensheathed the elements. The synapses are less elongate in immature stages but by 80% of pupal life have already attained the same frequency as that found in the adult (approx. 7-9 synapses/μm. receptor terminal segment) and are invariably associated with 4 postsynaptic elements each of which has a postsynaptic density not found in the adult. Some characteristic features of synaptic ultrastructure are however acquired between 80%-100% of pupal life. These include the platform component of a conspicuous T-shaped presynaptic density, a subsynaptic cisterna in each of two (L1 and L2) postsynaptic elements, the full complement of synaptic vesicles and the presence of nearby glial invaginations (capitate projections).

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798 THE "PHASE RESPONSE" CURVE OF THE LOCOMOTOR CIRCADIAN RHYTHM IN THE CRAYFISH. Beatriz Fuentes-Pardo, Angélica Alucema-Molina* and José Viccon-Pale*. Departamento de Fisiología, Facultad de Medicina, U.N.A.M., Apdo. Postal 70250, México 20, D.F., México.

The locomotor circadian activity was investigated in the intact crayfish under conditions of complete darkness and constant temperature. In order to obtain information about the sensitivity of the circadian system, among several possible experimental designs we chosed one that involves once only administration of a light stimulus to the organism under "free running" conditions. The light application was done along the twenty-four hours in different "circadian time". The results of such a series of experiments are phase advances or delays which have been summarized in the so-called "phase-response curve".

We observed that one complete oscillation of the phase response curve occurred during each circadian oscillation and that there is only one position of the circle during which the stimulus produces phase advances and another single one during which phase delay results. This unimodal phase response curve which exhibit different oscillators is essential for adaptive capability of "biological clocks" to periodical environmental changes.

799 DYE COUPLING INDICATES THE STRUCTURE OF ELECTROTONICALLY COUPLED NETWORKS IN SENSORY NEUROPIL OF THE CRAYFISH BRAIN. Raymon M. Glantz, Mark Kirk, and Howard Wood. Dept. Biol., Rice University, Houston, TX 77001.

Interneurons which arise in the visual and antennal neuropil of the crayfish brain descend to the more caudal ganglia via the circumesophageal connectives. These neurons are excited by stimuli to the sensory organs of the head and control or modify the activity of lower motor centers in thoracic and abdominal ganglia. Recent studies have shown that within each neuropil there is extensive reciprocal interaction between neurons with similar trigger features and receptive field (Glantz, R.M., J. Neurophysiol, 41:1297-1313, 1314-1327, 1978). Several lines of evidence indicate that many of the ensemble synapses are electrotonic: a) The synaptic delay is 0.1 ms or less; b) The synaptic potentials follow presynaptic spike trains at rates of up to 200 Hz without attenuation; c) The synapses transmit both depolarizing and hyperpolarizing injected currents. Morphological analysis of the ensemble interactions has been carried out with the flourescent dye Lucifer Yellow CH supplied by W.W. Stewart of the N.I.H. Stewart (Cell, 14:741-759, 1978) has summarized evidence that dye coupling is diagnostic for electrotonic coupling. Injection of single antennal interneurons has resulted in the dye spreading up to three additional cells. Transynaptic dye migration is observed in about 60% of the injected preparations. The soma of dye coupled cells may be adjacent to each other or separated by the width of the brain. The axons of the coupled cells frequently exit in each of the two circumesophageal connectives. Individual cells can possess up to three distinct dentritic zones spreading across the entire width and/or length of the brain. The dendrites are punctuated with multiple varicosities suggestive of chemical presynaptic endings. Central neurites of up to 28 μ in diameter are frequently observed.

The reciprocal coupling promotes strong synchronization in the discharge of the parallel descending axons. Since the individual EPSPs are generally subthreshold, the impulse synchronization of a given pair of neurons depends upon the coactivation of other elements in the network. Different stimulus conditions coactivate different subpopulations of cells. The stimulus thus specifies both the activated subpopulation and the spatial pattern of coordination within the active subpopulation (Wood, H. Ph.D. thesis, Rice Univ., 1978).

800 TRANSFER OF HABITUATION FROM ONE PATHWAY TO ANOTHER IN APLYSIA. J. Goldberg* and K. Lukowiak* (SPON: W. L. Veale). Div. Med. Physiol., Fac. of Med., Univ. of Calgary, Calgary, ALTA T2N 1N4

The gill withdrawal reflex and its subsequent habituation in Aplysia can be evoked by repeated stimulation of two distinct sites. Tactile stimulation of either the gill or the siphon evokes these gill behaviors. It has been suggested that the neural pathway which mediates these gill behaviors evoked by siphon stimulation is physiologically independent from the neural pathway which mediates the reflex behavior evoked by gill stimulation. We found that habituation of the reflex evoked in one pathway generalizes or is transferred to the evoked reflex in the other pathway. Thus, our results indicate that an interaction does exist between the two pathways. This supports the idea that the neural circuitry mediating gill withdrawal behaviors involves an integrated system. The following experiments were done: 1) Tactile stimuli (1g) were presented to the gill once every 20 minutes and the reflex did not habituate. 2) If the siphon was stimulated (1g) 10 times (ISI=30s) (which resulted in habituation of the reflex) just before a stimulus was presented to the gill, it was found that the amplitude of the reflex evoked by the gill stimulus was significantly smaller than the control. 3) It was further found that the rate of habituation evoked by repeated gill stimulation was faster following the interposition of the siphon stimuli than in control habituation sessions. 4) Transfer of habituation was also apparent at the neuronal level. The number of action potentials evoked in gill motor neuron L7 by a single tactile stimulation of the gill was reduced following a siphon stimulation habituation run. 5) Similar results were obtained when the gill stimuli and siphon stimuli were reversed. 6) The central nervous system (abdominal ganglion) is not necessary for the transfer of habituation to occur, since similar results were obtained in experiments where the abdominal ganglion was removed. These data show that there are at least 2 sites where the transfer of habituation can occur; centrally at the sensory-gill motor neuron synapse, and peripherally in the gill.

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801 CARDIOREGULATORY NERVES IN A SPIDER. F. Gonzalez-Fernandez* and R. G. Sherman. Dept. Biol., Clark Univ., Worcester, MA 01610 and Dept. Zool., Miami Univ., Oxford, OH 45056.

The heartbeat in spiders is initiated and coordinated by a thread-like cardiac ganglion situated along the dorsal mid-line of the heart. The ganglion generates bursts of impulses which are conducted throughout the myocardium to initiate the heartbeat via neuromuscular synapses. There is considerable evidence that the heart receives input from the central nervous system, but no one has yet demonstrated conclusively that cardioregulatory nerves exist in spiders.

Careful dissection of the anterior region of the abdomen of a tarantula (Eurypelma marxi Simon) and subsequent staining with methylene blue revealed the presence of a branch of abdominal nerve VIIIb extending to the cardiac ganglion just anterior to the first pair of ostia. Nerve VIII is paired; each member of the pair arises from opposite sides of the main abdominal ventral nerve and gives rise to three branches one of which is VIIIb. Nerve VIIIb further branches and one such bundle is the cardioregulatory nerve. Consequently, there is a pair of cardioregulatory nerves, each innervating the cardiac ganglion at about the same level, but from opposite sides.

Conclusive demonstration that VIIIb contains cardioregulatory neurons was obtained by stimulating it electrically while simultaneously recording the activity of the cardiac ganglion and the contractions of the heart. Both increases and decreases in the rate and amplitude of the beat could be evoked under appropriate stimulation conditions. The frequency of the cardiac bursts and their duration were altered accordingly. Therefore, both cardioinhibition and cardioacceleration can be achieved by activity in these nerves.

There appears to be only one excitatory and one inhibitory axon in each cardioregulatory nerve. Only one voltage dependent response of each type could be found. Therefore, our tentative conclusion is that there are two excitatory and two inhibitory neurons that arise in the CNS to modulate heartbeat activity.

The cardioinhibitory effect becomes evident at frequencies above 10 Hz; the effect is maximal at about 25-30 Hz and it does not increase appreciably above these frequencies. The excitatory effect first appears at 10 Hz; it becomes maximal at about 40-50 Hz. The cardioinhibitor predominates when the inhibitor and excitor are stimulated simultaneously at the same frequency.

802 DOPAMINERGIC INHIBITION OF BURST FIRING IN CELL R 15 OF APLYSIA CALIFORNICA: ESTABLISHMENT OF A DOSE-RESPONSE RELATIONSHIP. Sidney M. Gospe, Jr., and W. A. Wilson. Dept. Pharmacol., Duke Univ. Med. Ctr., Durham, N. C. 27710, and Epilepsy Center, V.A. Hospital, Durham, N. C. 27705.

Studies of the physiology of the bursting process of cells L2 - L6 and R15 of Aplysia californica have shown that the oscillatory currents responsible for burst firing are endogenous to the cell, but that synaptic input can modify the bursting pattern. Recently, it was demonstrated that the inhibition of bursting by acetylcholine in L2 - L6 and by dopamine (DA) in R15 was due to the abolition of the negative resistance region in the current-voltage (I-V) relationship of the cell (W. A. Wilson and H. Wachtel, Science 202:772, 1978).

Since the effect of DA on cell R15 is stable over long time periods and reversible with washing, it was possible to bath apply the neurotransmitter to the ganglion and determine the dose-response relationship.

Abdominal ganglia were constantly perfused with artificial sea water. Double the normal Mg^{++} concentration was used in order to eliminate naturally occurring IPSPs that also affect negative resistance. Different concentrations of DA and other pharmacologic agents were selectively added to the perfusate. The I-V relationship of cell R15 was determined under voltage clamp conditions using the single micro-electrode technique.

The threshold DA dose for an effect on the I-V relationship is 10^{-4} M. With increasing doses, the negative resistance region of the curve is progressively reduced. Doses of 6×10^{-4} M DA tend to linearize the curve and effectively abolish bursting activity. Washing the ganglion with sea water restores the I-V relationship to the control conditions. Addition of imipramine or pargyline to the perfusate in doses shown to affect DA metabolism in molluscan systems does not change the characteristics of the dose-response relationship. Experiments using putative dopaminergic agonists and antagonists to characterize the receptor mediating this response will be discussed.

803 AXONAL SPROUTING IN AN IDENTIFIABLE LARVAL LOBSTER MOTONEURON. C.K.Govind and Joanne Pearce*. Scarborough Coll., Univ. of Toronto, West Hill, Ontario, M1C 1A4, Canada.

Axonal sprouting is a well known but poorly understood phenomenon of the nervous system which should ideally be examined in a single identifiable neuron. The excitor motoneuron to the limb receptor muscle in lobster, Homarus americanus, provides this opportunity when examined in a larval stage. The distal accessory flexor muscle is innervated by an excitatory and an inhibitory axon which are recognizable in electron micrographs by the shape of their synaptic vesicles. The former are more spherical than the latter. This permits identification of the excitatory motoneuron in the 1st larval stage when it sprouts vigorously to innervate the rapidly growing muscle. Consequently almost two thirds (60μm) of the entire length of the motoneuron was examined by serial section electron microscopy. In the 1st larval stage there is little or no inhibitory innervation.

The excitor axon however traverses the width of the muscle and sprouts branches toward the tendon and exoskeleton ends. This primary sprouting occurs in regions of the axon which differentiate into terminals by contacting muscle granular sarcoplasm and accumulating agranular synaptic vesicles. The terminals possess presynaptic dense bars which are regarded as active sites of transmitter release, either by themselves or as part of fully formed synapses. The newly formed sprout is in contact with muscle sarcoplasm, is filled with synaptic vesicles and has synapses; in effect it is a terminal. The primary sprout gives rise to secondary sprouts which have similar characteristics. Thus sprouting in an identifiable larval motoneuron occurs only when it is in contact with muscle sarcoplasm. This suggests that the target tissue (muscle) may induce sprouting which would be a mechanism for regulating the innervation of the muscle during development and growth.

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804 COMPARISON OF SEROTONERGIC CEREBRAL CELLS OF HELISOMA TRIVOLVIS WITH THE METACEREBRAL CELLS OF SOME OTHER GASTROPODS. Bonnie Granzow, Stanley B. Kater, Dept. Zool., University of Iowa, Iowa City, IA 52242, and C.H. Fraser Rowell. Dept. Zool., University of California, Berkeley, CA 94720.

As previously demonstrated, a bilaterally symmetrical pair of serotonergic neurons in the cerebral ganglia of Helisoma trivolvis have an excitatory influence over the feeding motor program of the buccal ganglia. This excitation is manifested in the cells' ability to initiate and maintain patterned bursting in buccal ganglia motoneurons (Granzow and Kater, Neuroscience 2:1049-1063, 1977).

These cerebral neurons of Helisoma have characteristics in common with the Metacerebral Cells of other gastropods including their content of serotonin, their axonal distribution with branches extending into buccal ganglia nerve trunks, and their ability to influence buccal ganglia neurons. These commonalities indicate that the cerebral cells of Helisoma might be homologous to the Metacerebral Cells. However, we have found certain physiological properties of Helisoma cerebral cells which differ from those of the Metacerebral Cells of Helix and Aplysia. These properties include, among others, a high spontaneous firing rate (approx. 2 spikes/sec), a predominance of tonic inhibitory synaptic potentials, and a linear I-V relationship, i.e., no anomalous rectification of the membrane. The behavioral significance of these differences has not yet been determined. However, the cerebral cells of Helisoma strongly effect the pattern of the buccal ganglia feeding motor output, whereas the effect of activity in the Metacerebral Cells of Aplysia is most apparent as a potentiation of intensity of motoneuron activity. Since the serotonergic cerebral cells may have somewhat different roles in various gastropods, it is not surprising to find differences related to the integration processes of these cells.

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805 DIURNAL CHANGES IN LYSOSOME-RELATED BODIES IN THE CRAYFISH PHOTORECEPTOR CELLS. G.S. Hafner, G. Hammond-Soltis* and T. Tokarski*. School of Optometry, Indiana University, Bloomington, IN 47405.

The effect of illumination on the degradation of microvillar membrane in the invertebrate photoreceptor cell has been correlated with the appearance in the cytoplasm of certain structurally distinct lysosome-related bodies. Three types of bodies can be clearly distinguished in the retinula cell cytoplasm of the crayfish using the electron microscope, large (4.2-1.5 microns) and small (1.4-0.3 microns) multivesicular bodies (MVB), combination bodies (CB) and lamellar bodies (LB). The structural details of these bodies have been previously described in both crustaceans and insects by Eguchi and Waterman (1967) and White (1967). To determine the relationship between diurnal lighting and the appearance of these bodies in the cell, the numbers of each type of inclusion were determined at various times during a 12:12 lighting cycle.

Under the diurnal lighting conditions significant temporal differences were found in the appearance of all lysosome-related bodies. Small MVB increase rapidly after the onset of light and peak within 30 minutes. Thereafter they decline rapidly to a constant level during the remaining light phase. During the dark phase of the cycle they again increase reaching high levels by 6 hours. The large MVB begin to increase at 30 minutes and reach a peak 4 hours after the onset of light. Thereafter they decline in numbers to near zero by 12 hours. Combination bodies and LB do not begin to increase until 1 hour after light onset but then rise rapidly to reach maximum values at 6 hours. Both CB and LB decline rapidly to near zero by 8 hours. This low level persists throughout the remaining time in the light and no increases are seen during the dark phase of the cycle. Control animals maintained in the dark during the normal light phase show a slight increase in small MVB through 10 hours. These levels reached values near the basal values for small MVB seen in the light. The combined peak production of all three lysosome-related bodies occurs between 4 and 6 hours in the light. This peak corresponds with the minimum rhabdome diameter which also occurs between 4 and 6 hours. The maximum rhabdome diameter occurs during the dark phase of the cycle.

These data support the hypothesis that light produces microvillar membrane breakdown resulting in the initial production of MVB which undergo degradation to form CB and finally LB. This process appears to be completed within the first 6-8 hours after the onset of light.

806 MORPHOPHYSIOLOGY OF AN IDENTIFIED AXON BUNDLE IN THE BUCCAL GANGLION OF NAVANAX INERMIS. D.H. Hall*, D.C. Spray, M.S. Cappell and M.V.L. Bennett. Dept. Neurosci., Albert Einstein College of Medicine, Bronx, NY 10461.

Serial reconstruction of 3μ sections through the buccal ganglia of Navanax inermis has revealed a group of large axons (10-50μ diameter) in a bundle which runs mediolaterally through both ganglia in a linear fashion and crosses the midline commissure. Most or all of these large axons belong to physiologically identified motoneurons innervating radial musculature, which are responsible for pharyngeal expansion. Some of this group of motoneurons were not previously recognized as motoneurons physiologically. However, their morphological similarities to known motoneurons prompted further studies which confirm that they do cause local expansions of the buccal mass. Presently, there appear to be six to eight identifiable expansion motoneurons in each buccal ganglion innervating partially overlapping or discrete regions of the buccal mass. These motoneurons can be identified morphologically in the reconstructed series by comparison to a catalogue of physiologically identified expansion motoneurons that had been injected with the dye Lucifer Yellow CH. The morphological criteria for identification include the size, shape and position of the soma and the number, size and distribution of the neurites, e.g. do axons exit only ipsilaterally or both ipsilaterally and contralaterally.

The expansion motoneurons are known to be electrotonically coupled to one another by way of the largest members, the G (giant) cells. This coupling can be blocked by synaptic input from a population of inhibitory neurons at least some of which are sensory. The coupling and uncoupling synapses may be localized within or very near the bundle of large axons. Intracellular recordings show stronger electrotonic coupling from soma to postsynaptic axon than from soma to postsynaptic soma. Also, IPSPs in the G cell are larger with faster rise times in the axon than in the soma. Examination of the axon bundle in thin section reveals a heavy glial sheath which prevents contact between axons. There are however nearby specializations between primary branches of the axons which resemble gap junctions in some respects and extracellular lanthanum treatment is being used to further characterize these appositions. Chemical synapses are also found on the axons and their primary branches in this region. These synapses may mediate uncoupling of expansion motoneurons.

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807 Saxitoxin Binding to Sodium Channels from Wild-Type and Mutant *Drosophila melanogaster*. Linda M. Hall, Jane Gitschier* and Gary R. Strichartz*. Dept. Biol., 16-713, MIT, Cambridge, MA 02139 and Dept. Physiol. Biophys., SUNY, Stony Brook, NY 11794.

The voltage-sensitive sodium channel in *Drosophila melanogaster* has been studied using two neurotoxins, tetrodotoxin and saxitoxin, which are known to bind specifically to sodium channels and block the action potential. These toxins are identical in their ability to block nerve conduction in *Drosophila* larval motor nerves; 200 nM tetrodotoxin blocks conduction within 20 minutes. [^{3}H]-saxitoxin binds specifically to *Drosophila* head extracts. The high-affinity interaction (K_D=1.9 nM) between the toxin and extract is reversible, noncooperative, and inhibited by tetrodotoxin. The number of saturable saxitoxin binding sites in *Drosophila* is 6.4 fmol per mg wet weight head. A comparison between the physiological and biochemical studies in *Drosophila* and other organisms suggests that the *Drosophila* saxitoxin receptor is part of the voltage-sensitive sodium channel. Binding and electrophysiological studies were done on a mutant, ttx^s, which is abnormally sensitive to dietary tetrodotoxin. The ttx^s mutation does not appear to affect the density or structure of sodium channels since there is no difference between mutant and wild-type with regard to physiological sensitivity to tetrodotoxin and [^{3}H]saxitoxin binding parameters. These studies provide techniques which can be used to identify mutants with defects in the sodium channel.

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808 ADDITIONS TO THE PHYSIOLOGY OF THE STOMATOGASTRIC GANGLION (STG) OF THE SPINY LOBSTER, Panulirus interruptus. - D.K. Hartline, D.V.Gassie*, C.D. Sirchia*, Békésy Lab., U. Hawaii, Honolulu, HI.

We present additional properties and connections of specific cells in isolated STG to aid understanding pattern generation by a simple network (some results confirmed or extended in collaboration with D. Russell ("*") or K. Graubard and J. Raper ("†")).

AB Neuron compared to PDs (intrasomatic records): a) often has twice as many spikes due to firing twice on each summed PD electrical EPSP; b) receives a smaller IPSP from LP; c) often has a smaller excursion of interburst pacemaker potential relative to driver-potential amplitude.

LP Neuron has a rectifying electrotonic connection to PLs.†

PY Neurons are of two types, PEs ("early") and PLs ("late"): a) after strong stomatogastric nerve (Stn) stimulation, PEs fire at early phase, often overlapping with LP; PLs fire later, rarely overlapping; b) PEs but not PLs get EPSPs from HD; c) PLs but not PEs put strong IPSPs on LP and IC; d) PEs primarily innervate the posterior pyloric muscles (p10, p12); PLs anterior (p2, p8); e) PE somata are often smaller than PL*; f) PLs are electrically interconnected*†; g) axon counts show 4-6 PEs; 2-4 PLs. Variability in individual PYs may reflect a spectrum of types.

VD Neuron appears to receive major inhibition from AB and little from PD. With AB bursting weakly, VD activity can increase during PD burst and terminate during PD repolarization, likely due to its electrical coupling to PD. Addition of AB inhibition can lead to a gap in VD pattern, with two VD bursts per cycle. Transmitter release from PD/AB via electrotonic paths may explain weak delayed VD IPSPs on LP, PL, and IC, blocked by hyperpolarizing PD.

LG Neurons: These gastric motoneurons send axon branches in the internal pyloric nerve and appear to innervate pyloric muscle p6.

HD Axon: Hepatopancreas-duct nerve stimulation gives: a) antifacilitating unitary EPSPs in AB, PD, PE; b) higher pyloric cycling rates; c) intense PE firing. EPSPs in PEs show post-tetanic potentiation*. The axon may arise from a multi-polar cell (sensory?). It travels in the lateral ventricular nerve, synapses in STG, and exits via Stn and superior oesophageal nerves.

Delaying conductance: In PY, PD, and GM* cells, a hyperpolarizing conditioning pulse followed by a suprathreshold step depolarization produces an unusually long delay in recovery to beginning of repetitive firing as compared to other cells (LP, VD, CP). In PYs this is associated with a rapidly developing slowly decaying conductance increase and a membrane potential trajectory with a characteristic rapid hyperpolarizing "hook" followed by a slow depolarizing ramp. These properties appear similar to those of the Connor-Stevens "A-conductance". This mechanism may be activated by inhibitory inputs (e.g. PD/AB) producing a phase-delay of activity in a burst cycle. Support: NIH NS13138; NSF GJ 43177.

809 ACTIVITY OF CLAW OPENER MOTOR NEURONS DURING HABITUATION AND DISHABITUATION OF THE CRAYFISH DEFENSE RESPONSE. R.D. Hawkins and J. Bruner. CNRS, Gif-sur-Yvette and Université de Picardie, Amiens, France.

A neural mechanism which has been proposed for behavioral habituation is an increase in the activity of inhibitory neurons. We have investigated this hypothesis for habituation of the crayfish (Astacus) defensive claw opening response. Claw opening is caused by contraction of the abductor of the dactylopodite, which is innervated by only two motor neurons: one excitor and one inhibitor. In partially restrained animals we recorded simultaneously the activity of these two neurons, the electromyogram of the opener and closer muscles, and movement of the claw. Tactile stimulation of the thorax produces phasic activity in both the excitor and inhibitor motor neurons, and opening of the claw. If this stimulation is repeated at 30 second or 1 minute intervals, the claw opening response becomes progressively smaller. This habituation is due to a progressive decrease in the evoked excitor activity, while the activity of the inhibitor remains unchanged.

The habituated claw opening response can be dishabituated by tactile stimulation of the claw or head, or by visual stimulation. Dishabituation was found to be due to (1) an increase in the evoked excitor activity, (2) a decrease in the evoked inhibitor activity, and (3) PTP at the excitor neuromuscular junction. PTP was demonstrated in isolated claw preparations using physiological parameters of stimulation, and shown to be of sufficient magnitude and duration to contribute to dishabituation.

Thus, at the level of the motor neurons, habituation of this response is due to a decrease in evoked excitor activity while the inhibitor activity remains unchanged. Dishabituation, on the other hand, involves both an increase in excitor activity and a decrease in inhibitor activity, as well as PTP at the excitor neuromuscular junction.

810 AFTERDISCHARGE IN BAG CELL NEURONS IS INITIATED BY PEPTIDES FROM THE ATRIAL GLAND OF APLYSIA. E. Heller*, L. K. Kaczmarek, M. Hunkapiller* and F. Strumwasser. (SPON: W. R. Adey). Division of Biology, California Institute of Technology, Pasadena, CA 91125.

The neuropeptidergic bag cells in the abdominal ganglion of Aplysia are able to initiate egg laying and its associated behavior by secreting egg laying hormone (ELH) during a long lasting afterdischarge. This afterdischarge may be triggered by electric stimulation of a presumed afferent pathway from the head ganglia. Copulation may be a natural stimulus for egg laying and it has been shown that the atrial gland in the reproductive tract of Aplysia contains a factor that is able to induce egg laying behavior when injected into recipient Aplysia (Arch et al., J. Comp. Physiol., 1978). We have now found that extracellular application of crude extracts or of purified peptides from the atrial gland produces long lasting afterdischarges in the bag cells. This effect is also observed with isolated pleuroabdominal nerve preparations which contain asomatic bag cell neurites. Two preliminary lines of evidence suggest that these peptides may act by activating the afferent input from the head ganglia. i) No effects were observed on isolated bag cells in primary cell culture, ii) In experiments with the entire intact nervous system, in which the abdominal ganglion and the head ganglia were maintained in separate chambers, application of these factors to the head ganglia alone could induce bag cell afterdischarge.

We have purified two peptides, A and B, that are able to induce both egg laying behavior in recipient animals and bag cell afterdischarges. These peptides were isolated from homogenates of the atrial gland by a combination of ammonium sulfate precipitation, agarose gel filtration and cation exchange chromatography. Each peptide has 32 amino acid residues. Microsequencing revealed the following sequence for peptide A: H-Ala-Val-Lys-Leu-Ser-Ser-Asp-Gly-Asn-Tyr-Pro-Phe-Asp-Leu-Ser-Lys-Glu-Asp-Gly-Ala-Gln-Pro-Tyr-Phe-Met-Thr-Pro-Arg-Leu-Arg-Phe-Tyr-OH. Peptide B differs from A in only four positions. The first nine residues of B are: Ala-Val-Lys-Ser-Ser-Ser-Tyr-Glu-Lys- while residues 10-32 of B are identical to those of A. Neither sequence resembles that of ELH. The calculated Mw of A is 3714 and that of B is 3822. The pI of A is about 8 and that of B is 9. Gel filtration of atrial gland homogenates on agarose in the presence of urea reveals material that can induce egg laying in the excluded volume in addition to those fractions containing peptides A and B. We are currently determining the relative potencies of these differing fractions. [Supported by NIH grant (NS 15183) to F.S.]

811 NEURONAL NETWORK OF THE EYE OF APLYSIA: INTRACELLULAR AND DYE MARKING ANALYSIS. Jon W. Jacklet. Dept. Biology, SUNYA, Albany, NY 12222.

A circadian clock resides in the isolated eye of Aplysia. In culture medium and complete darkness endogenous compound action potential (CAP) activity from the optic nerve waxes and wanes rhythmically with a periodicity of about 26 hours. The circadian aspects of the eye activity have been studied extensively (i.e. Jacklet, Science 198, 69, 1977).

Intracellular recording with KCl electrodes of 60-70 MΩ and injection of Lucifer yellow with similiar electrodes filled with the dye has allowed a better understanding of the eye neuronal network. Three basic neuronal response types were obtained. One, R, had resting potentials of 70 mV and gave a graded depolarizing receptor potential to illumination. Light adaptation reduced this response from the maximum of 70 mV in the dark adapted state. Some had depolarizing and others hyperpolarizing afterpotentials. These cells were usually silent during endogenous optic nerve CAP activity but sometimes biphasic electrotonic potentials followed the CAP. Injection of Lucifer yellow revealed this cell situated in the pigmented retina, with a distal photoreceptive apparatus and an axon contributing to the optic nerve. Thus, the R type appears to be a primary receptor making weak electrical connections with neurons that produce the endogenous CAP. A second response type is H. This had resting potentials of 30-45 mV and always actively spiked (up to 90 mV) when impaled. Illumination caused initial depolarization and spiking followed by prolonged hyperpolarization. Spikes in this cell appear in optic nerve but do not contribute to the CAP. Dye injection shows it in the retina near the R type. A third type is D. These cells have 30-40 mV resting potentials and attenuated spikes of 5-20 mV. They spike in synchrony with the endogenous and evoked CAP. Several spike potentials of different amplitudes are often observed in these neurons suggesting that neighboring D neurons are electrically coupled. Apparently the spike initiation zone and site of coupling are near the origins of the optic nerve some distance from the soma. Lucifer yellow injection shows this neuron type has a large axon in the optic nerve and a non-receptor soma. There is extensive but weak electrical coupling among the D neurons which accounts for the synchronous optic nerve CAP. These results provide evidence for three basic types of neurons in the retina even though the total number of neurons is about 5000. Continued analysis should yield the complete network of a neuronal circadian clock.

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812 STRUCTURES AND CHARACTERISTIC PROPERTIES OF SWIMMERET COMMAND FIBERS IN CRAYFISH. G.A. Jacobs and B. Mulloney. Dept. Zoology University of California Davis, Davis, CA 95616

Rhythmic swimmeret beating in crayfish can be driven by stimulating any one of five pairs of command interneurons (Hughes and Wiersma, 1961; Ikeda and Wiersma, 1964). We have further distinguished these pairs of fibers by using both physiological and structural criteria.

We investigated the effects of proprioceptive feedback on the period of the motor pattern by interfering with the movements of individual swimmerets. Experiments were performed on Procambarus clarkii (Girard) and Pasifasticus leniusculus. The abdomen was separated from the body and pinned in a Sylgard lined dish which allowed the swimmerets to move freely. Activity of the motor neurons was monitored with extracellular pin electrodes. Bundles of axons containing command fibers were stripped from the ventral nerve cord anterior to the second ganglion and stimulated with suction electrodes. When a command fiber was located and a regular motor pattern produced, we interfered with one swimmeret's movement with a pin held in its path. The swimmeret was held in the fully retracted position which occurs at the end of a power stroke for several bursts then allowed to move freely. We observed four different kinds of results. Either the period decreased, did not change, increased, or bursting was inhibited. We think these differences reflect the individual command fiber isolated for stimulation.

Swimmeret motor neurons abruptly stop bursting when stimulation of some command fibers stops. However when stimulation of others stops, motor neurons burst for several more cycles.

Bundles of axons each containing a command fiber, were filled with Co^{++} to discover the fibers' structure. The most obvious structure seen when bundles containing command fibers are filled is a large diameter axon (20-30μ) which has one or two branches which ramify ipsilaterally in swimmeret neuropil; medial command fibers project laterally and lateral fibers project medially. The axon narrows as it enters the ganglion, branches and flares again in the posterior connective. The structure of a fiber is similar in successive ganglia. No contralateral projections have been observed.

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813 PROTEIN PHOSPHORYLATION DURING AFTERDISCHARGE OF THE NEUROENDOCRINE BAG CELLS IN APLYSIA. K. R. Jennings,*, L. K. Kaczmarek and F. Strumwasser. (SPON: C.A.G. Wiersma) Biology Div., Caltech, Pasadena, CA 91125.

The neuroendocrine bag cells in the abdominal ganglion of Aplysia generate a long-lasting afterdischarge upon brief electrical stimulation of a pleurovisceral connective nerve. Evidence has been presented that activation of adenylate cyclase may be involved in the genesis of afterdischarge (Kaczmarek, Jennings and Strumwasser, P.N.A.S. 75: 5200, 1978). Experiments were therefore conducted to determine whether the actions of cAMP might be mediated by a protein kinase. Intact abdominal ganglia were incubated at 14°C in the presence of $Na_2H^{32}PO_4$ overnight (Levitan and Barondes, P.N.A.S. 71: 1145, 1974). Experimental ganglia were either electrically stimulated to afterdischarge or incubated for 15 minutes in the presence of 8-benzylthio cAMP. These treatments resulted in 51% (N=9) greater ^{32}P incorporation into TCA-insoluble material from the bag cell somata and surrounding connective tissue, which contains many bag cell neurites. Increased phosphorylation into one protein band was observed with either electrical stimulation or treatment with cAMP analogue when compared with controls. Microdensitometric analysis of the gel autoradiograms showed a 45% (N=10) increase in phosphorylation of this protein. The m.w. of this phosphoprotein was estimated to be 22,000 daltons by its migration on SDS polyacrylamide gels. The phosphorylation of the 22,000 m.w. protein in the bag cell region occurs within 2 minutes of the onset of afterdischarge. We have also observed phosphorylation of two high m.w. (approx. 120,000 and 125,000 dalton) proteins in the bag cell region. The long time course of the excitability changes observed in the bag cells (30 minutes) may allow correlation of the time course of protein phosphorylation with the onset and termination of the afterdischarge. (K.R.J. is a Gordon Ross Medical Research foundation fellow.)

814 CYCLIC AMP ANALOG GENERATES AFTERDISCHARGE IN APLYSIA BAG CELL NEURONS. L. K. Kaczmarek, K. R. Jennings* and F. Strumwasser. Division of Biology, California Institute of Technology, Pasadena, CA 91125.

A long lasting afterdischarge can be generated in the neuropeptidergic bag cells of the abdominal ganglion of Aplysia following brief electrical stimulation of a pleuroabdominal connective. The afterdischarge can also be elicited by the extracellular application of the cAMP analog, 8 benzylthio cAMP (8BT-cAMP). We have now shown that 8BT-cAMP is able to induce afterdischarge in dissociated bag cells in primary cell culture. Normally, cultured bag cells will fire repetitively in response to depolarizing current pulses but do not discharge spontaneously. The extracellular addition of 8BT-cAMP (0.5 mM), however, induces the cells to discharge after a delay of 8-55 min. This "afterdischarge" may continue for 2-4 hr and is correlated with an increased membrane resistance (1.5- to 3-fold), spike broadening and, occasionally, a regenerative hyperpolarizing response that can be blocked by 12 mM Cs^+. These effects are consistent with the hypothesis that cAMP induces a decrease in the membrane conductance to K^+ ions.

The mean duration (MD) of an electrically stimulated bag cell afterdischarge in a freshly dissected (Day 1) intact abdominal ganglion is 29.6 min (N=11) after which the cells remain refractory to further electrical stimulation for several hours. After 16-24 hr (Day 2) full recovery takes place (MD=32.0 min, N=11). If, however, the ganglion is incubated continuously with the protein synthesis inhibitor anisomycin (3×10^{-6} M) during the first afterdischarge and its recovery period, full recovery fails to occur (MD for Day 1 = 54.8 min, N=5; MD for Day 2 = 4.0 min, N=5). Previous work has shown that bag cell neurites in the connective tissue of the pleuroabdominal connectives retain the ability to afterdischarge after they have been surgically isolated from their somata. Full recovery of afterdischarge in response to electrical stimulation also fails to occur in these asomatic neurite preparations (MD for Day 1 = 30.2 min, N=16; MD for Day 2 = 4.6 min, N=10). For both the pharmacological and surgical treatments, control experiments showed that full length afterdischarges occur on Day 2 if no stimulation is given on Day 1. Furthermore, when after either treatment, electrical stimulation failed to produce any afterdischarge, vigorous afterdischarges could still be obtained in response to 8BT-cAMP (0.5 mM). This data suggests a role for de novo protein synthesis in the control of bag cell excitability, possibly in the synthesis of proteins regulating cAMP levels. (K.R.J. is a Gordon Ross Medical Research Foundation Fellow. This work was supported by grants to F.S. from the NIH [NS 13896, NS 15183].)

815 INNERVATION OF THE DIRECT FLIGHT MUSCLES IN DROSOPHILA. David G. King. School of Medicine, Southern Illinois University, Carbondale, IL 62901.

In insects such as flies whose wing-beat is powered by large indirect fibrillar muscles, the orientation of the wing during flight is controlled by several smaller muscles attached to the wing base, the direct flight muscles. The abbreviations for these muscles used here will be those of Zalokar (1947, Revue Suisse de Zoologie 54:17-33) who described the thoracic musculature of Drosophila melanogaster, followed in parentheses by those of Heide (1971, Zool. Jb. Physiol. 76:87-137) who described the functions of these muscles in Calliphora. Nerve abbreviations follow Power (1948, J. Comp. Neurol. 88:347-409). The direct flight muscles are innervated by the ADMN and the MAN. These nerves both cross a notch in the pleural apophysis so that at this point they form a single nerve with axons running in opposite directions. Individual axons were traced with light microscope through serial thin sections (½μm) from the thoracic ganglion to specific muscles. Axons from the ADMN innervate muscles pa1 (I1), pa3 (b2), pa4 (b1), pa5 (b3), pp1 (hg1), pp5 (I2) and the anterior portion of pp4 (III3,4?). Axons from the MAN innervate muscles pa2 (III1), pp2 (hg2,3), pp3 (hg4), the posterior portion of pp4 (III2?) and sp1 (ps1); the latter muscle receives two axons.

The 10-15μm diameter of the ADMN axon to muscle pa4 (b1) makes this axon one of the largest in Drosophila, comparable only to the cervical giant fibers and the motor axon to the tergotrochanteral muscle. The large diameter of this axon suggests a function that requires rapid impulse conduction. Such rapid conduction would be important if this axon were to participate in an escape response, such as the initiation of flight. Since in Calliphora muscle pa4 (b1) begins firing before the start of spontaneous wing-beat and since this muscle functions to abduct the wing, this muscle may serve to extend the wing in preparation for flight. In Drosophila a giant fiber pathway (King, in preparation; Tanouye, in preparation) activates the tergotrochanteral muscle and the dorsal longitudinal flight muscle; both of these actions contribute to the initiation of flight in response to sudden visual stimulation. Thus, since the large axon to pa4 (b1) appears to participate in rapid activation of another aspect of flight initiation, this axon may also be part of the same giant fiber pathway. Synapses of the pa4 (b1) motor neuron with the cervical giant fibers are now being sought.

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816 CHARACTERISTICS OF AND INTERACTIONS BETWEEN SEPARATE BRANCH SPIKES FOUND IN CRAYFISH SENSORY INTERNEURONS. Mark Kirk and Raymon Glantz. Dept. Biol., Rice University, Houston, TX 77001.

Intracellular recording from neuropilar processes of the crayfish brain and abdominal ganglia reveal action potentials which can be distinguished as spikes originating from separate branches of the same cell. Each branch spike is usually seen to have a distinct threshold to injected currents. They may occur in a variety of temporal sequences with respect to each other. Often EPSPs precede either event when they occur separately or alternatively one branch spike may lead into the other. In special cases, such as during a step of depolarizing current, one spike is seen to entrain the other's discharge. The branch spikes may have overlapping or completely separate sensory receptive fields, and in the extreme case may subtend different sensory modalities. During recordings from neurons of the supraesophageal ganglion, in which the branch spike corresponding to the cell's descending "main" axon discharge can be uniquely identified, it can be seen that summation of the "dendritic" spike with other depolarizing events (injected outward current, PSPs) is required for the main axon to each threshold. In some instances the occurrence of the axon spike is invariably associated with the sequential activation of the "dendritic" spike on the descending phase of the axon spike. Conversely, several observations have been made of neurons in which the "dendritic" spike and main axon spike were completely independent. The latter observation suggests that the "dendritic" spikes may have a role in communication or integration that does not involve the axonal process at all.

817 A MAGNESIUM- AND BARBITURATE-SENSITIVE INHIBITORY SYNAPTIC POTENTIAL IN LEECH GANGLION. Anna L. Kleinhaus and Scott Brand*. Dept. Neurology and Section of Neuroanatomy, Yale Med. Sch., New Haven, CT 06510.

Stimulation of the leech mechanoreceptive cells sensitive to pressure (P cells) evoked hyperpolarizing postsynaptic potentials in a neuron of unknown function located near the annulus erector motor neurons on the ventral surface of the ganglion. This potential had an amplitude of 3-5 mV at resting membrane potential and a stable onset latency of 5 msec measured from the peak of the presynaptic action potential. Its reversal potential was -71.8 +- 1.4 mV (n = 20) and was unaffected by removal of 90% of the Cl from the bath. Changing external K from 10 mM to 1 mM shifted the reversal potential about 35 mV in the hyperpolarizing direction. At rates of stimulation above 0.5 Hz the amplitude of the postsynaptic potential was depressed by about 80%. At lower rates amplitude was stable and there were no failures of transmission. Magnesium 20 mM reversibly blocked the synaptic response.

Electron micrographs made after injection of horseradish peroxidase into the pre- and postsynaptic neurons showed that they were in direct contact; the regions of contact had the morphological characteristics of synapses described in leech neurons by other workers.

The amplitude of the hyperpolarizing potential was greatly decreased by 0.5 mM Na phenobarbital, a concentration which had no effect on the pre- or postsynaptic action potentials. Thus, at least one inhibitory postsynaptic potential in leech ganglion is depressed by a barbiturate, in contrast to other preparations in which such potentials have been reported to be unaffected or enhanced by this class of drugs.

818 METABOLIC CONSEQUENCES OF CHRONIC DEAFFERENTATION IN THE CRICKET CNS. T.E. Knox*, M.R. Meyer, and J.S. Edwards. Dept. Zoology, Univ. Washington, Seattle, WA. 98195.(SPON: R. Haschke)

Chronic removal of the cercal appendages of the cricket Acheta domesticus throughout postembryonic development results in loss of known afferent input to identifiable giant interneurons (GI) in the CNS of the terminal ganglion (TG) (Proc. R. Soc. Lond. B.,1974,185:105). Such prolonged deafferentation retards the growth of GI dendritic processes (J.Comp.Neur.,1975,159:407) which depends on presence, rather than activity of intact presynaptic input (Soc. Neurosci. Abstr.,1977,7:186). These findings suggest the likelihood that growth of the GI's may be trophically dependent upon presynaptic sources.

To assess what effects deafferentation might have on metabolism in the TG, we compared incorporation of [^{3}H]-leucine into TG between normally reared (control) crickets and those receiving bilateral deafferentation throughout development and changes in amount of TG protein content after removal from adults. Chronic deafferentation resulted in statistically significant reduction (>30%) in TG protein content when compared to control TG's. Such decreases were in accord with marked reduction in neuropile volume observed in histological sections of deafferented TG. However, when adult crickets were subjected to short-term deafferentation following development through instar 9, only a slight (<8%) decrease in total TG protein was noted, probably ascribable to degeneration and loss of cercal sensory axons, terminals, and glial elements.

We then compared in vitro incorporation of [^{3}H]-leucine into TCA insoluble protein between control and chronically deafferented TG, which resulted in a marked (60%) decrease in incorporation in the deafferented TG's. When data was normalized for decreased protein content of deafferented TG's, TCA insoluble activity was still significantly lower (>40%) in the experimentally treated TG's. In contrast, TG taken from crickets receiving a short-term deafferentation regimen showed no decreases in incorporation. These data indicate that chronic removal of presynaptic input to the TG does depress protein metabolism within the CNS. We cannot say which elements of the CNS are principally affected. A generalized injury response to deafferentation, however, seems unlikely.

In an attempt to determine whether loss of input directly affects metabolism of known target GI's, autoradiographic analysis is being performed on serially sectioned, unilaterally deafferented TG. Qualitative results show decreased grain density over the somata and dendritic processes of deafferented GI's.

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819 RECIPROCAL INHIBITION BETWEEN DISTINCT BEHAVIORAL ACTS IN PLEUROBRANCHAEA, Mark P. Kovac* and W.J. Davis. Thimann Laboratories and Center for Coastal Marine Studies, Univ. of Calif., Santa Cruz, CA 95064.

Distinct behaviors in Pleurobranchaea are organized into a priority sequence, or behavioral hierarchy, which governs rudimentary choices in this organism (J. Comp. Physiol. 90, 207, 225, 1974). Several legs of the behavioral hierarchy involve unidirectional suppression of one behavior by another; thus, feeding behavior suppresses righting, but righting does not suppress feeding. In contrast, we have found that one leg of the hierarchy, feeding versus withdrawal from tactile stimulation, involves reciprocal rather than unidirectional inhibition. To demonstrate this reciprocal inhibition, 32 specimens were used to analyze quantitatively the interaction between the two behaviors. In each animal, withdrawal was elicited by delivering a controlled, graded tactile stimulus, and monitored visually. The sensory stimuli for the two behaviors were presented separately (control trials) and simultaneously (experimental trials), and each varied in a graded manner over a large range. Analysis of the behavioral data showed that as previously reported (J. Comp. Physiol. A 117, 199, 1977) the withdrawal amplitude declines from control levels during active feeding. Conversely, feeding declines in intensity during withdrawal, and the suppression is graded with the intensity of withdrawal behavior. Thus, the two motor systems are reciprocally inhibitory.

Plots of feeding intensity versus withdrawal stimulus intensity are not linear, but instead show several turning points and inflections. Analog electronic modeling of reciprocally inhibitory neural networks resembled the behavioral data best when: 1) the models employed feedback rather than feedforward inhibition; and 2) when cross excitation was also added. These two features of the models have been largely confirmed by neurophysiological investigations showing 1) an inhibitory connection from a corollary discharge cell in the feeding network onto a command-like cell in the withdrawal network (Science 198, 632, 1977; J. Neurophysiol., in press, 1979) and 2) an excitatory/inhibitory connection from the withdrawal network to the paracerebral cell, a command-like neuron in the feeding network. The study introduces a new integrative variable into considerations of how the nervous system regulates animal behavior, namely reciprocal inhibition between motor systems subserving distinct behavioral acts.

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820 TRANSMISSION BETWEEN CRAYFISH GIANT FIBERS AND NON-GIANT MOTONEURONS IS NOT PRIMARILY MONOSYNAPTIC. F.B. Krasne, A.M. Roberts,*† A.P. Kramer,†† Dept. Psychol., UCLA, Los Angeles, CA 90024. J.J. Wine, Dept. Psychol., Stanford, Palo Alto, CA 94305.

The mechanism for generation of the motor patterns that produce giant fiber escape reactions in crayfish is believed to be particularly simple: The medial and lateral giant command neurons (MG/LG) are thought to distribute excitation directly to relevant fast flexor motor neurons via monosynaptic connections in each bodily segment, and these connections are believed to be the sole or paramount cause of motoneurons firing in appropriate extent and sequence. The direct connections of the command neurons to the motoneurons is important for at least two reasons: (1) These connections have been a source for the discovery of principles of neuronal communication such as electrical transmission at MG/LG - motor giant (moG) junctions and amplification by dendritic spikes at MG/LG - nongiant fast flexor motoneuron (FF) junctions. (2) The direct activation of motoneurons by command neurons has been viewed as an unusual form of motor system organization. Prevailing conceptual schemes picture the neuron that triggers or turns on a behavior (ie. the 'command neuron') as activating an independent pattern generating network; it is believed that command neurons may commonly bias motoneurons via monosynaptic connections but not fire them. In this view crayfish escape seems to be anomolous.

We have found that in each hemiganglion, in parallel with the monosynaptic pathway between MG/LG and the FFs, there is a disynaptic pathway in which a neuron we call the segmental driver (SD) is interposed. This disynaptic pathway is absolutely necessary and usually sufficient for driving of the FFs by MG/LG. The monosynaptic connections between MG/LG and FFs are weak and quite incapable of firing the FFs by themselves.

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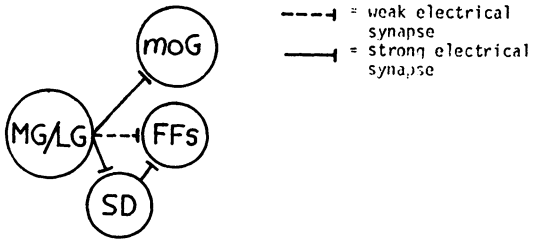

821 INCREASED ELECTROGENICITY OF CRAYFISH NEURONS FOLLOWING AXOTOMY: INVESTIGATIONS OF THE SIGNAL AND MECHANISM. John Y. Kuwada, Grace C. Hagiwara, & Jeffrey J. Wine. Department of Psychology, Stanford University, Stanford, CA 94305.

The peripheral inhibitor (FI) to the fast flexor muscles of the crayfish abdomen has a spiking axon and a nonspiking cell body. Axotomy or partial axotomy induces a transient increase in the electrogenicity of FI somata. Overshooting action potentials can be recorded in the soma from 2 days to 2 weeks following axotomy (Kuwada & Wine, Soc. Neuro. Abs., 4, 1978).

We would like to discover the signal that initiates the increase in excitability and clarify the nature and mechanism of the change. With regard to the signal, we find that (1) axotomy of other crayfish efferents (the phasic extensor motoneurons) also induces soma spikes; (2) axotomy of crayfish interneurons does not induce soma spikes; (3) cutting one branch of an efferent axon induces the same transient increase in soma excitability as cutting the whole axon; (4) after excitability has waned, recutting the stump of a previously cut axon will not reinstate excitability, but (5) cutting another, intact branch of the axon will reinstate excitability. Finally, preliminary evidence suggests that the onset of soma excitability occurs slightly sooner if the axon is cut proximally rather than distally. These results, plus the ability of colchicine to mimic axotomy-induced changes in excitability (Ritman, Tweedle & Cohen, Science, 178, 107 (1972), are consistent with the hypothesis that the signal is a sudden decrease in a factor transported along the axon from muscles to the neuron soma.

With regard to the nature of change in membrane excitability, we find that (1) the axotomy-induced soma spikes are sodium dependent; (2) like many other cells, FI has latent, electrogenic calcium channels that can be unmasked with tetraethylammonium ions; but (3) no changes in calcium conductance are detected following axotomy; and (4) increases in membrane excitability are not confined to the soma but can also be detected in the neuropilar processes of FI.

The mechanism of the change is unknown, but involves a temperature dependent process, in which a 10°C temperature increase shifts time of onset from c. 36 hours to less than 24 hours after axotomy, and offset from c. 21 days to c. 11 days. Systemic injections of cycloheximide, a protein synthesis inhibitor, prevent or retard the increase in excitability if given during the period following axotomy. Cycloheximide did not affect normally spiking neurons.

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822 SEROTONIN-INDUCED HYPERPOLARIZATION OF APLYSIA NEURON R15 IS MEDIATED BY CYCLIC AMP. Irwin B. Levitan and Alan H. Drummond*. Friedrich Miescher-Institut, P.O. Box 273, CH-4002 Basel, Switzerland.

Serotonin elicits alterations in the endogenous bursting activity of neuron R15 in the abdominal ganglion of Aplysia californica. Bath perfusion of serotonin at concentrations greater than 0.05 μM enhances the interburst phase of the burst cycle; both the depth and the duration of the interburst hyperpolarization are increased. Higher concentrations of serotonin (10-100 μM) cause complete cessation of bursting and "chemically clamp" the cell to about -70 mV. These effects are mimicked by bath application (1-500 μM) or intracellular injection [Treistman and Levitan, NATURE 261:62-64 (1976)] of cyclic AMP analogs such as 8-parachlorophenylthio-cyclic AMP, and by activation of adenylate cyclase in R15 following intracellular injection of guanylyl-imidodiphosphate [Treistman and Levitan, PNAS 73: 4689-4692 (1976)]. Furthermore the serotonin-induced hyperpolarization is potentiated by phosphodiesterase inhibitors; in the presence of 1 μM RO 20-1724, 0.1 μM serotonin can abolish bursting completely.

Serotonin stimulates adenylate cyclase activity in membranes from abdominal ganglion, or from isolated R15 cell bodies. It also causes cyclic AMP to accumulate in R15 in intact ganglia. A pharmacological analysis indicates that the threshold concentration of serotonin and its analogs, necessary to alter bursting, correlates well with the concentrations necessary to stimulate adenylate cyclase. All the criteria necessary to assign a role for a cyclic nucleotide in a physiological response have been satisfied for this case. Accordingly we conclude that the hyperpolarization induced in R15 by serotonin is mediated by cyclic AMP.

823 DIPHENYLHYDANTOIN DEPRESSES FIRING IN THE APLYSIA GIANT NEURON BY BLOCKING A SLOW INWARD CURRENT. D.V. Lewis*, K.L. Zbicz* and W.A. Wilson. (Spon: P.B. Bennett). Div. of Ped. Neurol., Duke Univ. Med. Ctr., Durham, N.C. 27710 and Epilepsy Ctr., V.A. Hosp. Durham, N.C. 27705.

Injection of constant depolarizing current into the Aplysia giant neuron, R2, produces a train of action potentials with an initial high firing rate followed by a gradual adaptation with slowing of the firing rate. Diphenylhydantoin (DPH)(3.8 to 10.0 x 10^{-5}M) markedly slows the initial firing rate as does lanthanum (La^{+3})(1-3mM). Voltage clamp techniques were used to analyze this effect of DPH on firing rate. When the membrane potential of R2 is held at resting level (-50 to -60 mV) and then depolarized to between -35 and -45 mV for many seconds (sec), a changing current is seen. Initially, current is inward and peak magnitude is attained in 1 sec. This inward current rapidly declines to zero by 3 to 4 sec, after which a slowly and persistantly increasing outward current is seen. On a current voltage graph of one sec. current values, the inward current is reflected by a negative slope resistance region of the curve. Both DPH and La^{+3} block this inward current at concentrations which reduce early firing frequency as described above. Also, after 1 hour in calcium free 10 mM cobalt sea water, the inward current is markedly reduced. The blockade of inward current is reflected in the current voltage curve as disappearance of the negative slope resistance region and increased magnitude of outward current values. The reduction of inward current by DPH may explain the slowing of action potential generation by this drug. The similar effects of La^{+3} and zero calcium with cobalt suggest that calcium may carry a portion of the inward current, and that DPH may be blocking this calcium flux.

824 RESETTING THE CIRCADIAN CAP RHYTHM IN THE APLYSIA EYE BY LL TO DD TRANSITIONS, I: THE EYE AND BRAIN INTERACT. Marvin E. Lickey and Robert G. Prichard* (SPON.: Richard Young). Dept. of Psych., U.of Oregon, Eugene, OR 97403.

Many circadian systems can be reset to a precisely defined phase by switching the system from continuous light (LL) to continuous darkness (DD). The circadian rhythm of compound action potentials (CAP) in the Aplysia eye is no exception. Intact Aplysia were first exposed to LD 12:12 and then, beginning at dawn, switched to LL varying in duration from 1 to 62 h. The eyes were then removed and placed in culture for recording the CAP rhythm in DD for 3 or more cycles. When LL>24 h the phase of the CAP rhythm was always reset by the LL to DD transition (LL/DD) such that the midrise point of the rhythm occurred about 12 h after LL/DD. This fixed phase lag between the CAP rhythm and LL/DD occurred regardless of the initial phase of the CAP rhythm. The reset, or phase shift ($\Delta\phi$) was not caused by the procedure of eye removal or setting up for recording; if LL was continued in vitro for 3 to 12 h, the phase of the rhythm was determined by the time of LL/DD and not by the time of the set up. The optic nerve contains efferent fibers from the cerebral ganglion and we investigated whether these fibers can influence resetting of the eye by LL/DD. The eyes together with the brain were removed and the optic nerve of only one (detached) eye was cut; the other (attached) eye was left free to interact with the brain. When $12 \leq LL \leq 24$ the detached eye was not reset by LL/DD but the attached eye was; i.e. the duration of LL pretreatment required to produce resetting by LL/DD was less in the attached than in the detached eye. The brain, via the intact optic nerve, must participate in resetting the phase of the CAP rhythm when $12 \leq LL \leq 24$. We discount the possibility of hormonal interaction between the eye and brain because the attached and detached eyes were side by side in the recording medium. The critical signals in the optic nerve might represent the output of photoreceptors signalling LL/DD. To test this we reversibly blocked activity in the optic nerve with isotonic sucrose for 3 h beginning just before LL/DD. Resetting was not prevented. Resetting was prevented, however, by a 3 h nerve blockade beginning 3 h after LL/DD. The long delay between LL/DD and the effectiveness of nerve blockage suggests that the critical nerve signals are not from photoreceptors. In summary, following $12 \leq LL \leq 24$ h the eye and brain interact in vitro in DD to cause a phase shift of the CAP rhythm. This interaction is mediated by the optic nerve. The interaction is not continuous and does not occur until several hours after LL/DD. In another abstract we further explore the timing of the critical signals in the optic nerve and suggest that the eye-brain interaction may best be explained by two separate timing mechanisms in the eye-brain system. NSF 28251, NS 12374.

825 CENTRAL PEPTIDE-CONTAINING NEURONS MODULATE GUT ACTIVITY IN TRITONIA. Philip E. Lloyd* (SPON: A.O.D. Willows). Dept. Zool., Univ. Washington, Seattle, WA 98195

The paired buccal ganglia of Tritonia are largely responsible for the neuronal control of feeding. On the dorsal surface of each ganglion, there is a large neuron (100-150 μm diameter) that appears white when epi-illuminated (termed the large dorsal white cells or LDWC). Both electrical stimulation of nerve trunks and back-filling with Co^{++} indicate that each LDWC sends its axon out the ipsilateral gastro-esophageal nerve. These nerves run parallel along the foregut and contribute to a complex plexus on the surface of the stomach.

Using an in vitro snail heart assay (Lloyd, J. Comp. Physiol. 128: 269, 1978), it was found that these neurons contain large quantities of a cardioactive peptide. This assay is sensitive to as little as 0.005 the contents of a single LDWC. A minimum of 95% of the cardioactivity present in a LDWC is destroyed by incubation with trypsin or Pronase. Furthermore, a survey of other large identified neurons (12 orange, 11 white, under epi-illumination) demonstrates that none contain protease sensitive cardioactivity. The peptide found in LDWCs co-elutes with a small cardioactive peptide found in the brains of Helix and Tritonia when subjected to both molecular sieve and ion exchange chromatography. It has an apparent MW of under 1,000, and an extremely basic isoelectric point (pI of approx. 11.0).

Intracellular recordings from semi-isolated preparations (buccal ganglia-foregut) reveal that in "non-feeding" ganglia, the LDWCs are tonically active, firing as slow beating pacemakers with weak phasic synaptic modulation. However in "feeding" ganglia (feeding-like activity induced by stimulation of nerve roots), the LDWCs fire high frequency bursts at short intervals. These bursts are in phase in both LDWCs, but the two neurons are not synaptically interconnected.

The response of the foregut to electrical stimulation of the LDWCs is biphasic. The initial response to a high frequency burst is a contracture of the gut. This response is highly refractory. A much longer lasting response is the inhibition of the gut's spontaneous activity. Experiments are currently underway to more precisely determine the role of the LDWCs and the peptide during feeding in Tritonia (supported by NSF grant BN575-1359 7A02.

826 IMMUNOHISTOCHEMICAL LOCALIZATION OF A SPECIFIC SECRETORY MEMBRANE GLYCOPROTEIN IN IDENTIFIED *APLYSIA* NERVE CELL BODIES. B.W. Lubit, R.T. Ambron, and J.H. Schwartz. Div. Neurobiol. & Behav., Depts. Anat. & Physiol., Columbia U., New York, N.Y. 10032, U.S.A.

A small number of membrane glycoproteins become rapidly labeled when identified neurons of *Aplysia* are injected with ^{3}H-sugar precursors. Rapid synthesis may imply that these membrane components play an important role in the physiological activity of the cell. In order to relate biochemical information to physiological functioning, we have been studying the localization of individual ^{3}H-glycoproteins to specific organelles within the neuron. We had previously shown in the giant cholinergic neuron R2 that a glycoprotein with a molecular weight of 180,000 daltons (Component-I) is preferentially retained in the cell body, whereas the lower molecular weight membrane glycoproteins are exported into the axon for rapid transport. Using both quantitative electron microscope radioautography and subcellular fractionation, we found that Component-I is a constituent of somatic vesicle membranes. Recent analyses of glycoproteins in other identified cells have suggested that Glycoprotein-I may be a common constituent of *Aplysia* neurons. We have now examined the distribution of this membrane glycoprotein by direct immunofluorescence using a specific antibody raised in rabbits.

Component-I was purified by extraction of neuronal membranes with lithium diiodosalicylate; it was then further purified by SDS polyacrylamide gel electrophoresis and the region of the gel containing Component-I emulsified with complete Freund's adjuvant. Antibody was detected by Ouchterlony immunodiffusion and rocket immunoelectrophoresis using an SDS membrane extract as the test antigen. The antibody was shown to bind selectively to ^{3}H-Component-I isolated from an R2 injected with ^{3}H-N-acetyl-galactosamine. There was no binding to other labeled glycoproteins. The antibody was purified by adsorption with acetone-powders of liver and of *Aplysia* body wall muscle, and was then coupled to fluoroscein isothiocyanate. In cryostat sections of ganglia treated with the antibody, fluorescence was confined to neurons: cytoplasm of nerve cell bodies appeared intensely fluorescent. There was little staining of nuclei and, consistent with biochemical studies, no staining of axons, neuropil or glial cells was observed. Examination of the fluorescence in tissues other than the nervous system indicates that Component-I is a universal constituent of *Aplysia* secretory cells. Thus both the salivary gland and the winding gland stain intensely, whereas there was no staining of muscle or connective tissue. Using the antibody as a specific probe, experiments are now in progress to determine the role of this membrane glycoprotein in neuronal activity.

827 FOOD INCREASES CNS SUPPRESSIVE CONTROL OVER GILL REFLEX BEHAVIORS IN APLYSIA. Ken Lukowiak* (SPON: L.K.Vaughan). Div. Med. Physiol. Fac. of Med., Univ. of Calgary, Calgary, ALTA. T2N 1N4, Canada.

The central (CNS) and peripheral (PNS) nervous systems interact and are parts of an integrated system which mediates adaptive gill reflex behaviors evoked by siphon stimulation. In this system the CNS exerts suppressive and faciltatory control over gill behaviors. I now report that continuous exposure to food significantly increases the CNS's suppressive control which results in a significantly smaller reflex amplitude and a significantly faster rate of gill reflex habituation. The experimental group (n=10) had continuous exposure to food while the control group was fed once a week. The following data were obtained: 1) In controls the amplitude of the reflex evoked by a 1g. siphon stimulus with branchial (Br), ctenidial (Ct), siphon (Sn) nerves and PNS intact was not significantly different than the reflex with only the PNS intact (19.3mm ± 2.6 vs. 20.1mm ± 2.2). The reflex was significantly enhanced if only Br was cut (33.0mm ± 2.4). The rate of habituation with Br, Ct, Sn and PNS intact (-0.55) was not different from that with only PNS intact (-0.56). 2) In the experimental group the reflex amplitude with Br, Ct, Sn and PNS was significantly smaller than with only the PNS intact (3.0mm ± 0.5 vs. 20.3mm ± 4.1). Again the reflex was enhanced following only Br cut (36.5mm ± 6.0). The rate of habituation with Br, Ct, Sn and PNS intact (-1.81) was significantly faster than with just PNS (-0.53). 3) The reflex with Br, Ct, Sn and PNS intact in the experimental group was significantly smaller than the reflex with everything intact in the controls (3.0mm vs. 19.3mm). Yet the reflex amplitudes with only the PNS present were not significantly different nor were the amplitudes with only Br cut. Moreover, the rate of habituation in the experimental group with everything intact was 3x faster than in the controls. But the rates were not different when only the PNS was intact. 4) The neural activity evoked in L7 by the siphon stimulus was less and decremented faster in the experimental group than in controls. 5) In young Aplysia there were no observable differences between control and experimental animals. It is concluded that continuous exposure to food alters the central "state" of the preparation by increasing the CNS's suppressive control over gill behaviors. This CNS control is absent in young Aplysia. Thus, to completely understand the neural mechanisms of habituation, it is necessary to take into consideration CNS suppressive and faciltatory control over gill behaviors. (Supported by the MRC of Canada.)

28 THE NUMBER OF NEURONS IN SEGMENTAL GANGLIA OF THE LEECH. Eduardo R. Macagno. Dept. Biological Sciences Columbia University, New York, N.Y. 10027

The number of neurons was measured in specific segmental ganglia from the nerve cord of four different species of leeches. Quantitative data were obtained by means of computer-aided techniques for the analysis of nerve structure from serially-sectioned or whole-mounted tissue (Macagno et al,1979, Ann. Rev. Biophys. Bioeng. 8). The species studied were Hirudo medicinalis, Macrobdella decora, Haemopis marmorata (family Hirudinidae) and Haementaria ghilianii (family Glossophoniidae).

Two sets of ganglia were studied in each species: middle ganglia (9, 10 and 11) and sex ganglia (5 and 6). The middle ganglia, as well as the rest of the 21 segmental ganglia excepting 5 and 6, are thought to be quite similar. The sex ganglia are associated with the sexual organs and, visually appear to have more neurons.

The data to be presented show that: (a) the number of neurons in a specified ganglion varies by one to two percent from animal to animal of a given species; (b) the middle ganglia of a particular leech have approximately the same number of neurons, with a variation also within one or two percent; (c) the middle ganglia of Hirudo, Macrobdella and Haemopis have three to four hundred more neurons than their middle ganglia, with the exact number varying according to the species, but the sex ganglia in Haementaria have only about 20 more neurons than its middle ganglia.

829 **THE MYOCHORDOTONAL ORGAN IMPOSES THE CONTRACTILE PROPERTIES OF ITS RECEPTOR MUSCLE ONTO THE FLEXOR MUSCLE OF THE MERUS-CARPUS JOINT OF THE CRAYFISH.** **John D. Marrelli*, James L. Larimer. Dept. Zool., U. Texas at Austin , Austin , Texas 78712**

The Myochordotonal Organ (MCO) is a muscle-sensory organ whose distal tendon spans the Merus-Carpus joint (MC) of the crayfish claw. Its sensory cells are in series with the small Accessory Flexor muscle (AFm) and in parallel with the large working Flexor muscle (Fm). Thus the MCO is stretched by AFm contraction and released by Fm contraction. Voluntary flexion of the MC joint is normally produced by co-activation of Fm and AFm. When AFm and Fm contract simultaneously, the resulting stretch of the sensory ending is proportional to the difference between the extent of contraction of AFm and Fm. The rate sensitive MCO cells thus record the differential AFm-Fm contraction velocity. During imposed movements of the MC joint, some MCO cells are extension sensitive. During voluntary flexion, these units gain additional properties: First, they discharge before any detectable movement of the MC joint (10 um resolution). Second, they discharge during flexion , but only during flexion deceleration. Ablation of the AFm motoneurons eliminates these responses leaving only the extension sensitivity. These additional properties are thus emergent properties of the sensory motor loop. Selective electrical stimulation of these sensory units produces one-for-one activation of the working muscle, Fm. The following sequence accompanies voluntary flexion : Co-activation of Fm and AFm; Fm fatigue or inadequate Fm tension causes Fm contraction to slow down relative to AFm contraction; MCO responds in proportion to this differential contraction; Fm receives additional activation and the limb speeds up; MCO output declines as Fm rate of contraction approaches that of AFm.

This system is a negative feedback control system in which the contractile speed of Fm is forced to conform to the contractile speed of AFm. This kind of control of intrinsic working muscle properties is important because Fm growth, injury, fatigue, heterogeneity of muscle fibers, motoneuron recruitment and external loads can alter limb movements arising from a stereotypic motor program. These factors are less likely to influence the contractile properties of the AFm because of its location, homogeneity of muscle fibers,size and simpler motor innervation.

The MCO sensory-motor loop thus acts to reduce the complexity of Fm response to motor input, insuring that successive motor programs produce similar limb movements.

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830 Swallowing and Regurgitation in the Isolated Nervous System of Pleurobranchaea: Distinguishing Features and Higher Order Control. Andrew D. McClellan* (SPON: P. Sheafor). Dept. of Anatomy and Dept. of Biomed. Eng., Case Western Reserve University, Cleveland, Ohio 44106

In Pleurobranchaea regurgitation has several features in common with feeding. For example, both behaviors display rhythmic radula movement, a kinematic component commonly thought to be exclusive to feeding. Alternating buccal root activity, which underlies this rhythmic radula movement, is therefore not an adequate criteria for identification of feeding in the isolated nervous system. The motor programs for swallowing and active regurgitation can be selectively elicited in isolated nervous system preparations by extracellular stimulation of various nerves at low and high levels, respectively. Since swallowing is one of the phases of both the feeding and regurgitation responses, it presently can be behaviorally classified only in more intact preparations, where natural stimulation is possible.

In so far as feeding and regurgitation have similar motor programs, the behavioral specificity of some of the putative feeding "command" cells is in question. The metacerebral and paracerebral cells are thought to be command cells specific for feeding. Although these cells are sometimes active during the regurgitation motor pattern, particularly at its termination, they do not elicit the response when stimulated. This result suggests that these cerebral cells may perform a non-command function during regurgitation. Several cells in the buccal ganglia do appear to specifically evoke active regurgitation. One class of cells, some of which are "ventral white cells", elicits the complete motor pattern for regurgitation when stimulated and actively bursts when the same response is evoked by nerve stimulation. A second class of cells is also capable of commanding regurgitation, although the membrane potential of these cells is only modulated during regurgitation elicited by other means.

This study implies that the higher order command cells for swallowing and regurgitation reside in the buccal ganglia, while the higher order cells for consumatory feeding are located in the brain. The results also indicate that rigorous criteria must be developed for identifying feeding in isolated gastropod nervous systems; rhythmic, alternating buccal root activity does not appear sufficiently specific for feeding.

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831 HETEROGENEITY OF EXCITATORY INNERVATION ALONG SINGLE MUSCLE FIBERS IN THE LOBSTER. D.E. Meiss and C.K. Govind. Scarborough College, University of Toronto, West Hill, Ontario, Canada M1C 1A4.

Crustacean neuromuscular synapses arising from a single excitor axon are known to be well differentiated between different fibers but little is known about their condition along single fibers. Focal recording techniques were used to examine the quantal transmitter release and facilitation properties of synapses located at opposite ends of single muscle fibers in the singly excitatory innervated distal accessory flexor muscle of the lobster, Homarus americanus. Synapses were reliably differentiated with respect to quantal output such that those located near the tendon end were 1.15-4.12X greater than those at the opposite, exoskeletal end ($p < 0.01$, paired t-test). Similar differences were seen in the amount of facilitation determined from twin pulse experiments. The fine structural basis for these differences were determined by serial section electron microscopy of 10 µm segments at each end to ensure that the area of focal recording was sampled. No quantitative differences were found in the terminals or synapses in the two regions. Instead, the physiological diversity is correlated with number and size of presynaptic dense bars. Thus, the tendon end had a greater number and larger mean surface area of dense bars compared to the exoskeletal end. This heterogeneity of excitatory multiterminal innervation is correlated with the axonal branching pattern. Thus, the main axon and the larger primary axon branches lie in close proximity to the tendon end of the muscle fibers whereas the exoskeletal end is innervated by smaller secondary and tertiary axonal branches. This proximity to the large axonal branches of the larger quantal output synapses at the tendon end may be regulated by some neural influence including a timing of innervation and/or access to greater amounts of metabolites in the larger branches which may be conducive to forming larger transmitter synapses.

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832 EXPERIMENTAL ARREST OF CLAW MUSCLE TRANSFORMATION AND ASYMMETRY REVERSAL IN ALPHEID SHRIMP. DeF. Mellon and P.J. Stephens*. Dept. of Biol., Univ. of Virginia, Charlottesville 22903.
In alpheid shrimp the first chelipeds are asymmetrical and consist of a small pincer claw and an enlarged snapper claw on opposite sides of the animal. Reversal of claw asymmetry follows loss of the snapper (Wilson, E.B., Biol. Bull., 4 (1903) 197-210) in which the pincer claw is transformed to a snapper. Transformation also occurs in response to damage to the nerve supply of an otherwise intact snapper (Mellon, DeF., Stephens, P.J., Nature, 272 (1978) 246-248). The transformation process entails profound functional as well as morphological changes in the original limb and requires as many as eight consecutive moult cycles to complete (Stephens, P.J., Mellon, DeF., J. Comp. Physiol. (1979) In Press). The mass of the claw musculature increases by as much as ten-fold and is brought about solely by an increase in the diameter and length of existing muscle fibers. Sarcomere length in fibers of the major closer muscle increases by as much as five-fold, apparently by a process of gradual myofibrillar reorganization. In addition, the properties of synaptic release at the neuromuscular junctions undergo modification during claw transformation. Amplitudes of individual ejp's increase from 1-5 mV in the original pincer to as much as 20 mV in the transformed snapper. Facilitation index during the same period is doubled. Pincer transformation does not occur if its nerve supply is interrupted (Wilson, E.B., op. cit.) and, we now find, can also be arrested by specific experimental procedures that prevent the genesis of maximal contractile tension in the closer muscle of the pincer claw. Tenotomy of the major closer muscle of the pincer in many cases prevented transformation of the pincer following snapper amputation. Tenotomy of the pincer opener muscle, an operation which restricts normal length increases of the closer muscle, also resulted in cases in which pincer transformation was arrested. Finally, banding the pincer claw in the fully closed position following snapper amputation prevented claw transformation in most cases. None of these procedures was effective in initiating transformation when they were performed on snapper claws. These results support the hypothesis that transformation of the pincer closer muscle requires a sustained increase in contractile tension, possibly initiated and maintained directly by changes in the functional properties of its motor neurons. The loss of muscle tension in the snapper claw, however, is apparently not implicated in the triggering of transformation.
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833 SPATIAL DISTRIBUTION OF SYNAPSES ONTO A SINGLE IDENTIFIED MOTONEURON: AN ULTRASTRUCTURAL STUDY. Thea Mendelson* (Spon: E. L. Gasteiger) Section of Neurobiology and Behavior, Langmuir Laboratory, Cornell University, Ithaca, NY 14850.
The fast flexor motoneurons control tail flexion in the escape response of crayfish. The neural circuitry for this response and the electrophysiology of individual neurons in the circuit have been well studied (Zucker, J. Neurophys. 35, 1972). The structure of the motoneurons has been elucidated by means of single-cell staining with cobalt (Mittenthal and Wine, J. Comp. Neur. 177, 1978). A single one of the fast flexor motoneurons in the 1st abdominal ganglion exits anteriorly in isolation from the other motoneurons. Therefore, this preparation is particularly suitable for studying the role of a single cell in the escape response. Since many inputs converge on each motoneuron, an ultrastructural study was undertaken to provide the morphological basis for the electrical activity of this individual neuron.
Horseradish peroxidase was used to backfill the soma, dendritic arborization and proximal axon of the motoneuron. Subsequent treatment with dianisidine produced deposits of electron-dense benzidine reaction product throughout the neuron. Thus the fine dendritic processes could be identified in sections cut through the ganglionic neuropil.
The pattern of dendritic branching and the form of the fine secondary dendrites were compared in whole mounts and in reconstructions from serial sections. The ultrastructure of the electrotonic junctions formed by both medial and lateral giant interneurons on this particular motoneuron was determined with electron microscopy. The ultrastructure of these synapses was compared with that of electrically rectifying synapses between the lateral and motor giant neurons and with that of the septal electrotonic junctions within the lateral giant interneurons.
Chemical synapses were also found on the dendrites of the motoneuron. Their spatial distribution in relation to the electrotonic synapses and to the configuration of individual dendrites is described.

834 MUSCARINIC CHOLINERGIC BINDING SITES IN TERMINAL GANGLION OF THE CRICKET CNS. M.R. Meyer, J.S. Edwards, and R.E. Morrissey*. Dept. Zoology, Univ. Washington, Seattle, WA. 98195.
Cholinergic binding sites have been reported to be present in the insect CNS and, in most cases are thought to be either nicotinic or mixed nicotinic-muscarinic in nature. Recently, muscarinic sites have been detected in homogenates of whole Drosophila heads (J.Neurochem.,1978,32:543). Although electrophysiological studies indicate that acetylcholine may serve as a neurotransmitter at specific synapses in the terminal ganglion of some insects, little is known of the characteristics of the receptor sites in this area of the CNS. We now report evidence for the presence of sites in the terminal ganglion (TG) of the cricket Acheta domesticus that specifically bind the potent muscarinic antagonist 3-quinuclidinyl benzilate (QNB).
Using a modified [^{3}H]-QNB filter-binding assay, homogenates of isolated TG were shown to specifically bind the ligand with high affinity. [^{3}H]-QNB binding was displaced 70-90% by 10^{-6} M scopolamine, while 10^{-5}M scopolamine or prior heat treatment of the tissue reduced binding to near background levels. Incubation of TG in 10^{-5} tubocurarine and 10^{-4}M oxotremorine reduced binding by 40 % and 86% respectively. Binding was shown to increase linearly with increasing concentrations of TG in the assay mixture. When increasing concentrations of labeled ligand were employed in the assays binding was observed to saturate at ca. 6 nM [^{3}H]-QNB. Scatchard analysis of binding curve data yielded an apparent dissociation constant (K_d) of 5.4 x 10^{-9}M. The maximum number of binding sites (B_{max}) was calculated to be ca. 480 fmol mg^{-1} protein (96 fmol mg^{-1}wet weight tissue). Hill plot data gave a Hill coefficient (n_H) of 0.93, indicating essentially non-cooperative binding. The K_d calculated from the Hill plot data was 5.1 x 10^{-9}M.
Although, at this time, cholinergic binding sites in the cricket TG have not been localized by autoradiography, physiological data generally support the notion that cholinergic transmission occurs in certain neuronal pathways in the ganglion neuropile. Our findings demonstrate a rather high density of binding sites in the cricket CNS that fulfill at least some of the prerequisite requirements for a muscarinic receptor candidate (e.g. high affinity, saturability, specificity). Additional studies are now in progress to further characterize the pharmacology, physiology, and localization of these binding sites.
Supported by a grant from the Graduate School Research Fund, Univ. of Washington and NIH NB 07778.

835 A REFLEX PATHWAY FOR EVALUATING CENTRAL NERVOUS REGENERATION IN A SNAIL. Stacia Moffett. Dept. Zool. WSU, Pullman, WA 99164
Anatomical evidence for regeneration of central nervous connections has been reported for the pulmonate snail Melampus bidentatus by Price (Cell Tiss. Res. 180, 529-36, 1977). In the primitively unfused nervous system of this snail, it is easy to sever the cerebral commissure or the cerebro-pleural or cerebro-pedal connectives. Small diameter nerve connections form in a matter of days to weeks and progressively shorten and thicken to an approximation of their normal condition. The appropriateness of the connections that are formed is evaluated by testing reflex responses to tactile stimulation in a small cerebral nerve, labial nerve 2. This nerve was chosen because it has two large efferent units that are easily recognized in extracellular recordings by their relative spike heights and response patterns. These units in the right or left labial 2 nerve receive tactile information from both sides of the posterior and medial foot and mantle regions primarily through the ipsilateral connectives, with connections through the contralateral connectives and the cerebral commissure making a small contribution to the reflex in normal animals. The cerebral commissure is the pathway through which tactile information from one side of the anterior foot and head region elicits activity in the contralateral labial nerve units.
In snails with regenerated connectives, normal responses to tactile stimulation of the posterior foot and mantle region are present after the contralateral connective pathway has been eliminated. Electrical stimulation of the regenerated connectives also elicits the reflex response. In instances in which no obvious regenerated connective is present, the nerve fibers appear to have wandered extensively; no reflex response could be detected in these animals.
Cerebral commissure transections are more readily repaired than connective transections and both electrical stimulation of the regenerated commissure and tactile stimulation of contralateral head regions elicit the reflex response. Preliminary evidence indicates that an auxillary pathway between the cerebral ganglia via the connectives and ring ganglia is formed or enhanced in some snails as a result of cerebral commissure transection. In these snails stimulation of one side of the head elicits a small reflex response in the contralateral nerve after the regenerated commissure has been cut.
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836 FREQUENCY DEPENDENT RESPONSE PROPERTIES OF AN IDENTIFIED AUDITORY INTERNEURON IN THE CRICKET TELEOGRYLLUS OCEANICUS. Andrew Moiseff and Ronald R. Hoy. Section of Neurobiology and Behavior, Cornell University, Ithaca, New York 14853.

The response properties of an identified auditory interneuron (Interneuron-1)[1] were measured over a broad range of stimulus carrier frequencies (3kHz to 100kHz) for male and female T. oceanicus. Interneuron-1 was tonically excited by tones at carrier frequencies of 10-100kHz. The minimum threshold intensity occurred at 25kHz (61.0 dB SPL; n=10, standard deviation= 9.7).

Between stimulus presentations, interneuron-1 is spontaneously active. At frequencies of 3-6kHz this spontaneous activity is suppressed (or inhibited). Suppression was most accurately measured through two-tone suppression experiments. A standard excitatory stimulus was delivered simultaneously with a test tone. The intensity of the test tone (3-6kHz) was varied until the number of spikes recorded in response to this two-tone stimulus was one-half of the number recorded in response to the standard excitatory tone alone. Using this criterion of 50% suppression, it was determined that the peak sensitivity of the "inhibitory response" is at 5kHz (50% criterion met at 62.5 dB SPL; n=11, standard deviation= 5.7).

A single ear was demonstrated to mediate the excitatory and inhibitory responses in the ipsilateral interneuron-1 (n=6), and inhibitory responses alone in the contralateral interneuron-1 (n=3).

We believe that the function of this interneuron is the detection and lateralization of ultrasonic sound signals produced by predators (e.g. insectivorous bats).[2]

1) Casaday, G.B. and Hoy, R.R. (1977) J. comp. Physiol. 121, 1-13.

2) Moiseff, A., Pollack, G.S., and Hoy, R.R. (1978) Proc. Natl. Acad. Sci. USA 75, 4052-4056.

837 INTRACELLULAR pH REGULATION IN CRAYFISH NEURONS STUDIED WITH ION-SENSITIVE MICROELECTRODES. William Moody. University of Bristol, Bristol, BS8 1TD,England.

The intracellular pH (pH_i) of crayfish motoneurons was measured using recessed-tip pH microelectrodes. Experiments were performed on both the motor giant and flexor inhibitor neurons of abdominal ganglia; no differences were noted between the two cells. The resting pH_i of the cells in nominally CO_2-free Ringer (pH 7.4) was 7.05 - 7.2. Since the resting membrane potential was -65 to -80 mV, intracellular H^+ activity is maintained well below its equilibrium value. Upon application of 5% CO_2 - 40 mM HCO_3^- Ringer at constant external pH, pH_i decreased rapidly by 0.2 - 0.4 units. This lower pH_i was not maintained, however, and within 1-2 minutes pH_i began increasing back toward its pre-CO_2 level with an approximately exponential time course. The maximum rate of recovery of pH_i ranged in different cells from.05 - .13 units/min. Since the electrochemical gradients for H^+ and HCO_3^- favor further acidification during prolonged CO_2 application, the recovery of pH_i presumably represents active H^+ efflux and/or HCO_3^- influx. When 5% CO_2 was applied in Na-free (Li or BDAC replacement) Ringer, the rate of recovery of pH_i was reduced by 80-100%. In some experiments, pH_i was lowered by removal of NH_4Cl after a brief exposure. Virtually no recovery from this acid load was seen in Na-free HCO_3^--free Ringer. Relatively rapid recovery of pH_i was stimulated when Na was replaced, even in nominally HCO_3^--free Ringer; external HCO_3^- further increased the rate of pH_i recovery. Experiments are presently in progress to attempt to detect Na entry during pH_i recovery using intracellular Na-sensitive microelectrodes, and to assess the degree of coupling between the movements of various ions involved in pH_i regulation.

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838 ADAPTATION OF THE TOBACCO HORNWORM (MANDUCA SEXTA) CENTRAL NERVOUS SYSTEM TO NICOTINE. Catherine E. Morris * (SPON: D.L. Gilbert). NIH, Bethesda, MD 20205.

Manduca sexta caterpillars, when feeding on tobacco, chronically tolerate haemolymph levels of 2.3 X 10^{-5}M nicotine (Self et al.,1964,J Ins Phys 10:907), a concentration several orders of magnitude greater than that required to alter synaptic function in some insects. This implies either that the Manduca CNS is intrinsically insensitive to nicotine or that a blood-brain barrier system operates against the toxin. In vitro electrophysiological studies (comparing Manduca to Periplaneta americana) established that Manduca CNS is susceptible to nicotine, but, 2 orders of magnitude less sensitive (indicated by drug-evoked discharge monitored in connectives and ganglionic polarisation measurements) than Periplaneta. During 5 min exposures, desheathed Manduca nerve cords have a threshold for response at 5 X 10^{-5}M, whereas that for intact cords is between 1 and 2 X 10^{-3}M nicotine. The cholinergic agonists muscarine, lobelline and ACh are all effective at 10^{-5}M or less. Studies with radioactive nicotine show: a) the blood-brain interface is not efficient in excluding nicotine passively from the CNS, since uptake ratios are the same in Manduca and the cockroach for incubations of 2-30 min b) nicotione is rapidly metabolised by Manduca's CNS to water-soluble compounds that are almost certainly conjugates of the hydrolysis product(s) of nicotine c) nicotine uptake has a saturable component which may reflect an alkaloid transporting system capable of removing nicotine from extracellular spaces and d) nicotine and its CNS metabolites wash out of the Manduca CNS according to 2 exponentials, whereas 3 exponentials are found in Periplaneta, suggesting that a "nicotine space" (viz. a deep extracellular compartment) is missing in Manduca. Conjugate formation from nicotine would require a mixed-function oxidase system plus conjugating enzymes - these are largely associated with smooth ER, an ultrastructural feature abundant in the perineurium and glia of Manduca. Also relevant to a barrier system is the configuration of the perineurium; in Manduca larvae, individual cells of the innermost layer extend over such a large area of ganglion that a very few cells (forming lateral tight junctions) would encapsulate the ganglion. Consequently, intercellular diffusion of nicotine at the blood-brain interface must be severely restricted so that transcellular permeation probably accounts for the observed nicotine entry, allowing nontoxic conjugates to be formed intracellularly before nicotine can diffuse through to the extracellular spaces. The fact that a limited amount of metabolism occurs in the cockroach CNS indicates that the rate of detoxification relative to influx is the critical factor in conferring protection on Manduca. (Study carried out in Dept. Zool., Univ. of Cambridge, U.K.)

839 ACTION OF ETHANOL ON IDENTIFIED NEURONS IN THE CNS OF ACHETA DOMESTICUS. R. E. Morrisey* and J. S. Edwards. Dept. Zool., Univ. Washington, Seattle, WA 98195.

Giant fiber activity elicited by mechanoreceptor input to identified interneurons of the abdominal nerve cord in the house cricket Acheta domesticus was used as an assay for the action of ethanol on electrophysiological function of an insect sensory system.

Giant fiber activity is affected by perfusion with ethanol. Concentrations above 0.20% caused a 2-4 fold increase in number of giant fiber spikes in response to cercal stimulation within 30 seconds of perfusing the body cavity. The site of action was localized by means of vaseline barriers which isolated specified regions of the CNS. Only perfusion of the terminal ganglion caused an increased spike number comparable to that of wholly perfused nervous systems. Extracellular records from the afferent neurons in the cercal sensory nerve during stimulation were unaffected by alcohol treatment. Similarly perfusion of anterior connectives and segmental ganglia did not cause a significant increase of giant fiber responses to cercal stimulation. The principal site of action of ethanol appears to be in the neuropile of the terminal ganglion, and probably at the sites of cercal nerve input to the giant fiber arborizations.

Spatial localization of point sound stimuli as manifested in giant fiber spike number has been quantitated and can be used as an assay for neural function. Perfusions of ethanol at concentrations of up to 2% induces increased spike number but does not affect directional response. At higher perfusion concentrations directional responses are skewed and distorted. The circuitry of this system is not fully elucidated but the results point to an alteration in relative excitation/inhibition of the giant interneurons. This work was supported by a grant from the Alcohol and Drug Abuse Institute, Univ. of Wash.

840 IDENTIFIED NEURONS OF THE STOMATOGASTRIC GANGLION HAVE DIFFERENT, CHARACTERISTIC MEMBRANE TIME CONSTANTS. Brian Mulloney, Kate Skinner, and Donald H. Edwards, Jr.* Zoology, University of California at Davis, Davis, CA 95616.

The gastric system of the stomatogastric ganglion includes ten motor neurons and one interneuron which interact to generate a characteristic motor pattern. These neurons are identifiable in terms of the muscles they innervate, the nerves their axons travel, and the synapses they make within the ganglion (Mulloney and Selverston, 1974). As part of an effort to simulate the pattern-generating mechanism, we have measured the time-constants (τ_m) of the membranes of some of these neurons using Jack and Redman's (1971) brief current-pulse method, and discovered that these neurons differ characteristically in this parameter. The data on hand for these neurons are:

neuron	τ_m (msec)	R_{in} (Mohm)	R_m (Mohm cm^2)
DGN	140	21	.140
AMN	20	2	.020
LGN	---	7	---
MGN	---	10	---
LPGN	120	15	.120
GM	35	7	.035
Interneuron 1	14	--	.014

τ_m lets us calculate the specific membrane resistivity (R_m) of these neurons, since $\tau_m = R_m C_m$ and $C_m = 1\ \mu F/cm^2$. These neurons differ up to ten-fold in their specific membrane resistivities, and therefore in the density of ionic channels.

The input resistances of these neurons were also measured, in most cases in the same experiment with the same microelectrodes, by injecting long pulses of constant current and measuring the change in membrane potential. Many measurements were made using an active bridge circuit, but some were made with two microelectrodes. The values of R_{in} are given in the Table; R_{in} is not a simple function of R_m.

Chemical synaptic transmission onto these neurons should change both τ_m and R_{in}, since it will cause local changes in the membrane's conductance which our methods will integrate into resistance of the entire cell. When we measure τ_m in x5 Mg^{++} saline, its value increases about 50% in cells suspected to receive continuous synaptic input (Graubard, 1978; Mulloney, unpublished). We think the highest measured values of τ_m most closely approximate the true values.

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841 THE DEVELOPMENT OF DISTINCT SYNAPTIC TERMINAL ARBORIZATIONS IN THE CRICKET NERVOUS SYSTEM IS CORRELATED WITH BIRTHDAY AND POSITION IN A RECEPTOR ARRAY. R.K. Murphey. Dept. Biol., SUNY Albany, NY 12222.

An array of sensilla known as clavate hairs are located near the base of the cercus of the cricket (Acheta domesticus). These sensilla are equilibrium receptors which excite a small subset of interneurons which can be recorded in the ventral nerve cord. There are two groups of clavate sensilla, one located ventro-medially and one located dorso-medially on the cercus. Only the ventro-medial group will be discussed here.

The sensilla are arranged in rows running parallel to the long axis of the cercus. In general, a hair is added at the base of each row each time the animal molts. Thus, proximo-distal position within a row correlates roughly with birthday.

A method for staining the sensory neurons associated with individual hairs was then developed. We found that cutting the tip of a hair off and placing a bubble of cobalt over the hair would lead to staining of a single neuron. A similar method has been used by Anderson and Bacon (Dev. Biol. In Press).

First we examined the terminal arborizations of sensory neurons of different ages within a single row. We found that the oldest cells had the most profuse ramifications within the terminal abdominal ganglion, while younger neurons ramified in more restricted areas of neuropile nearer the cercal nerve. We conclude that the position of a hair along the proximo-distal axis has a correlate in position of its synaptic terminal in the ganglion - the most proximal receptors have arborizations which are the most distal (nearest the cercal nerve) in the neuropile.

If birthday were the only variable determining the differentiation of synaptic terminal arborizations then receptors with the same birthday should be virtually identical. In fact within a birthday group, each sensory neuron has a unique terminal synaptic arborization. Since the only variable which appears to distinguish these sensilla is position in the array, we conclude that positional values are assigned to the sensilla and these influence the growth of the terminal arborization.

We conclude that position and birthday of these sensory neurons are correlated with the morphology of the terminal arborization and its position in the ganglion. We intend to delete small numbers of sensilla in order to determine whether there are neighbor-neighbor interactions involved in growth of these terminal arborizations. We will also examine postsynaptic neuron responses in a search for physiological correlates of these distinctive synaptic arborizations.

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842 THE EFFECT OF DOPAMINE, ACETYLCHOLINE AND THE NEUROPEPTIDE FMRFamide ON A MOLLUSCAN MUSCLE. G.T. Nagle, D.A. Price and M.J. Greenberg. Dept. of Biological Sciences, Florida State University, Tallahassee, FL 32306.

The excitatory actions of the neurotransmitters, dopamine (DA) and acetylcholine (ACh) have been compared with those of the molluscan cardioexcitatory neuropeptide, FMRFamide, on the isolated radula protractor of Busycon contrarium. The effects of various blocking agents on the mechanical responses to these three agonists were also tested.

The single phasic contraction of the radula protractor muscle elicited by DA is in sharp contrast to the sustained contractures caused by ACh and FMRFamide. Norepinephrine (NE) and epinephrine (E) produce responses similar to those of DA, but the threshold doses are higher. The muscle can be desensitized to DA by repeated treatment with high concentrations. Such tachyphylactic muscles no longer respond to NE or E, suggesting that the three catecholamines may be acting at a common receptor. The effects of FMRFamide and ACh on the radula protractor are undiminished by DA desensitization.

Ergometrine ($3x10^{-4}M$) blocked the response to DA ($3x10^{-5}M$) and to FMRFamide ($10^{-8}M = ED_{50}$), but not to ACh. In addition, d-tubocurarine (dTc; $4x10^{-4}M$) blocked the response to DA ($3x10^{-5}M$) and ACh ($10^{-6}M$); but higher concentrations also antagonized the actions of low doses ($2\text{-}3x10^{-9}M$) of FMRFamide. As inhibitors of the responses of Aplysia neurons, the ergot alkaloids are relatively specific antagonists of DA (Ascher, J. Physiol. 225:173,1972), but dTc appears to block the ionic channels common to a class of receptor-activated Na^+ and Cl^- responses (Carpenter et al., J.Neurobiol. 8:119,1977).

We conclude that DA, ACh and FMRFamide are all acting at different receptors in the radula protractor muscle. (Supported by NIH grant HL-09283 to M. J. G.)

843 HORIZONTAL EYE MOVEMENTS GENERATED BY PROPRIOCEPTION IN THE CRAYFISH, PROCAMBARUS. Richard F. Olivo and Martha M. Jazak*. Dept. Biol. Sci., Smith College, Northampton MA 01063.

Animals with movable eyes stabilize their eyes by using sensory input from vision (the optokinetic response), gravity/motion detectors (the statocysts of crustaceans), and proprioceptors. In crayfish, it is known that stabilization during horizontal turns of the body (yaw) is driven only by vision and proprioception, and thus crayfish offer a good system in which to characterize the role of proprioception in stabilizing the eyes. The proprioceptors involved are in the legs, but their actual identity is not yet known.

Crayfish (Procambarus clarkii) were prepared for experiments by gluing a plastic post to the carapace and attaching a fine silver wire to the left eyestalk. The antennae were cut short and in most cases the left cheliped was autotomized. A capacitative transducer (Sandeman 1968) was mounted on the carapace post to record movements of the left eye, and the same post was clamped to a vertical rod to suspend the animal in space. To stimulate proprioceptors, a turntable with fine gravel glued to it was placed beneath the legs; to stimulate the optokinetic response, a striped drum was placed on a second, concentric turntable. The turntables were oscillated sinusoidally, either in the same direction (in phase) or in opposite directions (out of phase).

Eye movements in response to visual and proprioceptive stimuli were measured at 6 frequencies between 3.5 and 20 cycles/min (0.06 to 0.33 Hz), for several excursion amplitudes between 10^0 and 34^0. With a stationary platform beneath the legs and with the striped pattern covered (control), stimulation elicited no eye movements; with the stripes covered ("legs only") or with the platform placed beneath the legs ("eyes only"), stimulation elicited eye movements with a mean gain between 0.1 and 0.5. The optokinetic response was stronger at low frequencies and the proprioceptive response was stronger at high frequencies. With both stimuli presented in phase, a larger response was elicited that was approximately the sum of the responses to separate visual and proprioceptive stimulation at each frequency. Thus, the optomotor system appears to sum algebraically the visual and proprioceptive inputs. This conclusion is supported by experiments in which the stimuli were presented out of phase; in such experiments the responses were very small. Similarly, in experiments in which the two stimuli had different frequencies, the eye movements were again approximately an algebraic sum of the visual and proprioceptive stimuli.

844 INSTRUMENTAL CONDITIONING IN CRAYFISH: LEVER PULLING FOR FOOD. Gene C. Olson and Robert Strandberg. Dept. of Psych., UCLA, Los Angeles, CA 90024.

Crayfish are relatively adept at manipulating objects with their claws. It would be interesting to determine if they can learn manipulative tasks. Previous studies of learning in crayfish have concentrated on locomotion tasks, e.g. mazes or shuttle box paradigms. This study demonstrates that crayfish can be trained to pull a lever to obtain a food reward.

For testing the crayfish were loosely restrained in front of the response lever. A food delivery tube was positioned near the subjects mouth area. Each pull of the lever delivered .005 cc of ground brine shrimp through the food tube. The crayfish would often chew on the end of the food tube as they held the tube with one claw and pulled the lever with the other claw. To control for non-specific effects the trained animals were compared to yoked control animals that received the same amount of food in an identical apparatus, but with an inoperative lever (non contingent food) Four matched pairs of crayfish received 9 training sessions. Training sessions lasted 30 minutes and were separated by 24 to 72 hours. Over all 9 sessions the food contingent animals performed an average of 51.1 lever pulls per session, compared to 19.1 pulls per session for the yoked control animals ($p<.02$, two-tailed t test).

After this initial training the groups were reversed: the former control animals now received contingent food and vice versa. In the following 9 sessions the newly trained animals averaged 62.4 pulls per session, the new control animals (formerly food contingent) declined to 19.9 responses per session ($p<.02$, two tailed t test).

It should be noted that the trained animals had no consistent improvement after the second session; individual subjects showed marked variability in daily performance. One possible explanation of this variability may be variance in the level of motivation. Further refinements will be necessary to determine the true performance capabilities of crayfish.

These data provide evidence that crayfish can learn a manipulative task for a food reward. This paradigm may be useful in the study of learning in simple systems.

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845 IN TANDEM PHYSIOLOGICAL AND CHEMICAL STUDIES: USE IN IDENTIFICATION OF NEW HISTAMINERGIC NEURONS IN APLYSIA. Joyce K. Ono and Richard E. McCaman, City of Hope Nat. Med. Ctr., Duarte, CA 91010

We have modified a freeze-substitution technique used for isolating individual neurons for chemical assay to permit chemical measurements of physiologically characterized neurons. In a previous study, identified cholinergic, serotonergic, and histaminergic neurons from Aplysia californica were assayed for their respective transmitters after intracellular recording and staining (Ono & McCaman, Brain Res. 165:156). The histaminergic neurons (RC2 and LC2) proved to be particularly sensitive and adversely affected by the freeze substitution procedure used for their isolation. This freeze-substitution procedure utilized ethylene glycol (Giller & Schwartz, J. Neurophysiol. 34:93) and greatly facilitated isolation of "clean" individual somata. In the present study, substitution of propylene glycol for ethylene glycol in the freeze-substitution procedure permits excellent recovery of histamine (Hm) from C-2 neurons subjected to intracellular recording and staining.

Additional Hm-containing neurons have been discovered in the CNS of Aplysia using this new freeze-substitution procedure. These neurons are generally difficult to distinguish as individuals on the basis of visual inspection. Application of in tandem physiological and chemical techniques has resulted in the unequivocal identification of additional histaminergic neurons. The new histaminergic neurons, designated RC3 and LC3: (a) are located bilaterally on the ventral surface of the cerebral ganglion; (b) contain a concentration of Hm comparable to the previously identified C-2 neurons; (c) make monosynaptic connections with some of the same follower cells of the C-2 neurons; and (d) evoke the same types of postsynaptic responses as does C-2 in these followers.

Use of the propylene glycol medium also markedly improves the recovery of dopamine (DA) from Neutral Red-stained anterior ganglionic roots from the leech, Macrobdella decora. Previous studies with the ethylene glycol medium indicated a significant loss of DA from the rootlets during freeze substitution. It is anticipated that the use of the propylene glycol procedure will now make it possible to isolate and assay the single Neutral Red-stained neuron in these rootlets for DA.

In tandem physiological and chemical studies of individual neurons are essential for characterizing small, visually nondescript neurons and for examining the possible effects of physiological perturbations on endogenous transmitter levels. The use of the propylene glycol freeze-substitution technique promises to greatly facilitate such studies. [Supported by USPHS (NS05343) to J.K.O. and USPHS (NS9339) and NSF (76-06053) to R.E.M.]

846 MOTOR TERMINALS AND MUSCLES IN LIMB BUDS OF THE REGENERATING CUTTER AND CRUSHER CLAWS OF THE ADULT LOBSTER. Natalie G. Pascoe. Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543.

The lobster has dimorphic claws. The cutter claw is adapted for speed; the crusher claw for power. If these claws are lost due to injury, the animal has the ability to regenerate them. The claws regrow in the form of soft buds enclosed in a chitinous capsule. At the next molt, the capsule is shed with the carapace, and a small but functional claw appears. The system has potential for experimentation relevant to neurotrophic influence on muscle development.

Opener and closer muscles of the lobster differentiate early in the development of the bud and then increase in size. Electron microscopy shows that innvervation occurs early in the growth of the buds. Motor terminals appear active: they have dense bodies and well defined synaptic clefts. They are already well equipped with mitochondria and large numbers of vesicles, dense cored as well as clear.

Muscle fibers are at first contiguous. As the bud grows they come to lie further apart with spaces between them. Individual fibers increase in diameter, and there is apparent infolding of the surface leading to fiber splitting. Cross sections of paired buds show that the opener and closer muscles of the cutter are smaller than the corresponding muscles of the crusher. The tendons are similarly unequal. In addition, the closer muscle in each bud has histochemical and ultrastructural characteristics that identify the type of claw from which it originates.

Crustacean muscle can be typed on the basis of histochemical differences in respect to oxidative capacity by NADH-diaphorase, and "fastness" by myofibrillar ATP-ase pH 9.4 (Ogonowski and Lang 1978). The closer of the cutter bud, but not the closer of the crusher bud, contains fibers with high activity for ATPase at pH 9.4. However, very early cutter buds will not stain darkly for ATPase at pH 9.4, which may qualify the closers as slow muscles at that stage. On the basis of the two enzymes surveyed, closer fibers in the crusher bud are slow fibers of high oxidative capacity.

The sarcomeres of the "fast" fibers of the cutter bud are shorter and differ from those in slow areas by having thinner, less dense Z-lines, clear H-bands and lower filament ratios of actin : myosin.

847 THE MORPHOLOGY OF GIANT INTERNEURONS: A STUDY USING COBALT BACKFILLING IN THE COCKROACH, P. AMERICANA. S.P. Pazdziorko* (SPON: R.A. Bernard). Anatomy and Biophysics Departments, Michigan State University, East Lansing, Mich., 48824.

The ventral nerve cord of the cockroach contains seven bilateral pairs of giant fiber interneurons (GFs), which are arranged into dorsal and ventral groups consisting of three and four pairs respectively. Anatomical experiments were done to determine the distance traversed and to examine the branching patterns of the GFs anterior to and including the third thoracic ganglion. Cobalt (introduced in one of the connectives between the fifth and sixth (last) abdominal ganglia) revealed at least seven axons entering the supraesophogeal ganglion (brain). The absence of supporting cell bodies, aside from the ones in the sixth abdominal ganglion, indicates that these axons are the GFs and that they are continuous throughout the length of the ventral nerve cord.

Extensive branching of the GFs was seen in all the ganglia examined. Branching in all three thoracic ganglia was similar, occurring primarily (but not solely) ipsilaterally and in both dorsal and ventral regions of the neuropile. Close examination of branching in this region revealed serially homologous branching patterns (identical branching in the same axon in two or more ganglia). This phenomenon appears to be a characteristic common to most, if not all, of the GFs as well as several other axons that extend through this region. At present, three GFs and two non-GFs have been mapped and found to exhibit this characteristic. Branching of the GFs in the subesophogeal and supraesophogeal are solely ipsilateral. Processes in the subesophogeal extend into both dorsal and ventral regions of the neuropile, whereas in the supraesophogeal ganglion they are confined to the dorsal region.

Since previous work has shown the existence of almost identical large neuronal cell bodies in the three thoracic ganglia, it is hypothesized that the serially homologous branching patterns observed in the GFs may transmit similar information to all three ganglia.

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848 PHYSIOLOGICAL CORRELATES OF PHONOTAXIS IN FLYING CRICKETS. Gerald S. Pollack and Ronald R. Hoy. Section of Neurobiology and Behavior, Cornell University, Ithaca, New York 14853.

Tethered, flying crickets (Teleogryllus oceanicus) perform steering movements in response to electronically synthesized models of calling song played from loudspeakers located to the right or left. They attempt to turn toward song models with carrier frequencies similar to those found in cricket songs, but away from song models in which the carrier frequency approximates that of bat echolocation cries (Moiseff, Pollack and Hoy, Proc. Natl. Acad. Sci. U.S. 75, 4052-4056, 1978). We have studied steering behavior both by visual observations and by recording the electrophysiological activity of relevant nerve roots and muscles.

Steering maneuvers include lateral movements of the antennae, legs and abdomen, rotation of the head, and changes in the pronation of the forewings. The movements are rhythmic and reflect the temporal structure of the song stimulus. Discrete pairs of sound pulses in the song stimulus are followed by discrete steering movements. The temporal structure of the song is evident in the firing patterns of motor neurons and muscles as well. Electrophysiological recordings show that, in some motor neurons, the song pattern is superimposed on the regular flight rhythm.

The auditory receptors which mediate sound-activated steering are located on the front legs and project to the prothoracic ganglion. Motor neurons which accomplish many components of the behavior (e.g. wing adjustments, leg and abdomen movements) are located in the thoracic and abdominal ganglia. Section of both cervical connectives eliminates sound-activated steering, even though it does not disrupt any direct connections that might exist between the auditory and motor centers mentioned above. It is therefore possible that the neurons which provide phasic sound-related information to steering motor neurons originate in the head ganglia. Alternatively, steering motor neurons may be driven in a phasic, sound-related manner by neurons from the prothoracic ganglion, and the effectiveness of this pathway might be modulated by information descending from the head ganglia.

849 FINE STRUCTURAL AND AUTORADIOGRAPHIC STUDIES OF IDENTIFIED NEURONS R3-R14 OF APLYSIA. C.H. Price and D.J. McAdoo, Univ. Texas, Marine Biomedical Inst., Galveston, TX 77550.

We have traced the axons of R3-R14 to their peripheral terminals, using light and electron microscopy and autoradiography. The R3-R14 cell bodies in the parietovisceral ganglion are white, large (100-750 μm dia.), and contain dense core vesicles (1800 A dia.). Their axons can be traced in vivo by their white color and in fixed material by their large size (up to 40 μm dia.), extensive glial infolding, dense core vesicles, and specific labeling by ^{3}H-glycine. The terminals (several hundred/cell) are of two distinct types: (1) varicose, neurosecretory endings on hemolymph spaces; and (2) neuromuscular endings on or near vascular smooth muscle (in the walls of arteries, of the pericardial chamber, and of the gill vein). Terminals are free of glia, packed with dense core and larger clear vesicles, and have a rapid and specific uptake system for ^{3}H-glycine, as previously described for the R3-R14 cell bodies. Large en passant varicosities (up to 100 μm dia.) are formed by R3-R14 axons within and alongside the branchial nerve near the osphradial and branchial ganglia; these appear to be vesicle storage and processing sites.

Previous studies on these cells suggested that they are peptidergic neuroendocrine cells. Recent neurochemical and physiological evidence suggests that they may be glycinergic. Axonally transported ^{3}H-glycine in R3-R14 axons is in the free form and preliminary EM autoradiographic evidence indicates that the ^{3}H-glycine is localized to the dense core vesicles. The present morphological work supports a bifunctional role for R3-R14 since they possess endings in neurohemal release areas for secreting a long range messenger (possibly a peptide hormone) and neuromuscular junctions at which a short range messenger (possibly glycine) acts on vascular muscle. Research supported by DHEW grants NS05856 (CHP) and NS11311 (DJM).

850 RESETTING THE CIRCADIAN CAP RHYTHM IN THE APLYSIA EYE BY LL TO DD TRANSITIONS, II: EVIDENCE FOR TWO TIMING MECHANISMS. Robert G. Prichard* and Marvin E. Lickey. Univ. of Ore., Eugene, OR 97403.

In another abstract we reported that activity in the optic nerve can participate in causing a phase shift (Δφ) in the circadian rhythm of compound action potentials (CAP)in the Aplysia eye. We now report that (i) the timing of this nerve activity is determined by one photic cue while the magnitude of Δφ is determined by another and (ii)the critical photoreceptors for determining the magnitude of Δφ are in the eye itself. Aplysia were first exposed to LD 12:12 and then,beginning at dawn, switched to LL for 18, 21 or 24 h. The eyes and brain were then removed to a recording chamber, the optic nerve of one (detached) eye was cut and the entire preparation put into DD (LL/DD). After 1 to 14 h in DD the optic nerve of the other (attached) eye was also cut. On the following 2 or 3 cycles the CAP rhythm of the attached but not the detached eye showed a Δφ of the predicted size provided that the nerve remained intact for a sufficient time. If the nerve was cut too soon the Δφ did not occur at all; there were no partial Δφ 's. Thus activity in the optic nerve has an all or none effect on the attached eye. The minimum required duration of attachment (Dm) following LL/DD decreased linearly as the duration of LL increased. The duration of LL plus Dm always equaled 28±1 h;i.e.the critical nerve activity always occurred at a fixed interval following the last dawn seen by the intact Aplysia. The nerve activity, therefore, must be timed by a mechanism that is initiated or reset by dawns. Its timing is unaffected by LL/DD. As described in the previous paper, the magnitude of Δφ caused by the nerve activity establishes a 12 h phase lag between LL/DD and the midrise point of the CAP rhythm. This requires a second timing mechanism that, unlike the first, is initiated or reset by LL/DD. It is difficult to resist the hypothesis that these two mechanisms are two separate circadian oscillators, one reset by dawn, perhaps the CAP rhythm itself, and the other reset by LL/DD. In a second experiment we exposed Aplysia to several cycles of LD 12:12 terminated with LL = 18 h. The brain and eyes (one attached, one detached) were then removed to a special recording chamber in which the eyes and brain could be separately illuminated. LL was continued for another 6 h in vitro for either the eyes or the brain but not both. During the in vitro LL optic nerve activity was blocked with isotonic sucrose to prevent transfer of photoreceptor information between the eye and brain. The magnitude of Δφ in the attached eye was determined by the LL/DD applied to the eye and not that applied to the brain. Thus, dominant photoreceptors for the second timing mechanism, and probably the mechanism itself, are in the eye. The question of how the optic efferents contribute remains unanswered.Perhaps they facilitate coupling between two ocular oscillators. NSF 28251, NS 12374.

851 Role of the Pneumostome Motor System in Water Balance and Respiratory Function in the Terrestrial Slug, Limax maximus. David J. Prior, School of Biological Sciences, University of Kentucky, Lexington,KY.40506

A major problem faced by air breathing animals is the threat of dehydration due to evaporative water loss across respiratory surfaces. Many behavioral responses to dehydration result in reduction of the area of exposed respiratory surface. This however leads to a physiological dilemma; responses which reduce water loss likewise reduce oxygen uptake. Therefore such behaviors must be balanced responses to both dehydration and oxygen demand. Using the terrestrial slug, Limax maximus, we have begun a study of the neural control of the behavioral responses to dehydration and oxygen demand. In a hydrated Limax, the pneumostome is continually open. Following a specific level of dehydration(10% loss of body weight) the pneumostome begins to open and close rhythmically, an activity that can reduce both water loss and oxygen uptake. In that the onset of the pneumostome rhythm corresponds with a specific level of hydration the possibility exists that the sensory cue initiating the activity is increased hemolymph osmolality. To test this hypothesis the osmolality of hemolymph from slugs at varying levels of hydration was measured. Dehydration results in an increase in hemolymph osmolality that is essentially linear. Hence this variable could be involved in the onset of the pneumostome rhythm. This hypothesis is being further tested using a brain-pneumostome preparation that allows intracellular recordings to be made from abdominal ganglion neurons whose activity is correlated with the opening phase of the pneumostome rhythm. Their activity patterns are affected by varying the osmolality of the perfusion medium. An attempt is being made to study the responsiveness of these and other neurons to saline osmolalities in the range that corresponds to the initiation of the pneumostome rhythm. Supported by NSF #BNS 74-15217-A01 and an Alfred P. Sloan Fellowship.

852 MORPHOLOGICAL PARAMETERS OF THE SQUID GIANT SYNAPSE: RELATIONSHIP OF ACTIVE ZONE PARTICLES TO IONIC CONDUCTANCE. D. W. Pumplin, R. Llinás and T. S. Reese. Dept. Anat., Univ. of Md. Med. Sch., Dept. Physiol. Biophys., NYU Sch. Med. and Lab. Neuropath. Neuroanat. Sci., NINCDS, NIH, Bethesda, MD 20205.

Previous studies of the giant synapse of the squid Loligo peali (Pumplin and Reese, Neurosci. 3,685) showed this synapse to consist of a number of active zones (AZ) defined by: 1) parallel appositions between membranes of the pre- and postsynaptic giant axons, 2) electron-dense fuzz subjacent to both membranes and within the synaptic cleft, and 3) clusters of vesicles within the presynaptic cytoplasm. Freeze-fracture revealed patches of large, relatively-homogeneous, intramembranous particles in the cytoplasmic leaflet of the presynaptic membrane and in the external leaflet of the postsynaptic membrane. These patches were coextensive with each other and with AZ seen in thin sections. From the freeze-fracture data, the concentration of particles at the presynaptic AZ was $1.5\pm.3 \times 10^3$ (N=17) particles/μ^2. In addition, ultra-thin sections were taken at 50μ intervals through an entire giant synapse. The length of AZ appearing in a section at each interval was plotted vs. the distance along the synapse (total distance=900μ); the curve was relatively smooth, tapering off slowly toward the distal end of the presynaptic axon. The total area of AZ (area under the curve) was $1.3 \times 10^4 \mu^2$. By plotting the distribution of lengths of individual AZ in thin sections, and assuming that these lengths were sections through circular active zones whose diameters had a normal distribution, we estimated the average AZ area as $1.25\pm.4\mu^2$. From these figures, the entire synapse contained 2.0×10^7 presynaptic particles in 1.1×10^4 active zones with 1.8×10^3 particles each. Assuming that each presynaptic particle is, or contains, a voltage-sensitive channel for Ca^{2+}, the conductance of such channels may be calculated from the total synaptic conductance. The resulting estimates are 8.5 Ca^{2+}/spike/particle with a Ca^{2+} conductance of 0.2 picosiemens/particle. These estimates agree with values for the Ca^{2+} conductance of individual channels derived from noise analysis studies on molluscan neurones (Brown, Akaike, and Lee, Ann. N.Y. Acad. Sci.,1978). In addition, the number of postsynaptic particles is about equal to the number of presynaptic particles. From the total postsynaptic current, the calculated conductance is 1-10 picosiemens/particle, again in agreement with results of noise analysis on Na^+ channels. The agreement between these sets of data suggests that equating presynaptic active-zone particles with Ca^{2+} channels, and postsynaptic particles with channels carrying postsynaptic current, is quantitatively reasonable.

853 IDENTIFIED CELLS R2 AND P1 CONTROL MUCUS RELEASE IN *APLYSIA:* A MODEL SYSTEM FOR STUDYING THE DEVELOPMENT OF BEHAVIOR ON THE CELLULAR LEVEL. Stephen Rayport and Eric R. Kandel. Division of Neurobiology & Behavior, Depts. Physiology & Psychiatry, Columbia University, P & S, New York, N.Y. 10032

The cholinergic cells R2 and P1 are among the largest and the best studied identified cells in *Aplysia*. Despite this, their behavioral function has not been known. The cells send axons into all the pedal and parapodial nerves and receive sensory input from all surfaces of the body (Hughes, J Exp Biol 46:169, 1967). Although each cell sends axons bilaterally, ipsilateral input is more effective in stimulating each cell. The cells are output cells since they make no known central chemical synapses, and *in vivo* recording in the adult shows that all action potentials flow towards the periphery (Cobbs & Pinsker, J Neurobiol 9:121, 1978).

With this background, we considered two possible behavioral roles involving the body wall: control of motor output and control of mucus release. We ruled out a motor role by stimulation of R2 alone or in conjunction with a foot or parapodial motor neuron and saw no interaction of any sort. By contrast, tests for mucus release were positive. We severed the cerebral commisure so that each cell was only connected to its ipsilateral axons and compared one side of the animal against the other over time. We modified CM Lent's carmine red assay (Science 179:693, 1973) for use on *Aplysia* and saw a significant difference between stimulated and control sides with as few as a few hundred spikes in the stimulated cell.

In the adult, graded mucus release is primarily a defensive behavior that may serve a specific role in the hierarchy of defensive responses intermediate between withdrawal, mediated by low threshold, spontaneously active motor neurons which are not coupled electrically; and inking controlled by high threshold motor cells that are well coupled electrically, and silent except when they fire all-or-none bursts. R2 and P1 are typically silent, but readily activated by synaptic input, and functionally uncoupled electrically in the adult.

Recording from R2 is feasible at least from the time of metamorphosis and perhaps earlier. One can therefore use this cell and the afferent input to it to examine on the cellular level how a behavior and its capabilities for being modified (Kandel & Tauc, J Physiol 181:1, 1965) develop.

854 NEURONAL PATHWAYS INVOLVED IN TRANSFER OF INFORMATION RELATED TO LEG POSITION LEARNING IN THE COCKROACH, P. AMERICANA . Roger L. Reep and E.M. Eisenstein, Zoology and Biophysics Departments and Neuroscience Program, Michigan State University E. Lansing, Mi. 48824

Behavioral experiments have shown that transfer of information related to leg position learning occurs via the interganglionic connectives. Anatomical and electrophysiological experiments were done to identify specific pathways by which information transfers from the prothoracic to the mesothoracic ganglion. Phase and electron microscopy were used to map the projections of sensory fibers which carry shock information from the leg to the CNS. These fibers were found to terminate ipsilaterally and ventrally in the neuropile of the ganglion with which the leg was associated. Cobalt staining of leg motorneurons showed that their CNS branches are wholly contained within the ipsilateral portion of the ganglion from which their axon exits peripherally. Since neither sensory nor motor neurons have branches that project interganglionically, all transfer must be via interneurons in the connectives. Groups of interneurons were stained and found to have ipsilateral and contralateral branches in both the prothoracic and mesothoracic ganglion.

Suction electrode recordings of connective interneurons showed that some large fibers carry corollary discharges of leg motorneuron activity. There is a one-to-one copy of spikes in corollary units of flexor motorneurons and an approximate copy of spikes in corollary units of extensor motorneurons. Corollary units were found in ipsilateral and contralateral descending connective fibers for prothoracic flexor and extensor motorneurons and in ipsilateral and contralateral ascending connective fibers for mesothoracic flexor and extensor motorneurons.

We hypothesize that sensory information related to prothoracic leg shock and motor information related to prothoracic leg position are independently transferred (by interneurons) down each prothoracic-mesothoracic connective to the mesothoracic ganglion, where they are associated and lead to the observed behavior change in mesothoracic legs.

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855 FEEDING MODULATION IN LIMAX: BEHAVIOR AND PHYSIOLOGY. Stephen C. Reingold & Alan Gelperin, Dept. Biology, Princeton University, Princeton, N. J. 08544.

A central neuronal pattern generator underlies movements of buccal musculature used in feeding by the terrestrial mollusk Limax maximus. The activity of the pattern generator is independent of sensory feedback. However, feeding activity in intact animals and feeding motor program (FMP) in isolated nervous system preparations can be modulated by changes in food-related sensory stimuli. We examined the effects of load on buccal muscles, concentration of food substances, and gut feedback, on feeding in intact animals and FMP in isolated preparations.

To monitor feeding movements in intact animals, pellets of prepared food in an agar matrix were attached to the arm of a movement transducer. Sequences of individual "bites" were recorded and data analysed in terms of instantaneous bite frequency throughout a meal, and meal duration. By varying the amount of agar and amount of food extract in a pellet, hardness and chemostimulative quality of food were varied, and the effects on feeding determined.

Isolated preparations consisted of buccal and cerebral ganglia with attached chemoreceptive lips. Delivery of food extract to lips triggered bouts of FMP, recorded by extracellular electrodes. Innervated buccal muscles or esophagous and crop were included in some preparations. Effects on FMP of load on buccal muscles, concentration of food extract, or degree of esophagous/crop inflation were examined.

LOAD. Increasing hardness of a food pellet increased load on feeding apparatus in intact animals. Individual animals fed on a hard pellet (15% agar base) showed a lower instantaneous bite frequency than when fed on a soft pellet (3% agar base). Weights attached to buccal musculature in isolated preparations provided analogous load increase during FMP. Instantaneous FMP frequency was lower with muscles loaded than unloaded.

FOOD CONCENTRATION. In intact animals, increases in concentration of attractive food substance in a food pellet increased both number of animals which fed and duration of a meal once feeding had begun. Instantaneous bite frequency was unchanged. In isolated preparations, duration of FMP was similarly increased in response to more concentrated food substances, but instantaneous FMP frequency was constant.

GUT FEEDBACK. Intact slugs use satiety signals from gut distention to terminate feeding activity: an animal will eat the same amount of food (weight) on successive days, independent of the type of food consumed. In isolated preparations with esophagous and crop attached, inflation of the crop with saline injections served to terminate FMP as well as to reduce peak FMP frequency. Support:NSF BNS76-18792;NIH 5T01MH-13445;NIH 5f32NS05188.

856 EFFECT OF PAIRED STIMULATION OF GIANT INTERNEURONS IN THE COCKROACH PERIPLANETA AMERICANA. Roy E. Ritzmann Dept. Biol., CWRU, Cleveland, OH 44106

Recent evidence indicates that the 14 giant interneurons (GIs) of the cockroach Periplaneta americana can excite motor neurons which direct movements of the metathoracic (T3) legs (Ritzmann and Camhi, J. comp. Physiol. 125:305-316, 1978). Each of the GIs is excited by a specific set of wind directions (Westin et al., J. comp. Physiol. 121:307-324, 1977). The specificity of the wind input and the motor output of each GI suggests that they may play an important role in directing the wind mediated escape behavior (Camhi and Tom, J. comp. Physiol. 128:193-201, 1978). However, a wind stimulus from any direction will excite 8-12 GIs simultaneously. Therefore, in order to understand how the GIs function in response to natural stimuli, we must know how the output of these cells combine when more than one GI is excited.

With this in mind, I have investigated the motor response to intracellular stimulation of pairs of GIs. The GIs were impaled with microelectrodes filled with 4% Procion yellow as in previous experiments (Ritzmann and Camhi, 1978). They could then be stimulated with high frequency trains of current pulses while motor activity was monitored with extracellular electrodes on nerve branches 5r1 (containing primarily depressor axons) and 6Br4 (containing primarily levator axons) in one of the metathoracic legs. Subsequent to the recording session Procion yellow was iontophoresed into the GIs for histological identification. A paired T-test was used to compare the motor response to stimulation of each GI individually and the response to stimulation of both GIs together.

When two dorsal GIs were stimulated, the motor activity was summed. For example, GI-5 excites primarily depressor motor neurons, while GI-7 excites both levators and depressors. When GIs 5 and 7 were both stimulated the resulting depressor activity was greater than that from either GI alone. Moreover, when the GIs were paired, less activity in either GI was required to elicit a motor response than when they were stimulated individually. A similar situation occurred when two ventral GIs were stimulated. GI-2 enchanced the output of GI-1 when these cells were paired. However, when ventral and dorsal GIs were paired, the result was not as expected. GI-1 and GI-5 both excite primarily depressor motor neurons. Nevertheless, in the paired situation the depressor output was less than that from GI-5 alone. The ventral GI appeared to inhibit the output of the dorsal GI. This suggests that the dorsal and ventral GIs are not designed to operate together. Rather, they may represent two distinctly separate pathways.

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858 EFFERENT NEURONS TO THE RETINA OF OCTOPUS: ANATOMY AND PHYSIOLOGY. William M. Saidel* (SPON: T.H. Bullock). Dept. of Neurosci., Sch. Med., UCSD, La Jolla, CA 92093

The eye of Octopus bimaculoides is connected to the ipsilateral optic lobe by about 150 small retinal nerves. Each nerve contains both centripetal and centrifugal fibers. Using the proximal stump of a cut nerve as a wick for cobalt staining, the relationship within the optic lobe between photoreceptor axon terminations and optic lobe cells stained through a given nerve has been determined and confirmed by HRP eye injections. Photoreceptor terminations from a single nerve are found predominantly in an area of the optic lobe plexiform layer restricted along the anteroposterior axis. Most efferent cells whose axons travel in the same nerve are found within the inner granule layer directly below the area of photoreceptor terminals. A few other cells are found deeper within medullary cell islands. Efferent cells may be uni-, bi- or multipolar. A striking characteristic of many cells is the axon collateral projecting into the same plexiform level at which the type II photoreceptors end.

Physiological activity of these cells in restrained animals has been recorded with suction electrodes from intact and cut retinal nerves. Activity from these cells can be distinguished from that of photoreceptors by their shorter (1.5 vs. 5 ms) spike duration. Efferent cells show responses to visual, tactile or statocyst stimulation. Steps of increasing light cause a delayed, transient spike frequency increase followed by a steady state whose frequency depends on the amplitude of the step. Reverse steps cause a frequency decrease whose size is proportional to the amplitude of the step and also prior activity. Hysteresis occurs, depending upon prior retinal adaptation.

A water stream or stroking the mantle or web produce two kinds of responses: a transient burst or suppression of background activity. Suppression also spontaneously followed a mantle movement resembling swimming or escape movements seen in the wild. Bursts of spikes were induced by vibrating the experimental chamber and by angular rotation of the preparation presumably via disturbance of the statocysts.

Golgi material indicates that these cells terminate at or near photoreceptor inner segments. The anatomy and physiology of these cells suggest a local feedback loop from a portion of the retina to a restricted portion of the optic lobe and back to the retina.

(This work was supported by NSF and NIH grants to T.H. Bullock)

857 PURIFIED BAG CELL PEPTIDE MIMICKS SOME BUT NOT ALL RESPONSES OF CENTRAL NEURONS TO BAG CELL STIMULATION IN APLYSIA. B.S. Rothman, P. Brownell* and E. Mayeri, Department of Physiology, UCSF, San Francisco, CA 94143.

An electrically stimulated discharge of bag cells produces a variety of distinct effects, each lasting several hours, in other neurons of the isolated abdominal ganglion. Certain left upper quadrant (LUQ) cells (L2,3,4,6) are inhibited; left lower quadrant (LLQ) cells of the LB and LC clusters show a marked increase in tonic activity; and in cell R15, bursting pacemaker activity is augmented (Mayeri et al., 1979). Egg laying hormone (ELH), a peptide isolated from bag cells, is the putative mediator of the effect on R15, since application of ELH to the cell's soma mimicks the effect of bag cell discharge (Branton et al., 1978).

To further investigate the mediation of bag cell effects, we isolated a basic peptide from bag cells by extraction in formic acid, fractionation on G-50 Sephadex and elution from CM-25 Sephadex, a cation exchange resin. The peptide has an apparent molecular weight of 4200 on SDS-polyacrylamide gels and is 84% pure based on an amino acid analysis. In preliminary experiments (N= 2, F.E. Dudek, personal communication) at 0.1 μM it released eggs from isolated fragments of Aplysia ovotestis.

In desheathed ganglia, somatic application of the peptide not only augments burst activity in R15, but also increases tonic activity in LLQ cells. The threshold for both responses is about 1 μM, with increasing intensities of response at higher concentrations. At the highest concentration used (300 μM) no inhibition of LUQ cells occurred, even though at this dose the intensity of the R15 response was greater than normally seen after bag cell discharge. Injection of the peptide (0.1 to 300 μM) into the caudal artery of the ganglion, with sheath intact, produced almost identical results as those obtained by somatic application. The lack of LUQ response is not explained by inaccessibility of perfusates to critical target sites since 1) arterial application of peptide did produce responses in R15 and LLQ cells, and 2) arterial application of acetylcholine (100 μM) produced its expected inhibitory effect on LUQ cells.

These data suggest that ELH mediates two distinct excitatory responses in target neurons, although at present we cannot rule out the possibility of more than one active factor in our purified material. These data also suggest that the peptide(s) causing the excitatory responses does not directly produce the LUQ inhibition. Supported by NIH postdoctoral fellowships: NSO 7067 and NSO 5931 and NSF grant BNS 76-20978.

859 OPTICAL SPIKES FROM A SALIVARY GLAND.

B.M. Salzberg and D.M. Senseman*. Dept. of Physiology and Pharmacology, School of Dental Medicine, University of Pennsylvania and Monell Chemical Senses Center, Philadelphia, PA 19104.

The mechanism(s) underlying excitation-secretion coupling in gland cells is not presently understood. The small size of many of these cells has limited the utility of conventional intracellular microelectrode recording. Since optical measurement of membrane potential has proven possible in giant axons, invertebrate central neurons, and vertebrate skeletal and cardiac muscle, as well as a number of other preparations, we hoped that these techniques could be used to study electrical phenomena in glandular tissue. In an effort to establish the feasibility of this approach for endocrine as well as exocrine glands, we attempted first to use optical probes to follow electrical activity in an exocrine gland which is also amenable to microelectrode methods. The salivary gland of the snail Helisoma trivolvis has been shown to produce overshooting action potentials and, in the experiments to be reported here, the Merocyanine-oxazolone dye NK 2367 was used to record extrinsic voltage dependent absorption changes from extended regions of this gland while electrical activity in a single ascinar cell was monitored simultaneously with a microelectrode. Optical signals easily visible in a single oscilloscope sweep could be obtained whether the gland was superfused or perfused through the lumen with a Ringer's solution containing the dye. The wavelength dependence of the optical signal differed significantly from that exhibited by this dye when applied to voltage clamped squid giant axons or barnacle supraesophageal ganglion cells, but was similar to that found with a close Merocyanine-rhodanine analogue (Dye XVII) in cardiac muscle. The largest signal:noise ratio was measured at 676 nm, where there was an increase in absorption by the stained gland. Decreases in absorption were recorded at 540 nm and 720 nm. In squid giant axons, a depolarization resulted in a decrease in absorption at all wavelengths measured.

We attributed some observed variability in the amplitude of the optical signal to differential invasion of the gland, and, in order to study this phenomenon further, electrical activity in the salivary gland was monitored optically for 50 seconds at a time while intracellular microelectrode recordings were made from the primary neuroeffector cells in the buccal ganglion. Compared with its bilateral homologue, activity in the contralateral effector neuron seemed to evoke electrical changes in the gland which were accompanied by smaller optical signals.

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860 TRIMETHADIONE HAS DIFFERENTIAL EFFECTS ON NORMAL AND PROLONGED ACTION POTENTIALS IN NEURON R2 OF APLYSIA. O. Sartor*, W.M. King* and N.R. Kreisman (SPON: A. Epstein). Dept. of Physiology, Tulane U. Sch. Med., New Orleans, LA 70112.

Effects of trimethadione (TMO), the prototypical drug for treatment of petit mal epilepsy, were studied on somatic action potentials in neuron R2 of the abdominal ganglion of Aplysia californica. Intracellular stimulation and recording was accomplished by inserting two 3M KCl filled glass micropipettes into R2.

In artificial seawater (ASW), TMO (10 mM) increased the action potential repolarization time (RT) (Horn and Miller, J. Neurobiol. 8:399-415, 1977) by 37-60% in four experiments. This prolongation of RT occurred without any change in action potential amplitude. In 0 Ca^{++} ASW, 10 mM TMO produced a maximum increase in RT of only 10%.

The addition of 50 mM tetraethylammonium (TEA) bromide or chloride to ASW resulted in prolonged action potentials with RT's of usually 50-200 msec in different experiments compared to RT's of 1.6-2.6 msec in ASW. The RT of action potentials in all experiments was noticeably dependent upon the stimulus rate and the resting membrane potential value. TMO (10 mM) caused a 33-41% reduction in the RT of action potentials in ASW + TEA.

In addition to effects on action potential RT, 10 mM TMO also increased threshold for spike generation by 250-300% in ASW. Threshold was determined by measuring the magnitude of a 100-1000 msec constant currect pulse just sufficient to elicit a soma spike. All effects of TMO were reversible upon flushing with 20 times the chamber volume.

The effects of TMO on spikes elicited in ASW can be interpreted as an increase in gCa^{++} (see Klein and Kandel, PNAS 75:3512-3516, 1978). Our experiments in 0 Ca^{++} ASW tend to support this suggestion. The effect of TMO on the TEA spikes, however, would conventionally be taken to indicate that TMO decreases gCa^{++}. Possible explanations for this apparent discrepancy are that TMO enhances the Ca^{++} dependent increase in K^+ conductance (Meech, Comp. Biochem. Physiol. 42A:492-499, 1972) and/or enhances the Ca^{++} dependent inactivation of gCa^{++} (Tillotson, PNAS 76:1497-1500, 1979).

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861 PRESYNAPTIC INHIBITION AND THE CONTROL OF TRANSMITTER RELEASE BY PRESYNAPTIC MEMBRANE POTENTIAL: IONIC MECHANISMS FOR SYNAPTIC MODULATION IN APLYSIA. E. Shapiro*, V. Castellucci, and E.R. Kandel. Div. of Neurobiol. & Behav., Depts. Physiol. & Psychiat. Columbia University, P&S, New York, N.Y. 10032

We have examined the relationships between transmitter release and specific ionic currents of the presynaptic cholinergic double action neuron L10 by simultaneously voltage clamping the presynaptic cell body and assaying transmitter release with intracellular recording from the postsynaptic cell under various pharmacological conditions.

When, in the presence of TTX, cell L10 is voltage-clamped from a holding potential of -60mV graded transmitter release can be evoked by graded depolarizing command pulses in the range of membrane voltage (-35mV to +10mV) in which the Ca^{++} current is also increasing. Depolarizing the holding potential of L10 results in increased transmitter output (Shimahara & Peretz, 1978; Nicholls & Wallace, 1978). Two ionic mechanisms contribute to this form of plasticity. First, depolarization inactivates some K^+ channels so that command pulses recruit a smaller K^+ current. In unclamped cells the decreased K^+ conductance causes spike-broadening and increased influx of Ca^{++} during each spike (see Klein and Kandel, 1978). Second, small depolarization around resting potential (-55mV to -35mV) activates a steady-state Ca^{++} current which also may contribute to the modulation of transmitter release, since even with most presynaptic K^+ currents blocked (using Ba^{++} substitution for Ca^{++}, TEA^+, and 4-AP) varying holding potential still affects transmitter release (see also Nicholls & Wallace, 1978). In contrast, under these conditions, the transient inward Ca^{++} current evoked by depolarizing clamp steps is relatively unchanged from various holding potentials.

The output of cell L10 undergoes presynaptic inhibition in response to connective stimulation. Since depolarization (postulated for primary afferent fibers in vertebrates) cannot be responsible for this effect (see also Mudge and Fishbach, 1978), we examined changes in presynaptic currents during presynaptic inhibition in L10. The inhibition could still be obtained with pharmacological blockage of K^+ currents. Reduction in transmitter release is correlated with decreases in transient and steady-state presynaptic Ca^{++} current.

Our results show that these two forms of synaptic plasticity are due to modulation of the Ca^{++} current which contributes to transmitter release. This current can be controlled in at least 3 ways: 1) By membrane potential control of available K^+ channels which can broaden or narrow spikes and indirectly modulate Ca^{++} inflow. 2) By direct steady-state activation of Ca^{++} channels at more depolarized membrane potentials. And, 3) by transmitter-mediated decrease of Ca^{++} current.

862 ORDERLY SEQUENCE OF POLYSYNAPTIC SENSORY INPUTS TO CRAYFISH TAILFLIP MOTONEURONS. Dyane N. Sherwood and Jeffrey J. Wine. Dept. Psych., Stanford U., Stanford, CA. 94305

The only tailflip command neurons that are excited by sensory stimulation in the isolated crayfish abdomen are the lateral giants (LGs) (Wine & Krasne, 1972, J. exp. Biol.56:1). Following an LG command, all of the efferents to the fast flexor muscles receive synaptic inputs: the motor giants (MoGs) are directly excited by the LGs and inhibited via polysynaptic pathways (Furshpan & Potter, 1959, J. Physiol. 145:289); the nongiant motoneurons (FFs) are excited by electrically-mediated PSPs (Zucker, 1972, J. Neurophysiol. 35,638); and the peripheral inhibitors (FIs) are excited with a delay (Wine & Mistick, 1977, J. Neurophysiol. 40:904).

It had been thought that sensory stimulation plays only a triggering role, with motor organization left to the central command. However, we have now demonstrated a set of polysynaptic pathways that are excited by nearfield stimuli and excite or inhibit all three classes of flexor efferents. A notable feature of the sensory pathways is that the order in which their effects are delivered to the flexor efferents matches the order of firing imposed by the LG command cells. Specifically, the effects of these pathways are (1) inhibition of MoGs, (2) subthreshold excitation of FFs via chemical synapses, and (3) sub- and suprathreshold excitation of FIs. Sensory-derived PSPs can summate in the efferents with giant-evoked PSPs. Further, sensory stimuli can facilitate indirect pathways from the LGs to the flexor efferents.

Sensory inputs to each class of cell must arrive via at least partially independent pathways, in order to explain the selective timing. Further details of the pathways were obtained by simultaneous recordings from FIs and motoneurons in different ganglia. PSPs in different FIs are always correlated 1:1, whereas PSPs in different MoGs often are not. We interpret this to mean that FIs receive direct inputs from interganglionic interneurons, whereas local (segmental) interneurons may be in the pathways to the MoGs.

For giant-mediated tailflips, the decision to move is made at the command cell level, and its outputs dictate the appropriate sequence of activity in the flexor efferents; whether the additional sensory-motoneuron pathways play a role beyond reinforcing the giant commands is not yet known. However, many tailflips are mediated by so-called nongiant pathways (Wine & Krasne, 1972). For these behaviors, the locus of decision may shift to the motoneurons, and the sensory pathways may then play an important role in insuring the orderly sequence of efferent activity.

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863 NON-SPIKING INTERNEURONS AND CONTROL OF POSTURE IN LOCUSTS. Melody V.S. Siegler* (SPON: P. D. Evans) Dept. Zoology, University of Cambridge, Cambridge, CB2 3EJ, U. K.

The metathoracic ganglion of the locust Schistocerca americana gregaria (Dirsh) contains local non-spiking neurons which can effect graded changes in the membrane potential of identified motor neurons of the hind legs (1,2,3). This study examines the relationship between imposed changes in the posture of a hind leg and tonic changes in the effectiveness of non-spiking neurons in producing changes in the membrane potential of motor neurons.

Simultaneous intracellular recordings were made from non-spiking neurons and motor neurons (2). The coxa and femur of the hind leg ipsilateral to the penetrated motor neuron were fixed. Imposed movements of the tibia set the femur-tibial angle (FTA) at either 0^o (flexed), 90^o, or 140^o (extended). The following results were obtained when the set position of the tibia was altered at 15-30s intervals. (a) In some non-spiking neurons that affect tarsal motor neurons, the tonic change in membrane potential was 8-10 mV, but in others that affect tibial motor neurons, the change was only 1-2 mV. (b) Different interneurons were maximally sensitive to different ranges of tibial movement; e.g., some showed the greatest tonic changes when the FTA was reset between 90^o and 140^o, others when the FTA was reset between 90^o and 0^o. (c) Most interneurons showed hysteresis in their tonic response to changes in FTA. The tonic membrane potential at 90^o was different when this position was approached from 0^o than if approached from 140^o. (d) Tonic changes in membrane potential could be of opposite polarity to phasic changes which occurred as the tibia was moved. (e) The effectiveness of non-spiking neurons in producing changes in the membrane potential of motor neurons was dependent upon the position of the tibia; the curve relating presynaptic current to postsynaptic change in voltage was shifted by changing the FTA. This change appeared in part to be due to a tonic change in the membrane potential of the non-spiking neuron, and could not be explained by tonic changes in the membrane potential of the motor neuron.

It is hypothesized that tonic changes in the membrane potential of non-spiking neurons are important in maintaining posture. In addition, the effectiveness of other synaptic inputs to an interneuron could be modified or "gated" by changes in the posture of the animal.

1)Burrows&Siegler, Nature, 262,p222,1976;2)--J.Physiol.,285,p231, 1978;3)Siegler&Burrows, J.comp.Neurol.,183,p121,1979.

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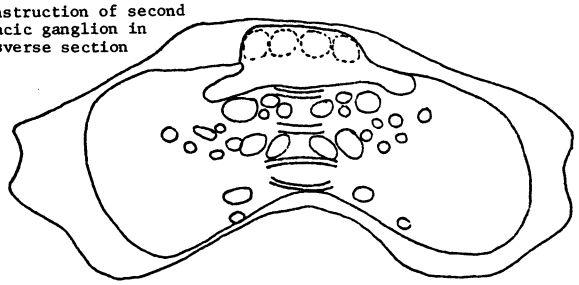
864 THE ANATOMICAL ORGANIZATION OF CRAYFISH SEGMENTAL GANGLIA. Kate Skinner. Dept. Zool., UCD, Davis, CA 95616.

The structural organization of crustacean ventral cord has been investigated using cobalt backfills and serial sections, silver-stained by the method of Rowell, of Procambarus clarkii thoracic and abdominal ganglia. The basic pattern of longitudinal tracts, cross-ganglion commissures and vertical tracts is being mapped. The major groups of cell bodies and the central projections of the peripheral nerves will be localized in terms of these landmark pathways.

The segmental ganglia are built on a sandwich plan of alternating longitudinal and commissural bundles of axons which form tracts through the ganglia. The most dorsal longitudinal tract contains the giant fibers and has the largest cross-sectional area. The most ventral longitudinal tract layer is the smallest in cross-sectional area. There are seven major commissures which cross the midline of the ganglion in four broad bands or layers. Between commissural layers three and four, in the ventral part of the ganglion, lies a dumbbell-shaped mass of very finely textured neuropil which may be homologous with the "ventral association center" of insect segmental ganglia (Pipa et al., 1959; Gregory, 1974; Tyrer, pers. com.). It is not yet known whether the layers carry comparable numbers of fibers or whether the neuropilar masses can be assigned behavioral or physiological functions.

Reconstruction of second thoracic ganglion in transverse section



The histological maps will make it possible to localize identified neurons more precisely within the ganglion (Stretton and Kravitz, 1973), to quantify the constancy and variability of individual neurons relative to stable "addresses" within the neuropil, to test ideas about the functional layering of invertebrate ganglia, and to propose homologies between related taxa.

This work was supported by NSF grant BNS 78-10516 and US PHS grant NS 12295.

865 THE EQUILIBRIUM SYSTEM OF A BURROWING COCKROACH: AFFERENT TO INTERNEURON CONNECTIVITY. Randall R. Stewart and H. Bernard Hartman. Dept. of Biol. Sci. Texas Tech Univ., Lubbock, TX. 79409.

In the equilibrium system of the cockroach Arenivaga, each of four interneurons are excited primarily by one of four rows of tricholiths. Tricholiths are spheroid-shaped sensilla, each about 15 μ in diameter, located in rows on the ventrobasal surface of the cerci. Two rows, a medial and a lateral, are found on each cercus. Extracellular recordings made from the connectives show that the interneurons signal the animal's spatial position. The larger interneuron, termed Positional Interneuron Ipsilateral or PII, receives its input from tricholiths on the medial row of the ipsilateral cercus; the smaller interneuron, called Postional interneuron Contralateral or PIC, is driven by tricholiths in the lateral row of the contralateral cercus.

Does a singular association exist between one row of tricholiths and one interneuron, or do the sensilla of a row contribute input whether excitatory or inhibitory to the other three interneurons? Three types of experiment were performed to answer this question. In the first, termed a cercal reflection experiment, recordings were made from both connectives of the ventral nerve cord during controlled changes in the animal's spatial position while the left cercus was moved anteriorly and posteriorly to alter the output of the PII's and PIC's driven by that cercus. Would changes in the output from the left cercus alter the response pattern of interneurons driven by the right cercus? The positional interneuron responses were graphed as a polar plot. No significant changes in the control cercus polar plot as a result of manipulation of the opposite cercus were observed indicating that a singular association does exist. In the second experiment, single rows of tricholiths were removed, and the effect of the removal on the responses of the remaining three position interneurons determined. Upon removal of either a medial or lateral row, the position interneuron response driven by the remaining row on that cercus increased. Thus, intracercal inhibition is suggested. No changes in output of interneurons driven by the opposite cercus could be detected. In the third experiment, with the animal tilted to a particular orientation, two or three tricholiths in a row were moved with a piezoelectric driven probe, and the output of all four interneurons observed. Medial row tricholiths on one cercus evoked consistent activity in the PIC driven by the other cercus suggesting intercercal interaction. No evidence could be found for intracercal inhibition with this method. Because of the conflicting results, improved manipulative methods are being employed to resolve the question posed.

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866 A CINEMATOGRAPHIC ANALYSIS OF FEEDING IN THE OPISTHOBRANCH MOLLUSC, NAVANAX. A.J. Susswein, M.S. Cappell, D.C. Spray and M.V.L. Bennett. Dept. Neurosci., Albert Einstein College of Medicine, Bronx, NY 10461

Motoneurons to feeding musculature in Navanax have been extensively characterized; however, the firing pattern of these motoneurons during feeding has not been studied. To understand the neural generation of feeding behavior, it is first necessary to describe the behavior. To this end, we have filmed feeding in Navanax. We have addressed ourselves to three questions: 1) What is the sequence of events in feeding? 2) Is the sequence a fixed action pattern, or can components of feeding occur without the entire sequence? 3) Is the sequence sufficiently unaffected by dissection that a feeding preparation is suitable for electrophysiological study?

Feeding begins with slow protraction of the pharynx, followed by expansion of the anterior pharynx and flaring of the lips around the prey. Removing prey during protraction or flaring stops feeding. Prey engulfment begins with a fast, lunging protraction, followed by lip closure that seals prey in the anterior pharynx. Engulfment continued with pharyngeal expansion and retraction. Removal of prey during sealing alters this sequence; retraction occurs without expansion. Expansion produces negative pressure, pulling prey into the pharynx. Holding the prey in place at the anterior pharynx does not eliminate posterior expansion; however, a second expansion superimposed upon the first may occur. Expansion is followed by contraction of circumferential musculature. There are both inward- and outward-directed waves of circumferential contraction around prey, as well as more localized movements. Motoneurons producing movements reminiscent of those observed in specific phases of feeding have been identified.

Essentially identical sequences of movement have been observed in animals in which progressively larger segments of body wall were removed for better visualization of the pharynx and CNS. Successful prey engulfment occurs even in radically dissected preparations. This insensitivity to operative procedures allows for recording from muscles and nerves in normally feeding preparations, which should confirm the functional role of specific motoneurons.

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DCS is a McKnight Scholar in Neuroscience.

867 LOCALIZATION OF DOPAMINE IN THE GILL OF APLYSIA: SOME PHYSIOLOGICAL IMPLICATIONS. John W. Swann, Martha G. Pierson*, and Annica Dahlström*. Neurobiology Department, Armed Forces Radiobiology Research Institute, Bethesda, MD 20014 and Institute of Neurobiology, University of Göteborg, Göteborg, Sweden.

The gill of Aplysia contains 3 ug of dopamine (DA) per gram of tissue. Physiological experiments utilizing the semi-intact gill preparation led one of us (JWS) to hypothesize that DA acts as a neurotransmitter for both motor and modulatory neurons thought to innervate the gill musculature (Swann et al., Br. Res. 157: 167, 1978). Additional experiments have suggested that L_9 neurons of the abdominal ganglion are dopaminergic gill motor neurons (Swann et al., Neurosci. Letters 10: 275, 1978). In experiments reported here, the distribution of DA within the gill is examined anatomically using fluorescence histochemistry for catecholamines. The Hillarp Falck technique was used.

Green fluorescing nerve fibers, with the same emission spectrum as that of DA, are distributed on muscle fibers throughout the gill with the notable exception of the bundles of longitudinal muscle fibers of the efferent vessel. Green varicosities, indicative of DA nerve terminals, cover muscle fibers in four areas of the gill: the afferent vessel, the pinnules, the efferent vessel trunklets and the circular muscles of the efferent vessel. These four areas of the gill contract when small quantities of dopamine are added to a gill perfusate and, with the exception of the circular muscles of the efferent vessel, all of them contract upon activation of L_9 cells. These observations support the contention that DA is a neuromuscular neurotransmitter in the gill and that L_9 cells are dopaminergic.

Contractions of the longitudinal muscle fibers of the efferent vessel are initiated by firing motor neuron LDG_1. These contractions are greatly enhanced by perfusing the gill with DA. Since these muscle fibers are not innervated by DA-containing nerve fibers, dopaminergic modulation of LDG_1 contractions cannot be mediated physiologically by dopaminergic modulatory neurons as originally proposed. Instead, DA modulation of LDG_1 contractions may be humoral in origin. We have found that a major portion of the DA content of the gill is located in highly fluorescent structures which are in close association with the microvasculature of the gill. The cellular origin of this potentially humoral source of DA will be discussed.

868 IDENTIFICATION AND MORPHOLOGICAL VARIABILITY OF A PEPTIDERGIC NEURON IN THE HAWKMOTH. P.H. Taghert* and J.W. Truman (Spon: M.D. Binder). Dept. of Zoology, UW, Seattle, WA. 98195.

We are investigating the control over the release of a specific peptide hormone by studying the individual neurons responsible for its production in the tobacco hawkmoth, Manduca sexta. The hormone - bursicon - functions to trigger the sclerotization of the wing vein cuticle following adult emergence. In response to combined hormonal and neural inputs, the peptide is released from specific segmental nerves of the abdominal nervous system (Truman (1973) J.Exp.Biol. 58:821). This release is rapid - 10 min. - and predictable - within 2 min. of a behavioral marker (Reynolds et al (1979) J.Exp.Biol. 78:77).

Several lines of evidence were used to identify the bursicon neurons and these included: surgery to assess the ganglionic source of the hormone, electron microscopy to determine secretory axons and pathways, neurosecretory and cobalt chloride staining and hormonal assays of individual cell bodies. The combination of these results has provided an unambiguous identification of a set of neurons with the following characteristics. There are four bursicon neurons per abdominal ganglion (two symmetric pairs). Each pair of cell bodies lies in a relatively consistent dorso-lateral position. The bursicon neurons consistently send their axons out the ipsilateral 'ventral' nerve; they terminate in the neurohaemal transverse nerve via an anastomosis between the two nerves.

Centrally, the neurons share a unique and characteristic dendritic morphology. However, when comparing homologous neurons between animals, variations in the basic pattern do emerge. The major dendritic branches are consistently ventral in the neuropile; fine branching exists in a series of 2 to 4 ventral to dorsal columns. While column position is constant, the routes taken to column positions by major dendritic branches and which positions are reached at all is subject to variation.

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869 MUTATIONS AFFECTING THE GIANT FIBER SYSTEM OF DROSOPHILA MELANOGASTER. Mark A. Tanouye* (SPON: R.J. Wyman). Biol. Dept., Yale Univ., New Haven, Conn. 06520.

Little is known about how the genome effects connections between neurons and establishes neuronal circuits. Although it is clear that there are not enough genes to specify each neural connection independently, there are no good theories which account for how single genes or gene combinations might provide the information necessary for proper nervous system connectivity. One method which can be used to investigate this problem is to use genetic mutations to disrupt neural circuits. The subsequent identification and analysis of these mutations would provide information about the nature of the disruption and perhaps ultimately an understanding of genetic contributions to neural connectivity.

In the present study, an attempt was made to identify genes which disrupt the proper functioning of a small neural circuit responsible for the jump response in D. melanogaster. The jump response is mediated via the cervical giant fiber whose connectivity has been described anatomically and physiologically in earlier investigations by our group. Genetic mutations affecting the giant fiber system were isolated in two steps. First, behavioral tests screened for mutants which couldn't jump. Second, electrophysiological screens identified those jumpless mutants with abnormal functioning of the giant fiber system.

The behavioral screen used visual and tactile stimuli to elicit jump behavior. Animals which jumped were eliminated and non-jumpers were saved. In the mutagenesis reported here, 56,805 X-chromosomes were screened behaviorally. These yielded 47 non-jumping mutant lines.

Giant fiber function was tested in non-jumping mutants by electrically stimulating the giant fiber and recording from thoracic muscles known to be driven by giant fiber spikes. A number of unusual responses were seen in the mutant lines including abnormally long response latencies and temperature sensitive paralytic phenotypes. In two mutant lines, thoracic muscles known to be driven by giant fiber spikes could not be driven. Results in these two mutants suggest a functional uncoupling of the giant fiber system.

870 LOCUST DORSAL OCELLI DETECT HORIZON DURING FLIGHT. Charles P. Taylor* (SPON: C. H. F. Rowell). Grad. Group in Neurobiology, Univ. of California, Berkeley, CA 94720.

The function of the ocelli, simple eyes located on the heads of many insects, was investigated in Schistocerca nitens and Schistocerca gregaria using neurophysiological and laboratory behavioral techniques. A survey of intracellular recordings of thoracic neurons revealed excitatory input to flight motor neuronsfrom ventral cord units activated by light flashes to the ocelli alone. This prompted behavioral investigations which made use of a visual horizon display (dark beneath, translucent above) which was rotatable about the animal's long axis. With compound optic lobes severed, the animal still tracked movements of the "horizon" with its head using the ocelli alone. A large tonically active axon in the ventral nerve cord consistantly showed correlation of spiking with ocellar-induced head motion toward one side and also had excitatory input from the cephalic wind-detecting hairs, and probably from other sensory modalities as well. This unit may represent a common neural pathway for roll axis orientation information from the head to the neck and thorax. The amplitude of head movements induced by the ocelli alone was small unless the animal was induced to tethered flight. During flight, however, the ocellar optomotor reaction was increased greatly in amplitude and was accompanied by corresponding flight steering behavior; the abdomen made rudder-like movements and the timing of wing depressor muscle unit (first basalar) firing changed so as to correct the visually detected "roll." Behavioral and optical evidence suggest the ocelli do not form a focussed retinal image, and that they probably detect horizon position by measuring average illuminance only.

The compound eyes alone can mediate a similar head-turning response which has been studied previously. However, with ocelli removed, the animal's ability to track movements of a diffuse horizon stimulus (sinusoidal light intensity gradient) during flight is greatly reduced. This may be because the extensive lateral inhibitory network of the compound eye, while enhancing sensitivity to sharp edges, makes it relatively insensitive to a continuous intensity gradient. Both ocellar and compound eye optomotor responses (measured separately) adapt to darkness sufficiently to operate on a dimly moonlit night. It is suggested that ocellar and compound eye-induced flight steering act synergistically, and posibly through a common neural pathway.

871 CONTROL OF SUPERFICIAL FLEXOR EFFERENTS BY ABDOMINAL CONNECTIVE FIBERS IN THE LOBSTER. Charlie S. Thompson* and Charles H.Page. Dept. Physiol, Bureau Biol. Res., Rutgers U., Piscataway,N.J. 08854.

The five excitatory motorneurons and the peripheral inhibitor which innervate the superficial flexor muscles (SFMs) in each half-abdominal segment of the lobster, Homarus americanus, were identified using electro physiological and cobalt staining techniques. Intracellular recordings from the cell bodies of the peripheral inhibitor (f5) and the largest motorneuron (f6) revealed three kinds of potentials: subthreshold depolarizing potentials (DPs), IPSPs and attenuated action potentials.

Abdominal connective fibers (ACFs) were divided into two groups depending upon their effects on the intracellular activity of the f5 and f6 neurons. First category ACFs elicited DPs in those neurons whose dendritic trees were located ipsilateral to the stimulated ACF. These DPs followed the stimulus with a fixed delay up to 50 Hz. The amplitude of the DPs varied with the stimulus frequency.

The second category of ACFs included those whose stimulation evoked DPs or IPSPs in f5 and f6 neurons which were not phase-locked with the stimulus. These fibers, which correspond to the extension and flexion command fibers (CFs) described in crayfish by Evoy and Kennedy (1967), produce strong bilateral effects when stimulated. Some fibers (extension CFs) elicit IPSPs in f5 neurons and an increase in DPs in f6 neurons while others (flexion CFs) produce an increase in DPs in f5 neurons and an apparent inhibition of DPs in the f6 neurons.
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872 LOCUST OVIPOSITION: A SYSTEM FOR THE STUDY OF MOTOR PATTERN GENERATION. Karen J. Thompson, Dept. of Biology, Univ. of Oregon, Eugene, OR 97403.

Locusts, and other Acridids, have ovipositors which are unique among insects, since the four terminal processes work by a divergent motion instead of sliding upon each other (Quadri,M.A.H.(1940) Trans. Roy. Ent. Soc. Lond.90(6):121-175). At oviposition these structures on the female abdomen tip are used to dig a deep hole into which the eggs are laid. The dorsal and ventral valves, although derived from serially homologous embryonic appendages, have different functions; the ventral pair levers the abdomen down while the dorsal pair excavates the hole (Vincent,J. F.V.(1975) J. Ent.(A)50:175-181). Specializations found only in the female allow the motive force of the ovipositors to stretch the abdomen from a normal length of 2.7 cm. to 8.3 cm. (data apply to Shistocerca nitens).

A single ganglion of the locust nervous system, the terminal abdominal ganglion, is capable of driving the rhythmic oviposition movements made by the dorsal and ventral valves. Furthermore, this rhythmic behavior is reliably elicited by severing the ventral nerve cord. Once released, movements continue autonomously for hours.

The movements of the ovipositor valves are entirely controlled by six pairs of bilaterally symmetrical muscles. The ventral valves, sternal appendages of the eighth segment, are innervated by the 8th sternal nerves. The dorsal valves, sternal appendages of the ninth segment, are supplied by a serially homologous pair of nerves, the 9th sternal nerves. The somata and dendritic fields of the neurons whose axons travel in these nerves are compartmentalized into two separate regions of the ganglion.

This behavior can only be elicited in sexually mature adult females. Extracellular nerve and muscle recordings were made and show an autogenic cyclic motor pattern which is comprised of two major classes of alternating burst activity. These experiments were designed as a framework for intracellular analysis of the mechanisms underlying initiation and maintenance of oviposition behavior.

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873 CORRECTION OF TURNING BEHAVIOR AFTER UNILATERAL CERCAL ABLATION IN THE COCKROACH. Noga Vardi* and Jeffrey M. Camhi. (SPON. F. Delcomyn). Section of Neurobiology and Behavior, Cornell, Ithaca, NY 14853.

Cockroaches (Periplaneta americana) respond to minute wind currents by turning away from the wind source and running. They use this wind-mediated response to escape from the strikes of natural predators (Camhi et. al. J. Comp. Physiol. 128, 203-212, 1978). The wind receptors are filiform hairs located on the cerci — two posterior abdominal appendages. These receptors excite 7 bilateral pairs of giant interneurons (GI's) which appear to be involved in mediating the directional motor output of the legs (Westin et. al. J. Comp. Physiol. 121, 307-324, 1977; Ritzmann and Camhi, J. Comp. Physiol. 125, 305-316, 1978).

We have studied the turning responses of the cockroach and the physiological responses of its giant interneurons to wind, following unilateral cercal ablation. In our behavioral experiments, standard wind puffs were presented to nymphal or adult cockroaches from different directions, and their turning responses filmed. Then the left cercus was ablated and the behavior was retested after 1 day, and again after 1 month and longer periods of time. During this time, the cockroaches did not regenerate a new cercus or any new cercal hairs. A few animals molted and developed a cercal bud which we ablated within two days. Prior to cercal ablation, all animals turned away from the source of wind stimulation. One day after ablation, on most trials, the animals responded to wind from either the right or the left by turning to the left. This presumably results from the fact that 6 of the 7 GI's on each side receive most of their sensory activation from the ipsilateral cercus. Thus removing the left cercus renders all but one of the right GI's more responsive than their left homologs. By one month the animals had corrected their turns in response to wind from the left. In most of the trials these animals made turns to the right in response to wind from the left. Statistically, this correction was highly significant (T test; χ^2 test).

To examine the cellular mechanisms of the behavioral correction, intracellular recordings are in progress from identified GI's before, during and after the period of behavioral correction. Immediately after covering or ablating once cercus, certain ipsilateral GI's become nearly or totally unresponsive to wind. These include GI's 1 and 3. So far, we have recorded only from these two GI's after long term behavioral correction. One GI 1 and three GI's 3 each gave about 1/3 the number of action potentials seen in normal unablated animals. The directionality of these cells was the same as in normal animals. This enhanced responsiveness of these GI's following ipsilateral cercal ablation could contribute to the observed correction of the turning behavior.

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874 PENICILLIN AND PENTYLENETETRAZOL DECREASE THE CONDUCTANCE OF SINGLE ACETYLCHOLINE-INDUCED CHLORIDE CHANNELS IN APLYSIA NEURONS. Ruth E. Wachtel and W.A. Wilson. Dept Pharmacol, Duke Univ Med Ctr, Durham, NC 27710 and Epilepsy Ctr, VA Hospital, Durham, NC 27705.

In Aplysia, the convulsants penicillin (Pen G) and pentylenetetrazol (PTZ) attenuate chloride-dependent responses to iontophoretically applied transmitters. At the same concentrations, they have little effect on sodium-dependent responses. This attenuation of chloride-dependent responses is independent of the transmitter used to elicit the response, suggesting that Pen G and PTZ are interacting directly with the chloride ionophore, rather than the transmitter-receptor complex (Science 197:912, 1977).

Individual sodium and chloride channels were studied by examining the current fluctuations produced by the application of acetylcholine (ACh). Steady-state responses were elicited by iontophoretic application of ACh to neurons of the pleural and pedal ganglia of Aplysia californica. Currents were recorded utilizing a single microelectrode voltage clamp which was modified for low noise measurements. Frequency response studies indicated that the clamp was effective to at least 400 Hz.

The elementary conductance of a single channel was determined from the relation $g_{el} = i_{el}/(V-V_{eq})$, where the elementary current i_{el} = variance of current fluctuations/mean current.

The elementary conductance of a chloride channel is 8×10^{-12} ohms^{-1} at 21°C. Pen G and PTZ reduced g_{el} in a dose-dependent fashion; the decrease in g_{el} paralleled the decrease in mean steady-state current evoked by ACh application. Concentrations of Pen G (1mM) and PTZ (2mM) which reduced the elementary conductance of chloride channels had little effect on the sodium channel, although higher concentrations of these drugs did produce a reduction in sodium channel conductance.

This data confirms previous studies that Pen G and PTZ depress transmitter-induced chloride responses at concentrations which spare sodium responses. It also supports the hypothesis that Pen G and PTZ attenuate responses by interacting directly with, and possibly physically blocking, the chloride channel.

875 ASSOCIATIVE LEARNING IN APLYSIA CALIFORNICA. E.T. Walters*, T.J. Carew and E.R. Kandel. Div. of Neurobiol. & Beh., Depts. of Physiol. & Psychiat., Columbia Univ., P&S, New York, N.Y. 10032

Aplysia has proven to be useful for cellular analyses of non-associative learning. We now report that Aplysia also is capable of associative learning.

The behavioral paradigm that we have used is based on Pavlovian fear conditioning (Rescorla & Solomon, 1967). 'Paired' animals (N=18) received a conditioned stimulus (CS, shrimp extract delivered to the oral veil) for 90 sec. Sixty sec after the onset of the CS an aversive unconditioned stimulus was applied to the head. The US was 30 sec of pulsed electric shock (400 ma) delivered through sea water via spanning electrodes. Explicitly 'unpaired' controls (N=18) received the CS 90 min after the US. 'Untrained' controls (N=12) received neither CS nor US. Training consisted of 3 trials per day for 2 days. The intertrial interval was 3 hours. Testing was carried out (blind) on the third day: the CS was delivered for 60 sec and then escape locomotion was triggered with a weak (50 ma) shock applied across the tail. Escape was measured as mean number of steps taken in 5 min following tail shock. Paired animals showed significantly greater escape ($\bar{X}$=11.22 steps) than unpaired ($\bar{X}$=2.22, $p<.005$) or untrained ($\bar{X}$=3.25, $p<.005$) animals. This experiment was replicated and the same results were obtained using blind training in addition to the blind testing procedure: paired animals (N=8) showed greater escape ($\bar{X}$=11.75 steps) than unpaired animals (N=8) ($\bar{X}$=2.13, $p<.005$). Thus, specific temporal pairing of the CS with a noxious US endows the CS with properties which can subsequently facilitate an escape response. By contrast, training with CS or US alone does not produce facilitated escape.

These results indicate that Aplysia can form a powerful, temporally specific association between a chemical CS and an aversive US. That the learned association can be demonstrated by testing the effects of the CS on a response system not directly involved in the training (escape locomotion to tail stimulation) supports the idea that associative learning exhibited by Aplysia may be analogous to 'learned fear' produced in vertebrates by Pavlovian fear conditioning.

Several other forms of associative learning have now been described in gastropod mollusks, including avoidance learning, baitshyness, and intersensory associations (Mpitsos & Davis, 1973; Mpitsos & Collins, 1975; Gelperin, 1975; Crow & Alkon, 1978). Thus, it may soon be possible to achieve cellular analyses of different types of associative learning in gastropods and to examine their relationships to each other and to nonassociative learning, both within a single species and across related species.

876 MOTOR OUTPUT TO A COCKROACH LEG IN RESPONSE TO WIND FROM DIFFERENT DIRECTIONS. Joanne Westin* and Roy E. Ritzmann, Dept. Biol., CWRU, Cleveland, OH 44106 (SPON: Leo S. Demski)

Giant interneurons (GIs) in the nerve cord of the cockroach Periplaneta americana are thought to be involved in triggering and orienting the cockroach's escape from a wind source. It is likely that several GIs are involved in controlling an escape, and that the ones involved vary with wind direction. Evidence for this is: 1) For any given wind direction 8-12 GIs are excited, and the ones excited vary with wind direction (Westin, et al., J. comp. Physiol. 121, 307-324, 1977). 2) More than one GI excites any given leg motor neuron, and different GIs excite different leg motor neurons to different degrees (Ritzmann and Camhi, J. comp. Physiol. 125, 305-316, 1978). 3) For turns of different directions, different leg motor neurons must be excited in any given leg (Camhi and Tom, J. comp. Physiol. 128, 193-201, 1978). My work is designed to determine which GIs are necessary to produce the motor outputs which turn the animal away from wind from different directions.

I recorded the responses of depressor motor neurons (in nerve branch 5r1) and levator motor neurons (in nerve branch 6Br4) of a metathoracic leg to wind puffs from different directions before and after cutting the ipsi- or contralateral half of the nerve cord. In an intact animal the output from depressor motor neurons to a metathoracic leg is always greater when wind is delivered from the ipsilateral rear than when the wind is from the contralateral rear. The output of levator motor neurons is generally greater when wind is from the contralateral rear than when it is from the ipsilateral rear. These results are as expected since a wind puff from the left rear generally produces an initial depression of the left metathoracic leg and often levation of the right metathoracic leg (Camhi and Tom, 1978).

Sectioning the ipsilateral side of the nerve cord greatly reduces the output of the depressors caused by wind from the ipsilateral rear, while having little effect on activity in the levators caused by wind from the contralateral rear. The converse is also true. Thus, it appears that some ipsilateral GIs (and/or smaller ipsilateral interneurons) provide the major input to the depressor motor neurons of the metathoracic leg when wind is from the ipsilateral rear, while contralateral GIs (and/or smaller interneurons) provide the major input to the levator motor neurons when wind is from the contralateral rear. Experiments are now in progress involving elimination of individual identified GIs.

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878 CHARACTERIZATION OF COMMAND INTERNEURONS EVOKING ABDOMINAL EXTENSION IN CRAYFISH. Benjamin J. Williams* and James L. Larimer. Univ. of Texas, Austin, TX 78712.

We have mapped the thoraco-abdominal (T5-A1) connective for "command" interneurons which evoke abdominal extension in P. clarkii. Preparations included behaving animals and/or animals with immobilized abdomens; up to 6 motor roots were simultaneously recorded. Over 80% of the 150 command fibers (CFs) examined were located in 6 loci in cross-section of a T5-A1 hemiconnective. CFs are characterized by loci-specific rostrally or caudally biased gradients of extensor output from ganglia 1 to 4. Two of the loci typically produced metachronous swimmeret beating in addition to extension; these loci correspond to those mapped by Wiersma and Ikeda (1964). Coactivation of 2 extension CFs at low (20-30Hz) frequency sometimes produced recruitment of previously inactive motoneurons. Interaction of strong flexor-biasing sensory input with an extensor CF at similar low frequencies revealed competition in the motor output. However, when the CF was stimulated at higher (75-100Hz) frequencies, the extensor drive was dominant irrespective of the frequency of sensory drive. Substituting one extensor CF for another after the first one was driven to fatigue resulted in immediate extensor motoneuron activation. This result may imply the presence of more than one driver interneuron. Furthermore, the most labile synapses are located at the connection between the CF to driver interneuron. Stimulating CFs in the T5-A1 connective and recording from the CFs in the 5-6 connective showed that at least 3 of the loci have uninterrupted axonal projections through homologous areas of the abdominal cord. No CF so isolated could be activated by stimulation of any other CF. In addition, CF stimulation produces little or no activation of other elements in the abdominal cord. Cinematographic analyses were performed on normal and cord-lesioned animals during extensions evoked by platform-drop stimuli. These data show that information must pass from a rostral site through the T5-A1 connective. Also, only one abdominal hemiconnective need be intact for the animal to produce extension equal in speed and amplitude to the normal animal. (Supported by NIH grant NS05423-15)

877 A SELF-INHIBITORY SYNAPTIC POTENTIAL ALTERS INITIAL FIRING FREQUENCY, BUT NOT SYNCHRONY, OF APLYSIA BUCCAL GANGLIA NEURONS. Roy L. White* and Daniel Gardner. Dept. of Physiology, Cornell Univ. Medical Coll., New York, N.Y. 10021

Each of the two buccal ganglia of Aplysia contains a coupled pair of identified cholinergic neurons. Each neuron monosynaptically inhibits itself, producing a self-inhibitory synaptic potential (SISP) after every action potential (AP). The SISP decrements with repetitive stimulation. The synaptic current decays with 43 msec time constant (Gardner, J. Physiol. 264:883, 1977).

As prelude to AP frequency studies, we measured synaptic current decrement under clamp, using a variable number of conditioning APs preceding each test AP and SISP. SISP current fell by 50% after 5 conditioning APs. To assess the SISP effect on firing patterns, constant current pulses lasting 1 sec were injected into resting cells and the resulting AP trains recorded digitally. Protocols in sea water (SW) were repeated with ganglia bathed in d-tubocurarine $5x10^{-4}$ to 10^{-3} g/ml (dTC) to permit comparison of AP trains with SISP intact to those with the cholinergic SISP blocked. The SISP prolongs initial interspike intervals (ISI) of each train, but declines in efficacy sharply over 300 msec, then slowly. ISI and post-stimulus time histograms show smaller interval variance within each train, and greater trial-to-trial regularity with SISP intact. AP frequency vs. injected current (f-I) curves in both SW and dTC show single-range near-constant slopes over the range 5-18 nA, with average threshold 7 nA. The SISP increases the initial instantaneous firing rate [1/(first ISI)] 6-fold without affecting firing rate at the end of each train.

The two cells in each ganglion receive common input and are electrotonically interconnected (Gardner, Science 173:550, 1971). Blocking the SISP with dTC reduces the hyperpolarizing component of the electrotonic coupling potential without affecting coupling coefficient (0.135), input resistance (1-2 MΩ) or common input. To determine the effect of the SISP on synchrony of firing of cell pairs, we depolarized cells near threshold with 10 sec constant-current steps and recorded the resulting AP trains in both neurons. Cross-correlation histograms from early or late times in the epoch with or without dTC were identical: a strong peak around t=0, reflecting high synchrony due to common input and the depolarizing component of the coupling potential.

We conclude: 1) The SISP prolongs initial ISIs, providing an early analogue to accommodation and ensuring a constant firing frequency for constant input current. 2) Neither the more regular firing nor the larger hyperpolarizing component of the coupling potential produced by the SISP contributes markedly to synchrony of the coupled neurons.

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879 THERE ARE UNIQUE, IDENTIFIABLE LOCAL NON-SPIKING INTERNEURONS. John A. Wilson, Department of Zoology, University of Cambridge, Cambridge CB2 3EJ, England.

A feature of simpler systems neurobiologists have taken advantage of is that certain neurons are unique and identifiable. In the locust Schistocerca gregaria many leg and flight motor neurons and some large interganglionic interneurons have been identified using a combination of anatomical and physiological criteria. Local interneurons constitute a large part of the population of neurons in insect ganglia, and a variety of local non-spiking interneurons have been described in the metathoracic ganglion (Siegler & Burrows, 1979 J. comp.Neurol. 183). It was not known whether or not any of these interneurons are unique. It will be shown that there are local interneurons in the locust mesothoracic ganglion which are unique and identifiable.

A distinct local non-spiking interneuron has been studied in several individuals. The neuron is the apparent serial homologue of the neuron shown in fig. 4 of Siegler and Burrows. It was impaled with a microelectrode and subsequently injected with cobalt ions after its effects on leg motor neurons were studied. In all cases, the interneuron strongly excited flexor tibiae, levator trochanter, and coxal motor neurons. After silver intensification of the cobalt stain, the neuron was seen to have its major region of dendritic branching on the side of the ganglion contralateral to its cell body. A region of dendritic branching ipsilateral to the cell body was also seen.

Examination of whole mounts of ganglia in which this neuron has been filled reveal the neuron's structure to be very similar in different individuals. Its primary neurite has been found to pass through the same neuropillar regions. Sections reveal that in the region between the connectives, two larger (greater than 10 μM) and four smaller neurons follow a similar course. No other processes this large or larger are found within 30 μM of these neurons. One of the two larger neurons was revealed to be this neuron and the other larger neuron its presumed contralateral homologue. Thus there are unique and identifiable local non-spiking interneurons.

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880 CONSTANCY AND VARIABILITY IN SYNAPTIC CONNECTIONS BETWEEN IDENTIFIED NEURONS. Jeffrey J. Wine & Grace C. Hagiwara, Department of Psychology, Stanford University, Stanford, CA. 94305

Variability of synaptic connections was recently demonstrated in Locusts (Pearson & Goodman, J. Comp. Neurol. 184, 141, 1979). We tested the hypothesis that synaptic connections among neurons are variable to the extent that they are redundant. By a redundant synapse we mean one which has no effect beyond increasing the safety factor for transmission. Redundancy is a graded property, such that a very weak synapse in parallel with a very strong, suprathreshold synapse is considered highly redundant. We have evidence for redundant synapses in the crayfish central nervous system and find that such synapses are often missing. In contrast, synapses judged to be crucial for behavior were invariably present in the population we tested. Two sets of giant neurons were studied.A pair of giant interneuron axons (LG axons) form electrical, 1:1 synaptic contacts with each other, and each axon contacts one member of a pair of giant motoneurons (MoGs). An impulse in one LG axon invariably excites the other LG axon and thus excites both MoGs, one directly,and one indirectly via the contralateral LG axon. The regions of synaptic contact between the LG axons and the MoGs can be visualized by filling the MoGs with cobalt sulfide. In prior studies, where a perfect correlation was obtained between anatomical and electrophysiological measures of synaptic transmission (Mittenthal & Wine, Sci. 179, 182, 1973), we saw extensive synaptic contact between ipsilateral LG and MoG axons, and occasionally saw tenuous branches from the MoG to the contralateral LG axon. We have now backfilled a large number of MoGs (n = 52) and intensified the wholemounts with a modification of the Timm's technique. We found connections with the ipsilateral LG axon in 100% of these ganglia, and with the contralateral LG axon in 33% of the ganglia. Homologous sets of LG axons and MoGs exist in the three anterior abdominal ganglia. By ganglion, the percentage of crossed (redundant) connections was: G1: 28% (5/18), G2: 44% (8/18), and G3: 25% (4/16). We conclude that the motoneurons can make synaptic connections with both ipsilateral and contralateral LG axons. The strong, ipsilateral connection has a high probability of forming, while the redundant, contralateral connection is usually omitted. Since the strong coupling between the LG axons renders the contralateral connection redundant, its absence should have no behavioral consequences. We suggest that nervous systems may be overconnected to a significant extent, and that redundant connections will tend to be variable, since natural selection can neither eliminate nor reinforce them.

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882 AN ORDERLY PROJECTION OF AFFERENTS IN INSECT PERIPHERAL NERVES. Sasha N. Zill*, Mary A. Underwood*, J. Carter Rowley,III* and David T. Moran* (SPON: W. Proctor). Dept. Anat., Univ. Colo. Med. Sch., Denver, CO 80262

Although many vertebrate sensory systems are somatotopically organized, the organization of afferents in invertebrates remains largely unexplored. Several early observations suggest that afferents may be discretely organized in insect peripheral nerves. Wigglesworth noted that sensory axons, which develop from cell bodies in the epidermis, follow preexisting afferents in their path to the central nervous system. Edwards and Palka found that removal of the cercus tip in crickets resulted in a defined area of degeneration in the cercal nerve. These studies imply that afferents from a defined body surface gather together and remain grouped in peripheral nerves.

Does this grouping produce an overall organization of afferents? To answer this question we took advantage of the fact that insect axons degenerate rapidly after separation from their somata and produce changes detectable by light and electron microscopy. Degenerative changes include clumping of axonal organelles, separation and expansion of glial wrappings and final resorption of axons. We used axonal degeneration to map sensory projections in the leg nerves of two orthopteran insects, the cockroach (Periplaneta americana) and the grasshopper(Melanoplus bivittatus). Mesothoracic legs of anaesthetized animals were transected at different leg segments. Seven days later, cuticle overlying the mesothoracic ganglion was removed and fixative perfused into the hemocoel. The main leg nerve (nerve 5) was cut at its entrance to the coxa and removed with the attached mesothoracic ganglion. After dehydration and embedment, thin and thick cross-sections were taken from the nerve's cut end.

Images of leg nerves of control animals showed a tightly packed, continuous array of axon profiles. In experimental animals, when the most distal leg segments were removed, a discrete area of degeneration was repeatedly found in the leg nerve along its posterior edge. More proximal ablations produced larger areas of degeneration that progressively extended into the anterior half of the nerve. Overlays of acetate copies of patterns of degeneration from different ablations showed a posterior-to-anterior layering that generally corresponded to a distal-to-proximal map of the leg. Remarkably similar patterns of degeneration were seen in both cockroaches and grasshoppers.

These experiments indicate that insect peripheral nerves possess a higher level of organization than had previously been recognized. We are currently investigating whether this arrangement is preserved in projections into the central nervous system.

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881 METHYLXANTHINE ENHANCEMENT OF INWARD AND OUTWARD CURRENTS IN APLYSIA GIANT NEURONS. Kerry L. Zbicz* and Wilkie A. Wilson. Dept. Pharmacol., Duke Univ. Med. Ctr., Durham, N.C. 27710 and Epilepsy Ctr., V.A. Med. Ctr., Durham, N.C. 27705

The methylxanthine phosphodiesterase inhibitors have been shown to increase membrane calcium fluxes and cause the release of calcium from intracellular storage sites. We have investigated the effect of two methylxanthines, caffeine and theophylline, on the slow currents regulating firing frequency in the giant neurons R2 and LPG of Aplysia californica. We have previously demonstrated the calcium dependence of the slow outward current underlying adaptation in these neurons (Zbicz & Wilson, Soc. Neurosci. Abstr., Vol. 4, p. 211, 1978). Voltage clamp studies have demonstrated that depolarization of the giant neurons to potentials more positive than -40 mV activates a transient inward current which is followed by a gradually increasing K^+ current which mediates adaptation. When the current-voltage relationship of a cell is plotted using current data taken at the time of maximum inward current (0.5-1.0 sec after the voltage step change), a negative resistance region is present at potentials more positive than -40 mV. Removal of sodium or calcium from the external solution depresses or eliminates the inward current and negative resistance region as does application of 1-3 mM lanthanum.

Both caffeine and theophylline (1-10 mM) increase the magnitude of the inward current, steepen the negative resistance region, and increase the development of the slow outward K^+ current. Neither the non-methylxanthine phosphodiesterase inhibitor papaverine nor the dibutyryl derivative of cAMP or cGMP enhance the inward transient current or steepen the negative resistance region. These substances are also ineffective in enhancing the development of the slow outward K^+ current.

The effects of methylxanthines on cell firing in response to stimulation by transmembrane current passage are complex, with enhancement of firing frequency generally occurring. In those neurons showing a large enhancement of outward K^+ current the initial firing frequency may be enhanced while the frequency after several seconds of stimulation is depressed. It is concluded that the methylxanthines can enhance firing in the giant neurons by increasing the magnitude of an inward current carried by sodium and/or calcium with depression of firing occurring only when the slow, calcium dependent K^+ current is greatly increased.

LIMBIC SYSTEM

883 EFFECT OF EXPERIMENTAL BILATERAL ISCHEMIA ON ENERGY METABOLITES IN THE MONGOLIAN GERBIL HIPPOCAMPUS. Marc S. Abel* and David W. McCandless* (SPON: J.W. Haycock). Dept. Neurobiol. & Anat., U. Tex. Med. Sch., Houston, Texas 77025.

Bilateral occlusion of the common carotid artery was used to produce ischemic attacks of varying duration in the Mongolian gerbil (Meriones Unguiculatus). The gerbil is uniquely sensitive to this procedure, perhaps due to the lack of a posterior communicating artery. In an attempt to discern the effect of ischemia on energy metabolism, levels of glucose, ATP and phosphocreatine were measured in the CA_1 and CA_3 regions of dorsal hippocampus. The hippocampus was chosen for its well-defined architectonic structure. Further, this allowed a comparison of the effect of ischemia to the well-known effect of hypoxia on Sommer's sector (CA_1). Ischemia was produced as follows: both common carotid arteries were loosely ligated with surgical thread while the animal was anesthetized. After recovery from anesthesia the vessels were occluded with Heifetz aneurysm clips. Animals were sacrificed by immersion in liquid nitrogen. This procedure freezes the brain at a rate of 2-3 sec/mm, thereby altering the dorsal hippocampal tissue environment sufficiently to stop metabolic activity within 5-10 seconds. After sectioning on a cryotome (-20°C), the tissue was lyophilized and further dissection was performed at room temperature. The samples were weighed on quartz-fiber fishpole balances. Levels of glucose, ATP and phosphocreatine were analyzed using enzymatic assays which take advantage of the fact that NADPH fluoresces while $NADP^+$ does not. The data presented describe how the energy metabolism in two regions of the gerbil dorsal hippocampus respond to the metabolic pertubation of experimental ischemia.

884 DISCRIMINATION LEARNING AND REVERSAL FOLLOWING ELECTROLYTIC MEDIAN RAPHE LESIONS. Karen E. Asin, David Wirtshafter and Ernest W. Kent. Dept. Psychology, University of Illinois at Chicago Circle, Chicago, Il 60680.

Anatomical and electrophysiological studies have demonstrated profuse interconnections between the median nucleus of the raphe and certain limbic structures. At past meetings of this society we (Asin et al., 1976, 1977, 1978) have noted that a number of the behavioral effects of median raphe lesions resemble those seen after damage to limbic structures, especially the septum and hippocampus. In the present report we extend our previous findings by describing the effects of median raphe lesions on the acquisition and reversal of several food reinforced T-maze discrimination tasks.

Similar to what has been reported following hippocampal damage, electrolytic median raphe lesions were without effect on the acquisition of a T-maze position habit, but severely impaired its reversal. It is likely that this deficit, in part, reflects damage to serotonergic elements since a similar, but less pronounced, deficit was obtained following treatment with p-chloroamphetamine (pCA) (2 X 10mg/kg). When trained on a simultaneous brightness discrimination and eight consecutive reversals, median raphe lesioned animals did not differ from controls. A similar dissociation between position and brightness reversal has been reported following septal and hippocampal damage. The acquisition of a successive brightness discrimination was extremely impaired following median raphe damage; reversal was not studied since a number of lesioned animals failed to reach acquisition criterion despite extensive training. This deficit may reflect damage to non-serotonergic elements since pCA altered neither the acquisition nor the reversal of this task. Impaired acquisition of successive discriminations has also been reported following limbic damage.

The current results provide further behavioral evidence for the anatomically derived concept of a limbic-midbrain circuit.

885 NEUROGENESIS IN THE RAT HIPPOCAMPAL REGION. Shirley A. Bayer, Dept. of Biol., Purdue Univ., West Lafayette, IN 47907

Neurogenesis in the rat hippocampal region was examined with ^{3}H-thymidine autoradiography. The rats in the prenatal groups were the offspring of pregnant females given two injections of ^{3}H-thymidine on consecutive days in an overlapping series: embryonic day (E) 13+14, E14+E15, ... E21+E22. The rats in the postnatal (P) groups were injected in a nonoverlapping series: the day of birth (P0) and P1, P2+P3, ... P18+P19. On 60 days of age, the percentage of labelled cells and the proportion of cells added during each day of formation were determined at several anatomical levels within each structure of the hippocampal region (entorhinal cortex, parasubiculum, presubiculum, subiculum, Ammon's horn, and the dentate gyrus). The neurons in each structure arise in overlapping, but still significantly different, waves: the entorhinal cortex between E15-E17; the para- and presubiculum between E16-E19; the subiculum between E16-E18; large cells in strata oriens, radiatum, lacunosum-moleculare of Ammon's horn between E15-E17; Ammon's horn pyramidal cells between E17-E19; large cells in the dentate hilus and molecular layer between E15-E19. Dentate granule cells begin to originate on E17, and 10% of the population forms after P18. There are three characteristic gradients of formation within structures: deep cells are generated before superficial cells; cells closer to the rhinal fissure are formed before those lying farther away ("rhinal to dentate" gradient); later forming cells are flanked by earlier forming superficial and deep cells ("sandwich gradient") in the entorhinal cortex, Ammon's horn, and the dentate gyrus. There is a "rhinal to dentate" gradient between structures: first, entorhinal cortex; second, the subiculum; third, field CA3 of Ammon's horn; fourth, the dentate gyrus. The para- and presubiculum are exceptions and form significantly later than the subiculum; CA1 forms significantly later than adjacent CA3 cells. This late neurogenesis may be related to prominent thalamic input to both structures.

Neurogenetic gradients between the cells providing laminated afferent input to the Ammonic pyramidal and dentate granule cells correlates with their order of termination: afferents from progressively later-originating cells terminate progressively closer to the cell body. Topographic hippocampal projections along the dorsoventral axis correlates with formation patterns in target structures: dorsal hippocampal fibers project to zones occupied by earlier-forming cells in the lateral septal nucleus and pars posterior of the mammillary body; ventral hippocampal fibers project to zones occupied by later-forming cells in these structures.

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886 EPILEPTIC PHENOMENA FOLLOWING INTRA AMYGDALOID INJECTIONS OF KAINIC ACID: AN ELECTROGRAPHIC, BEHAVIORAL AND NEUROPATHOLOGICAL STUDY. Y. Ben-Ari*, O.P. Ottersen*† and E. Tremblay* (SPON: D. Lawrence). LPN-CNRS Gif-sur-Yvette 91190 France and †Anatomical Institute, Oslo 1, Norway.

We have undertaken a series of experiments to explore the possibility that the distant lesions seen after intracerebral injection of kainic acid (KA) may be due to the epileptogenic action of the toxin rather than a direct action (i.e. through diffusion). Unilateral injection of KA (0.4-2 µg in 0.1-0.4 µℓ phosphate buffer, pH 7.4) into the amygdala of rats which had been chronically implanted with a cannula (0.2 mm i.d.) as well as cortical and deep electrodes, rapidly elicited severe recurrent motor and electrographic seizures for 2-6 h. Subsequently, irregular or regular spikes occurred continuously (for 4-30 h) without motor signs. The first brain damage to appear (after 2 h epileptiform activity) was restricted to the site of injection and the ipsilateral CA3 a field in the rostral pole of ammon's horn. The apical dendrite of the pyramidal neurons showed strong argyrophilia, the cell bodies were shrunken and distorted and the neurophil of stratum lucidum appeared vacuolated. At longer survival, additional damage was present in the contralateral amygdala and bilaterally in the claustrum, lateral septum, hippocampus (areas CA1 and 4), midline thalamic nuclei and neocortex.

Several observations suggest that the distant damage is causally related to the epileptiform activity induced by the toxin: 1) a positive correlation was found between the severity of epileptiform activity (in particular the post-ictal depressions) in the ipsilateral hippocampus during the first few hours and the subsequent CA3 pathology; 2) repeated administration of an anticonvulsant (diazepam, valium, Roche) reduced the distant damage without affecting the local lesion at the site of injection; 3) the rostral (septal) hippocampal pole was more vulnerable than the caudal (temporal) pole, even though the latter is closer to the site of injection; 4) thalamic nuclei contralateral to the injection site often displayed more damage than the ipsilateral nuclei; 5) control injections in structures closer to the rostral hippocampus (such as the septum) did not readily induce hippocampal lesions; 6) unilateral transection of the perforant path considerably reduced the distant brain damage without affecting the local (amygdaloid) damage. We conclude that a) intra-amygdaloid injections of KA constitute a particularly suitable model for investigating the relationship between epilepsy and brain damage; b) the perforant path may play a significant role in the propagation of epileptiform activity and its pathological consequences; c) that the widespread use of KA injections to produce circumscribed lesions is unwarranted, notably in chronic studies.

887 MOTOR EFFECTS INDUCED BY INTRA-ACCUMBENS INJECTIONS OF DOPAMINE IN THE SQUIRREL MONKEY. Sherry L. Berg*, Daniel L. Jones, Roy L. Dorris* and Russell E. Dill. Dept. of Microscopic Anatomy, Baylor College of Dentistry, Dallas, Texas 75246.

Naive male squirrel monkeys, Saimiri sciureus, were used to test drug-induced changes in motor activity. Drugs were infused through chronically implanted cannulae in the nucleus accumbens. Changes in motoric activity were indexed by computerized analyses of photocell interruptions in the four quadrants of the test cage. Animals were pretreated with RO 4-1284 and then infused with dopamine. The study is an extension of the findings of Dill, R.E., et al., Neurosci. Abst. '78, and is designed to assess the role of the nucleus accumbens in the integration of gross levels of motor activity. (Supported by NINCDS Grant NS 15020.)

888 RECIPROCAL ANATOMICAL CONNECTIONS BETWEEN CINGULATE-RETROSPLENIAL CORTEX AND ANTEROVENTRAL THALAMUS IN THE RABBIT STUDIED WITH HORSERADISH PEROXIDASE. Theodore W. Berger, Teresa A. Milner*, Gerald W. Swanson*, Gary S. Lynch and Richard F. Thompson. Dept. Psych., Univ. of Pittsburgh, Pittsburgh, PA 15260, and Dept. of Psychobio., Univ. of California, Irvine, CA 92717.

While previous investigations have established that reciprocal anatomical connections exist between the cingulate-retrosplenial cortices and anterior thalamic nuclei, few studies have utilized transport techniques. Data are particularly lacking with respect to transport documentation of the terminal fields for thalamofugal fibers.

Injections of 0.02-0.50 ul, 35% horseradish peroxidase (HRP) were centered in the anteroventral (AVT) nucleus of thalamus. Following 1-2 days of recovery, animals were perfused with 1.3% gluteraldehyde and 1.1% paraformaldehyde in phosphate buffer. Frozen sections were taken and reacted either with benzidene dihydrochloride or tetramethylbenzidene. Both orthograde and retrograde products were examined in posterior cingulate and retrosplenial cortices.

Orthograde and retrograde results were nearly identical for both limbic cortical areas. In addition, all results were described from animals showing heavy retrograde labeling of cells in ipsilateral mammillary nuclei. For both retrosplenial and cingulate cortices, HRP-positive neurons were almost exclusively restricted to layer VI. Retrograde-filled cells were sometimes, though very infrequently, identified in layer V, with no other limbic cortical layers showing the presence of retrograde HRP transport. Examination of orthograde HRP product revealed a highly localized terminal distribution that did not overlap with retrograde cell fields. Orthograde transport from AVT neurons was found to terminate in layers I and IV of both cingulate and retrosplenial areas.

These results clearly delineate the origins and sites of termination for cortical regions reciprocally connected with areas of anteroventral thalamus--AVT regions that are the target for mammillary efferent projections.

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889 DIFFERENTIAL EFFECTS OF PREOPTIC AND ANTEROVENTRAL SEPTAL LESIONS ON INTRASPECIFIC AGGRESSION IN MALE HAMSTERS. Alan Blau*, Michael Potegal and Murray Glusman (SPON: D.E. Hutchings). Dept. Behavioral Physiology, N.Y. State Psychiatric Institute, New York, NY 10032.

Septal lesions are reported to increase aggression among male hamsters (e.g. Sodetz & Bunnell, Physiol. Behav. 5:78, 1970) while preoptic area lesions decrease it among females (e.g. Hammond & Rowe, Physiol. Behav. 17:507, 1977). It would be of interest to compare these lesions in a single gender. Furthermore, these studies have involved unrestrained encounters in which a lesion's effects on the operated subject's aggressiveness is confounded with its effects on his aggression-eliciting characteristics vis-à-vis his opponent. We report here that anteroventral septal (AVS) and preoptic area (POA) lesions have differential effects on male hamsters' attacks on a standard, non-aggressive, muzzled and analgesic-treated male target hamster.

Twenty-four adult male hamsters selected for moderate preoperative aggressiveness were subjected to radiofrequency AVS or POA lesions or sham surgery. After a two week recovery, subjects were given (a) three 45 min sessions with a standard target, (b) one 45 min session with an estrogen/progesterone primed female and (c) one 45 min session on an activity meter. All tests took place in the subject's home cage with a 48 hr. intersession interval.

A statistically significant difference in attack rate among the groups was largely due to the elevated rate of AVS subjects (mean of 9.6 biting attacks/session) compared to POA subjects (1.1 attacks/session) and sham subjects (1.3 attacks/session). These differences in aggressiveness were confirmed by unrestrained pair encounters: AVS subjects defeated POA subjects in every contest. Twenty-eight per cent, 85% and 100% of the AVS, POA and sham subjects, respectively, copulated with the female. Their respective activity counts were 1501, 368 and 256. Activity counts and attack rates were significantly correlated for AVS subjects but not for the others. In contrast to these differential effects, AVS and POA subjects were similar in their deficiencies in nest-building and food-hoarding relative to sham animals.

Our aggression testing technique eliminates the possibility that AVS lesions act solely by altering the aggression-eliciting characteristics of operated hamsters but raise the possibility that attack rate increases are secondary to activity level changes. (Supported by an H.F. Guggenheim Foundation Grant)

890 EFFECTS OF ELECTRICAL STIMULATION OF THE SUBSTANTIA INNOMINATA UPON HYPOTHALAMICALLY-ELICITED AGGRESSIVE BEHAVIOR IN THE CAT. C.H.Block*, A. Siegel, H.Edinger. Departments of Physiology and Neurosciences, College of Medicine and Dentistry of New Jersey-New Jersey Medical School, Newark, NJ 07103.

The substantia innominata (SI) supplies afferents to the lateral hypothalamus, ventral tegmental area, and amygdala. Initiation or modulation of attack behavior can be generated by electrical stimulation of the three brain areas noted above. Accordingly, the present investigation was undertaken in order to determine the possible role of the SI in the control of aggressive responses.

Electrodes for both stimulation and recording were bilaterally implanted in SI and hypothalamus in ten cats. Postoperatively, the effects of stimulation of sites in SI upon either the quiet biting attack (QBA) or affective display generated by hypothalamic stimulation were determined by the use of a dual stimulation paradigm which compared stimulation of the hypothalamus alone with stimulation of hypothalamus and SI. The findings suggest a possible differentiation of function within SI. Specifically, stimulation of sites in lateral SI significantly inhibited QBA ($p < 0.02$) while stimulation of more medially placed SI electrodes facilitated the occurrence of QBA ($p < 0.02$). Stimulation of any portion of the SI alone produced no observable behavioral response. Those sites that significantly modulated QBA were further studied for their possible effects upon the sensory component of the attack response. It was observed that stimulation of SI sites that suppressed QBA also reduced the extent of the lipline that, when probed, would elicit a jaw opening response, while stimulation through electrodes associated with facilitation of attack produced an opposing effect. The effects upon motor components of QBA were also studied by probing the region of the midline of the lip that consistently elicited a jaw opening response during either single or dual stimulation and the latency for the response was measured. The possibility of a modulatory effect upon the motor component of the attack response was suggested as well.

The effects of SI stimulation upon affective display were tested from seven electrode sites and in no instance did stimulation significantly alter the response latency ($p > 0.1$).

Presently, these data imply that the SI differentially modulates the quiet biting form of attack and it appears to do so primarily by acting upon the sensory component of the response mechanism.

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891 DIFFERENTIAL EFFECTS OF LESIONS IN POSTERODORSAL SEPTUM AND DORSOMEDIAL FRONTAL CORTEX ON SPATIAL ALTERNATION AT TWO INTERTRIAL INTERVALS. G. N. O. Brito and G. J. Thomas, Center for Brain Research, School of Medicine & Dentistry, University of Rochester, Rochester, N. Y. 14642.

Successful alternation in a T-maze is not based on discriminations of currently available environmental stimuli but is based on a brain-constructed "cognitive map" involving a "memory" of the direction turned on the previous trial. Rats were trained preoperatively for 9 sessions (13T/day, 1 session/day) to perform reinforced alternations with a minimal intertrial interval (ITI, c.a. 6 sec). Then they were trained for 9 more sessions by running them in squads of 4 (ITI, c.a. 90 sec). By the 9th session all animals were running near perfectly. The rats were then divided into 3 matched groups. One group (N=9) received small bilateral stereotaxically guided electrolytic lesions in the posterodorsal septum (1 ma. for 7 sec). Another group (N=9) received strip-like bilateral lesions in the dorsomedial frontal cortex produced electrolytically (1 ma. for 10 sec at each location). The third group (controls, N=5) received bilateral strip-like lesions in either the dorsolateral frontal cortex (2 mm from the midline) or in posterdorsal cortex. After postoperative behavioral testing, lesions were evaluated from whole-brain photographs and celloidin-embedded, cresyl-violet stained histological material.

As has been reported previously (Thomas, JCPP 92-1128, 1978), and also found in the present results, rats with lesions in the posterodorsal septum, when tested 2 wk postoperatively, dropped to random choices during early testing, but by the 9th session, they recovered to perform near perfectly as did all controls. When subsequently tested in squads of 4 (c.a. 90-sec ITI), controls were slightly impaired but they quickly recovered to near perfect alternation within 9 sessions. Rats with posterodorsal septal lesions never achieved better than random choices. On the other hand, lesions in dorsomedial frontal cortex had no differential effect, compared with controls, on alternation at either short or long ITIs.

The data suggest that interference with septo-hippocampal circuitry temporarily reduces the ability of rats to cope with spatial alternation at short ITIs, but at long ITIs they are permanently (at least for 9 sessions) unable to perform above a random level. On the other hand, lesions in part of the cortical projection field of mediodorsal thalamic nuclei have no effect on the animals' "memory-for-directions" at either ITI.

892 ACQUISITION OF HIPPOCAMPAL SELF-STIMULATION IS FACILITATED BY KINDLING OF THE CONTRALATERAL HIPPOCAMPUS. Kenneth A. Campbell* and N. W. Milgram. Dept. Psychology, University of Toronto, Scarborough College, West Hill, Ontario, Canada.

We have previously reported that while rats are usually very slow to learn to bar-press for hippocampal stimulation, acquisition is markedly facilitated by a pretreatment of 22 single daily electrical stimuli to the hippocampal electrode, leading to a gradual development of convulsive activity (kindling) (Brain Research, 159, 458). When applied to various limbic and cortical loci, this repeated stimulation program is known to produce two types of long-term changes: increased excitability near the electrode tip (Racine, EEG clin. Neurophysiol., 32, 269) and facilitation of neuronal transmission to secondary sites (Racine et al., Brain Research, 47, 262). We were interested in separating these two neurophysiological effects and determining which is necessary for obtaining facilitated acquisition of hippocampal self-stimulation. The "transfer" paradigm provides such a means: the facilitation of transmission produced by repeated stimulation to one site is largely transferable to homologous contralateral loci, and this transfer is especially rapid in the hippocampus. While 20-30 daily electrical stimuli may be required to elicit convulsive activity from a primary hippocampal electrode, only 1-3 stimuli then applied to the contralateral hippocampus will elicit a seizure. This transfer effect cannot be explained in terms of local excitability changes, since it occurs even after lesion of the primary site.

Twenty rats were implanted bilaterally with electrodes in the dorsolateral hippocampi: 14 received 30 single daily stimuli in one hippocampus; 6 controls were handled identically but not stimulated. All animals were then tested for self-stimulation: 7 prestimulated rats were tested on the prestimulation electrode (ipsilateral group), and the other 7 on the contralateral electrode. Acquisition time was expressed as the number of 15 min periods required to reach a criterion of 25 presses in 15 min. As expected, the control group learned very slowly ($\bar{X}$ = 24.8) and the ipsilateral group much faster ($\bar{X}$ = 9.1). The contralateral group learned almost as quickly as the ipsilateral group ($\bar{X}$ = 9.7), suggesting that facilitated acquisition is not dependent on an excitability change in the tissue surrounding the prestimulation electrode tip. The results are consistent with the hypothesis that development of hippocampal reward depends upon increased neuronal transmission to secondary sites.

893 EVOKED POTENTIAL AND ELECTROENCEPHALOGRAPHIC CHANGES CHARACTERISTIC OF THE STAGES OF KINDLING. David B. Chandler*, Dorothy E. Woolley* and Stephen R. Overmann. Department of Animal Physiology, University of California, Davis, CA 95616.

Adult female Long-Evans rats were implanted under anesthesia with bipolar electrodes in the right prepyriform cortex (PPC) so that the tips straddled the pyramidal cells, in the right hippocampus with the short tip in CA1 and the long tip in the hilus of the dentate gyrus (DG), and bilaterally in the amygdaloid complex (AMYG). Following recovery each animal was kindled by a daily 1-sec tetanizing burst (TTB), consisting of 300 µA, 0.1 msec pulses at 62.5 Hz, to the right AMYG. Averaged evoked potentials (AEPs) produced by AMYG stimulation (300 µA, 0.1 msec pulses at 1/sec) were recorded from the PPC and DG and were averaged over 30-sec periods by a programmable computer. Behavioral parameters described by Racine (1971), EEG and AEPs were recorded for 2.5 min before and 10 min after the TTB during the various stages of kindling. The animals were sacrificed after they exhibited 5 successives days of Stage V seizures. Positions of electrodes were verified histologically. After the TTB the AEPs recorded from the CA1 and DG showed a progression of changes in waveform, amplitude and peak latencies associated with the stages of kindling, whereas the AEP recorded in the PPC showed only a slight increase in latency and amplitude. In Stage I the DG waveform was a single positive wave (P1) with a peak latency of 20 msec. With the appearance of Stage II this waveform increased slightly in amplitude and latency following the TTB. In Stages III and IV the amplitude and latency of P1 increased progressively. In addition, a second positive wave (P2) appeared in Stage III and became more prominent than P1 during Stage IV. Stage V was characterized by further increase in amplitude of P2 and marked oscillations in the waveform. As the number of Stage V seizures increased, the waveform following the TTB was depressed. Prior to kindling the AEP from CA1 was a negative wave with a peak latency a few msec shorter than that of the DG AEP. Although reversed in polarity from the potentials recorded in the DG, CA1 AEPs showed a similar progression with the stages of kindling. As the severity of the seizures increased, the duration of the EEG after-discharge following the TTB increased progressively to reach a maximum in Stage IV. The after-discharge showed a spike and wave pattern which increased in frequency to reach a maximum in Stage IV or V. The results demonstrate that changes in AEPs were greater in the hippocampus than in the PPC during kindling. The changes in hippocampal AEPs occurred predictably with the progression in the stages of kindling and indicate that kindling produces an increased effectiveness of the amygdala input to the hippocampus. (Supported by NIH grant ES01503.)

894 THE ACCUMBENS CONNECTION: INPUT FROM MIDBRAIN AND HINDBRAIN. R. B. Chronister, L. E. White, Jr., R. W. Sikes, J. R. DeFrance. Dept. Anat. Neurosc. Univ. So. Ala., Mobile, 36688 and Dept. Neurobiol. Anat., Univ. Texas Houston, Houston, 77025.

Injections of HRP (RZ-3.0) were made into nuc. accumbens in rats and rabbits. Delivery was made either via regulated pressure injections or iontophoretically. Following a 24 hr. recovery the animals were perfused, the brains removed, sectioned and processed with the TMB technique. All sections were counterstained with toluidine blue. One striking feature of the HRP positive neuron is the relationship of many of labeled systems to regions of the periaqueductal grey around sulcus limitans. This area is characterized by both retrogradely labeled neurons and presumed anterograde transport of the HRP. For example, HRP positive axons can be seen running at the level of the inferior colliculus from the central grey to a localized zone in the cuneiform nucleus. Associated with these fibers are retrogradely labeled neurons in a restricted focus. In addition, labeled neurons can be found scattered throughout the ventral regions of the central grey down to regions of the caudal medulla where the system continues as labeled neurons in nucleus solitarius and the dorsal motor nucleus of X. As stated above, labeled neurons can be found in the caudal regions of the medulla Most of these are in the parvocellular regions of nucleus solitarius and in the dorsal motor nucleus of X. The solitarius projection is impressive and indeed bilateral. The positive cell appear to diminish near the commissural nucleus and is restricted to the caudal solitarius region. Scattered HRP neurons are also found in the regions of the caudal ambiguus-ventral reticular nucleus. With the exception of isolated HRP positive neurons in the reticular nuclei, little else is seen in medulla. Pontine levels are characterized also by scattered cells in reticular nuclei. In rostral pontine levels, a few HRP positive neurons can be seen in the ceruleus system. HRP positive neurons are also found in the medial parabrachial regions. Throughout pons, scattered neurons or neuron-like cells can be found in the marginal zone surrounding the ventral portions of IVth ventricle. In mesencephalon two systems stand out. One is the midline raphe system and the other the tegmental area. The raphe system extends from centralis superior through the nuclei associated with the MLF (annularis and linearis) to the dorsal raphe. Indoing so it crosses the decussation of the superior cerebellar peduncle. The ventral tegmental area has its strongest focus in the paranigral nucleus (especially pronounced in rabbit). It extends cadually across the midline and is bilateral. At these caudal levels it extends also up into the fibers of the ventral tegmental decussation. As noted by others, HRP positive neurons can also be found in the subs. nigra. (NIMH 1RO3MH32418-01).

895 TRANSECTION OF DIRECT SUBICULO-ANTERIOR THALAMIC FIBERS: EFFECTS ON SEQUENTIAL LEARNING. ROBERT E. DAVIS AND ERNEST W. KENT. Dept. of Psych., Univ. of Illinois, Chicago, Illinois 60680.

In previous studies we have demonstrated that selective transections of the direct subiculo-anterior thalamic fibers after they exit from the postcommissural fornix produce behavioral changes similar to those reported following large hippocampal or total fornix lesions (Davis and Kent, 1979; Hirsh and Davis, 1979). As an extension of this work the effects of damage to these fibers on the acquisition and performance of an operant response sequence was examined (SLT). This task involved learning to press in any order 4 closely placed levers extending from one wall of an operant chamber. Only the first response on any given bar was reinforced. After all 4 bars had been depressed at least once a trial was complete and the levers were retracted to a position outside the chamber as a reset signal to the animals. The number of total and perseverative errors were recorded separatively for each trial.

Initial procedures consisted of 14 days of CRF training. Following this animals were run for 14 days on the SLT; 10 trials/day. Animals with transections of the subiculo-thalamic fibers (AT) were impaired only on the initial days of SLT making more total errors than normals. On later days the performance of AT animals was not different from operated controls (OC) on either error measure. However, further analysis of the response patterns of both groups revealed that AT animals were adhering to different response strategies than were OC animals. Usually brain damaged animals adopted rigid response chains which they repeatedly performed. Controls exhibited a much more flexible response pattern.

The addition of more easily discriminable manipulanda (patterned stimuli on the bars) transiently disrupted performance of both groups. The total number of errors made by all animals was elevated on the first day of this manipulation but quickly returned to baseline levels. Cuing also increased the frequency of perseverative errors on the first day of introduction. Thereafter, cues selectively reduced the number of perseverative errors made by AT animals. Taken together these results suggest that AT and OC animals learn this task in two entirely different ways.

896 A COMBINED INTRACELLULAR HRP AND GOLGI ANALYSIS OF THE NUCLEUS ACCUMBENS SEPTI. J.F. DeFrance, J.E. Marchand, R.B. Chronister, R.W. Sikes, and J.I. Hubbard. Dept. of Neurobiology and Anatomy, Univ. of Texas Medical School, Houston, Texas 77025 and The Dept. of Anatomy, Univ. of South Alabama, Mobile, Alabama 36688.

The nucleus accumbens septi (NAcS) is an important paralimbic structure standing in relation to both the limbic system and basal ganglia. The cytoarchitectural appearance of the NAcS resembles that of the caudate-Putamen complex (Fox, 1940), but receives numerous limbic system projections. Most prominent among these are the ventral subicular projection via the fimbria (IFim) (Siegel et al., 1971; Swanson et al., 1977) and the amygdaloid projection via the stria terminalis (ST) (DeOlmos, 1972). The present study was undertaken to determine the morphological appearance of the NAcS neurons receiving monosynaptic IFim and ST input.

Rabbits were acutely prepared under Urethane anesthesia. The cortex and corpus callosum were removed to allow for the visual position of the microstimulation and recording electrodes. The recording electrodes were filled with HRP (4%-10%) in Tris buffer plus 1M NaCl. Extracellular unitary responses were recorded following IFim and ST stimulation and characterized with power and paired-stimulus testing. The cells were then injected with HRP. For the correlative Golgi analysis, NAcS tissue was prepared according to the Rapid Golgi and Golgi-Kopsch techniques.

Monosynaptically excitatory input via the IFim and ST is largely restricted to dorsal-caudal halves of the NAcS. From the HRP analyses, there are two distinctly different cell types which are the recipient of both inputs. Firstly, there is a small (8μ-15μ) cell with spiny dendrites. The dendritic pattern is spherical with the dendrites themselves being delicate. The cell body is spine-free with spine appearance commencing on the primary dendrites. Secondly, there is a large cell type (12μ-20μ) whose dendrites are essentially spine-free. The dendritic pattern is again spherical, but the dendrites are thicker. These appear to reside more medially and ventrally in the NAcS.

The Golgi analysis confirmed that these two cell types indeed exist in the NAcS. It further shows that the small spiny neurons are the dominant cell type being scattered throughout the nucleus.

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897 KAINIC ACID AND ELECTRICAL STIMULATION OF THE LATERAL SEPTUM: CARDIOVASCULAR AND ELECTROMYOGRAPHIC EFFECTS. Ariel Y. Deutch, L. Scott Clark*, and Lelon J. Peacock. Dept. Psych., Univ. GA., Athens, GA, 30602.

At sites in the lateral septum in which electrical stimulation (0.5 - 1.0 sec train of biphasic waves; pulse duration, 0.5 msec; frequency, 100 cps; 2.0 - 14.0 V) produced cardiodeceleration in the anesthetized rat (Equithesin, .25cc/100g), subsequent injection of kainic acid (4 μg in 1.0 μl, injected at 0.2 μl/min) typically results in a pronounced tachycardia. Within minutes after the conclusion of the kainic acid administration, sustained myoclonus is observed bilaterally in forelimbs, jaw, and neck. Equivalent volumes of saline in these locations do not result in these effects. Chemical and electrical stimulation of the caudate nucleus (1.5 mm lateral to the septal sites) produces no observable cardiovascular or muscular responses. The effects of electrical stimulation of the lateral septum are in agreement with the findings of Holdstock (Psychon. Sci., 9:37-38, 1967). The effects of kainic acid administration to the lateral septum on cardiovascular and electromyographic variables have not been previously reported.

898 PERCEPTUAL CUES OF REINFORCING BRAIN STIMULATION. Philippe De Witte and Michel Meulders. Lab. Neurophysiol. Univ. Louvain, av. Hippocrate 54/49, B-1200 Brussels, Belgium.

Rats with rewarding electrodes in the lateral posterior hypothalamus were trained to an avoidance paradigm. A brain stimulation delivered through the rewarding electrode is used as conditioned stimulus (CS). Test for generalization to other brain stimuli (substitute stimulus, or SS) were made by modifying the values of the electrical stimulus parameters (i.e. intensity, frequency, pulse duration, and train duration).

The results show that two types of gradients were obtained. A two-way gradient was obtained for pulse frequency and for train duration while intensity and pulse width modifications produced a one-way gradient. Three hypotheses could explain the generalization and discrimination phenomena observed : (1) Generalization may occur to the quantity of charge offered by the substitute stimuli ; (2) Generalization may depend critically on one or more than one specific parameter of the electrical stimulus ; (3) The gradient of the rate of self-stimulation behavior for the same electrical parameters as those tested for generalization shows a close relationship with these generalization gradients. With other words, SS should be generalized in terms of the reinforcement induced. To test this hypothesis, we considered the percentage of avoidance responses as a scale of perceptions of the reinforcing stimulations. We then calculated the efficiency percentage of all the brain stimulations to elicit a self-stimulation behavior. The function relating the strength of the reinforcing sensation, as estimated by the percentage of avoidance responses during the generalization tests, and the rewarding intensity, as measured by the scaling of the self-stimulation behavior was then computed. In all cases, the data fitted a power function. It appears thus that the perception of the rewarding value through a reinforcing brain area follows the same law as with other sensory modalities. It should be noted that the value of the exponents does not deviate much from unit. These data thus indicate that the calculated functions are isometric. In other words, a relative change in the rewarding intensity elicited by brain stimulations is accompanied by the same relative change in avoidance response. This may serve to suggest that, in the brain, no noise occurs between the transformation from the input, i.e. the intensity of the brain stimulation, and the output, i.e. the reinforcing sensation as estimated by the generalization tests. Our experiments show that "degree of reward" is a perceived dimension and that it is being processed according to the psychophysiological laws.

899 DIRECT AND ORTHODROMIC ACTIVATION OF NEURONS IN THE PARAVENTRICULAR NUCLEUS: INTRACELLULAR RECORDING AND STAINING IN SLICES OF RAT HYPOTHALAMUS. F. Edward Dudek, Brian A. MacVicar and Glenn I. Hatton. Dept. of Zool.and Erindale College, Univ. of Toronto, Mississauga, ONT. L5L 1C6 and Depts. of Psych. and Zool., Mich. State Univ., E. Lansing, MI 48824.

Electrophysiological analyses of the afferent and efferent pathways of the paraventricular nucleus (PVN) have been restricted almost exclusively to extracellular techniques, primarily because of the difficulty in obtaining intracellular records in vivo from the mammalian hypothalamus. In vitro studies with slices of rat hypothalamus have revealed that many cells in the PVN fire spikes spontaneously and can be stimulated extracellularly while recording intracellularly (Hatton et al., Soc. Neurosci. Abstr. 4: 346, 1978). We have combined these techniques with intracellular staining to study the inputs and outputs of anatomically identified cells in PVN.

Coronally oriented, hypothalamic slices (400-500 µm)were prepared from mature rats. Intracellular recordings were obtained with micropipettes filled with 5% Lucifer Yellow (LY) in 1M LiCl. An array of tungsten, stimulating microelectrodes was positioned ipsilaterally between the fornix column (FX) and the supraoptic nucleus (SON).

Intracellular recordings (40-70 mV action potentials) have, so far, been obtained from >15 cells with LY electrodes; ten of these cells were injected with LY before loss of the impalement and six were successfully stained and identified. Four of the stained cells were magnocellular neurosecretory cells, one was a parvocellular neuron in anterior PVN, and another was a medium-sized cell in posterior PVN. All of these cells could be activated with extracellular stimulation. The parvocellular neuron fired an antidromic spike to stimulation midway between FX and SON, but not to stimulation near FX. The medium-sized cell in posterior PVN and two of the magnocellular elements could be activated orthodromically by stimulation near FX. It has also been possible to record, stain and stimulate cells in SON. Although these data must be considered preliminary until a larger number of cells have been studied with more rigorous controls for current spread and mode of activation, they do indicate that it is possible to study the functional anatomy of afferent and efferent pathways of PVN and other hypothalamic nuclei with intracellular recording and staining.

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900 RELATIONSHIP BETWEEN HIPPOCAMPAL RSA AND SNIFFING DURING REVERSAL ODOR DISCRIMINATION LEARNING. Howard B. Eichenbaum, Foteos Macrides and Karen J. Shedlack*. Dept. Biol., Wellesley College, Wellesley, MA 02181, Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545 and Dept. Psych. and Brain Sci., Massachusetts Institute of Technology, Cambridge, MA 02139.

In rodents, active investigation is characterized by vigorous sniffing and the presence of hippocampal rhythmic slow wave activity (RSA) in the theta range. These two cyclic activities have been observed to entrain while the animal appeared to be investigating an odor. Quantitative analysis of this phenomemon would be aided by a method which induced an animal to sniff at odorous stimuli on repeated trials and under conditions in which the chemical compositions and concentrations of the stimuli can be well regulated. For this purpose, a paradigm involving multiple reversal odor discrimination was devised and the relationship between hippocampal RSA and sniffing during reversal learning was characterized with retrospective computer analyses based on the Fast Fourier Transform (FFT). Rats were trained in a shielded arena with a port in one wall behind which clean air or odors, generated by a flow-dilution olfactometer, were passed. Proximity to the odor was recorded with a photocell beam at the port. An animal could initiate a trial by crossing another photocell beam located 36 cm from the port, after which a door covering the port opened allowing access to one of two odors. For an S+ odor stimulus the rat could hold its nose in the port continuously for 2 sec in order to obtain a water reward delivered at a well below the port. No reward was given for S- odor trials, and animals learned not to hold their nose in the port on such trials. Sniffing was monitored with a thermocouple placed in the nasal cavity via an implanted guide-tube, and slow wave activity was monitored in the dorsal hippocampus with implanted bipolar electrodes.

Rats rapidly acquired a discrimination of phenethyl alcohol versus geraniol, and with increasing rapidity acquired successive reversals. The animals typically investigated the stimuli with bouts of three or more successive sniffs whose repetition rate often corresponded with the principal frequency component of the ongoing hippocampal RSA. Moreover, the FFT analyses verified that the rats timed these sniffs with a preferred phase relationship to individual cycles of the hippocampal RSA. The entrainments did not occur on every trial during criterion-level performance, but were particularly prominent soon after the onset of a reversal and before criterion was achieved. This suggests that the entrainments are not critical for odor discrimination, per se, but may be associated with the assessment of an odor's behavioral significance.

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901 THE COMPARATIVE EFFECTS OF STIMULATION OF SUBSTANTIA INNOMINATA, INSULAR-TEMPORAL CORTEX, AND LATERAL OLFACTORY TRACT ON SINGLE-UNIT ACTIVITY IN THE AMYGDALA OF THE ANESTHETIZED CAT. Philip A. Femano, Henry M. Edinger, and Allan Siegel. Depts. of Physiology and Neuroscience, Coll. Med. Dent. NJ, NJ Med. Sch., Newark, NJ 07103.

The effects of electrical stimulation of the substantia innominata (SI) on amygdaloid unit activity were compared to those observed during stimulation of the sylvian gyri (SG) and lateral olfactory tract (LOT) in the ketamine anesthetized cat. Glass microelectrodes were used to record extracellular unit activity from the basal, lateral, central, cortical, and medial nuclei of the amygdala. Stimuli were delivered through stainless steel electrodes located in the SI, SG, and LOT.

In the basolateral group, many more units responded to SI stimulation than to any other site. The proportion of responses that were excitatory differed markedly depending on the source of stimulation. Of all responses elicited by SI stimulation, 90% were excitatory. Likewise, the percentages of excitatory responses obtained from SG and LOT stimulation were 70% and 65%, respectively. The relative invariability of the onset latencies of most of these excitatory responses, together with other criteria, imply monosynaptic activation of amygdaloid units.

Of the more than 100 units in the basolateral amygdala tested by stimulation of all three sites, 92% were affected by stimulation of SI and/or SG. Only 10% of these showed a response to stimulation from both sites. This suggests that the SI and SG project to different populations of cells. Furthermore, these populations appear to be anatomically intermingled since no topographical segregation of responses was observed.

It is concluded that the SI may have as much, if not more, excitatory influence over amygdaloid neurons than the sensory processing areas. This suggests that the SI may influence the amygdala in the limbic control of motivated behaviors.

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902 EFFECTS OF ELECTRICAL STIMULATION OF THE FORNIX AND HIPPOCAMPUS ON NEURONAL ACTIVITY IN THE SUBICULUM. David M. Finch and Thomas L. Babb. Brain Research Institute and Reed Neurological Research Center, UCLA, Los Angeles, CA 90024.

Male Long-Evans strain rats were prepared for acute neurophysiological experiments under Nembutal (50 mg/kg, ip) or Equi-Thesin (3 ml/kg, ip) anesthesia, supplemented as necessary. Twisted bipolar stimulating electrodes were implanted in the fimbria-fornix and hippocampus under physiological control. Micropipets (30-90 Mohms, filled with 4% Pontamine Sky Blue Dye in 1 Molar K Acetate) were lowered through overlying neocortex to the subiculum, where extra- and intracellular neuronal activity was recorded. Electrical stimulation was with 0.2 msec pulses, usually of 50-200 uA intensity, presented at 1/2sec.

The great majority of subicular neurons responded to electrical stimulation of the fornix or hippocampus with inhibition. Response latencies ranged from about 5 to 15 msec and response durations from 50 to more than 500 msec. Intracellular recordings showed prominent IPSPs that could be inverted by passing 1-3 nA of hyperpolarizing current through KCl-filled electrodes. Less common, but still reliable, were excitatory responses in subicular neurons after stimulation of the fornix or hippocampus. These responses were usually characterized by a single action potential, immediately followed by an inhibitory period. Neither fornical nor commissural afferents were necessary for the responses described here; both inhibitory and excitatory responses remained in animals that had received sections of the fornix and hippocampal commissures prior to (by at least one week) the neurophysiological experiments.

The prominent inhibitory responses were similar to those reported for cells of the hippocampus proper after fornix stimulation and we propose a similar mechanism; antidromic activation of subicular cell axons projecting via the fornix (e.g. Meibach and Siegel, 1977, Brain Research, 124, 197-224), with consequent activation of a recurrent inhibitory circuit. We propose that the excitatory responses reflected activation of the caudally directed hippocampal efferent system (e.g. Swanson et al., 1978, J. Comp. Neurol., 181, 681-715).

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903 INTRAHIPPOCAMPAL INFUSION OF NOREPINEPHRINE OR CARBACHOL INCREASES LOCOMOTOR ACTIVITY IN RATS. Charles Flicker* and Mark A. Geyer. Dept. Psychiatry and Neurosciences, Sch. Med., UCSD, La Jolla, CA 92093.

Intracerebroventricular infusion of either norepinephrine (NE) or the cholinergic agonist carbachol has been reported to increase spontaneous locomotor activity in rats. One site at which this response may be mediated is the hippocampus. A NE input to the hippocampus has been demonstrated via the dorsal noradrenergic bundle originating in the nucleus locus coeruleus. The septohippocampal pathway is an establised cholinergic input arising from the medial septal nucleus. In this study we endeavored to mimic the presumed effects of transmitter release from these two projections by the direct infusion of appropriate agonists into the hippocampus of awake, behaving animals.

Male Sprague Dawley rats (350-400g) were chronically implanted under Nembutal anesthesia with guide cannulas aimed bilaterally for the rostro-dorsal hippocampus. After a week of recovery from surgery the animals were connected to sham infusion lines and acclimated to the experimental chamber for 30 minutes. The experimental chamber consisted of a 12"x24"x15" holeboard box with 11 holes in the floor and walls.

A 30-gauge needle at the end of each infusion line was attached to each cannula, the needle's tip protruding 1.5mm deeper than the cannula's. Animals were then placed in the holeboard where - in addition to visual observation of behavior - locomotor activity and hole pokes, as measured by beam breaks, and rearings, as measured by electrical contacts with a steel wall plate, were monitored by computer.

After a 20 minute pre-infusion period, the animals received a 40 minute infusion of d,l-norepinephrine HCL, carbamylcholine chloride (3 µg/µl dissolved in saline), or physiological saline at a rate of 25 nanoliters per minute. Each animal received all three treatments in counterbalanced order at intervals of 5-7 days. Some animals were pre-treated with systemic injections of a muscarinic antagonist, either atropine sulfate or methyl atropine (i.p. 1 mg/kg).

The mechanical disruption caused by the insertion of a 30-gauge needle or the slow infusion of saline into the hippocampus of the rat frequently induces "wet dog" shakes which are commonly associated with electrical seizure activity in the structure. Despite these symptoms of abnormal baseline activity, infusion of either NE or carbachol into the hippocampus produced a transient increase in spontaneous locomotor activity as compared to saline controls. Results obtained with the various treatments are compatible with the hypothesis that this behavioral activation is mediated by a noradrenergic-cholinergic interaction at the level of the hippocampus.

904 OBSERVATIONS OF FEAR BEHAVIOR IN RATS RECEIVING NEONATAL HIPPOCAMPAL X-IRRADIATION. Dennis Gallant*, Robert B. Wallace, and Evan R. Susser*. Laboratories of Developmental Psychobiology, University of Hartford, West Hartford, CT 06117.

The purpose of this study was to determine whether there were significant behavioral changes in rats that have undergone hippocampal degranulation as contrasted to non-irradiated control animals in a fear provoking situation. Based upon the types of behavior observed, inferences could be drawn concerning the possible role of the hippocampus in mediating a fear response in rats. 30 male Long-Evans hooded rats, inbred in our laboratories, were randomly selected for this study; 15 received focal neonatal irradiation of the hippocampus while the other 15 served as non-irradiated control subjects. Testing began when the animals were 90 days old. Each animal was tested over a three-day period in an apparatus designed so as to moniter general activity as well as proximity of each rat to an enclosure containing a live cat. On Day 1 of testing, baseline measures were obtained - the cat was introduced into the apparatus on Day 2 and removed 24 hours later - day 3 then allowed for a second set of readings in the absence of the cat. A fear index was computed for each subject (Kim et al. 1970). At the conclusion of the study all animals were perfused transcardially and the brains prepared for histology. Examination of the dorsal hippocampus in matched sections showed about a 70% reduction in the granule cells of the dentate gyrus in the irradiated animals. Behavioral results indicated that the degranulated animals were significantly more willing to approach the part of the apparatus that contained the cat than were the control subjects; they were also willing to drink from a water bottle placed at that end of the apparatus whereas the control subjects would never do so. Based upon these observations we find support for the conclusions of Kim et al. (1971); not only were the animals in their study that had undergone gross aspiration lesions of the hippocampus less fearful in the presence of a live cat but this also seems to characterize the animal that has undergone hippocampal degranulation. Possible implications of these results in terms of the response suppression model of hippocampal functioning are suggested.

905 EFFECTS OF AMYGDALOID AND HIPPOCAMPAL LESIONS ON FREE-OPERANT AVOIDANCE LEARNING IN THE RAT. Michael G. Gaston* (SPON: E. H. Gregory). California State Univ., Los Angeles, CA 90032

Amygdaloid lesions have been shown to produce marked deficits in all forms of avoidance paradigms so far investigated. Hippocampal lesions decrement an animal's ability to acquire passive avoidance responses, but are often found to enhance performance in active avoidance tasks. In the usual procedure, the aversive stimulus (shock) is cued by an external warning signal or by distinctive situational stimuli. The present study explored the effects of amygdaloid and hippocampal lesions in a nondiscriminated active avoidance (Sidman-type) situation which did not entail the use of external cues. In this free-operant paradigm, the subject was shocked at regular (shock-shock) intervals unless it made the correct response (lever press), in which case shock was postponed for a specified period of time (response-shock interval).

Subjects were 48 male Long-Evans rats. Sixteen animals received bilateral lesions aimed at the basolateral amygdala, 16 subjects were bilaterally lesioned in the dorsal hippocampus, and 16 rats served as sham-operated controls. The apparatus was a commercially available Skinner box. The shock-shock and response-shock intervals were, respectively, 10 and 30 seconds. Thus, shock could be avoided entirely if the animal responded at least once every 30 seconds; complete failure to respond resulted in a 300 msec, 1 ma pulse of shock being delivered every 10 seconds. All animals received 24 hours of training, four hours per day for six consecutive days.

Amygdala lesioned rats were severely impaired--they made only about half as many avoidance responses as Controls and received about twice as many shocks. They displayed little improvement across days and showed deterioration of performance within sessions. In terms of number of shocks received, the hippocampal rats improved across days and within each four-hour session. Their performance, however, was characterized by its inefficiency. They received almost as many shocks as Controls, despite making nearly twice as many bar presses. Control animals learned to space out their responses--to delay responding until the latter part of the response-shock interval. Hippocampal rats showed little evidence of such temporal discrimination. They tended to emit bursts of responses, although only the first bar press in such a flurry appreciably postponed the next shock.

Amygdala lesions seem to interfere with an animal's ability to attach an appropriate instrumental response to the internal cue of conditioned fear. Further, the results are consistent with the view that the hippocampus mediates mechanisms of response inhibition.

906 DISCRIMINATION LEARNING IN ADULT RATS AFTER EARLY NEONATAL X-IRRADIATION OF THE HIPPOCAMPUS. Russell A. Gazzara* (SPON: L. J. Pellegrino). Dept. Biol. Sci., Purdue Univ., W. Lafayette, IN 47907.

Focal X-irradiation of the hippocampus during the early neonatal period (200 R on days 2,3; 150 R on days 5,7,9,11,13,15) in rats results in an 85% reduction in the number of granule cells normally forming in the dentate gyrus. Among the behavioral effects common to both reduction in dentate granule cells and classical hippocampal lesions are: deficits in passive avoidance learning and spontaneous alternation, hyperactivity in the open field, and facilitated acquisition of two-way active avoidance. One of the etiological factors underlying this syndrome of behavioral abnormalities could be some form of attentional dysfunction. A discrimination learning paradigm is well suited to an analysis of attentional dysfunction since it allows control over the stimulus environment of the subject. Three aspects of discrimination learning were studied: task difficulty (low, moderate, or high), sensory modality (visual or tactile), and cue property (localized or diffuse). Seven experiments assessed the effect of dentate granule cell reduction on discrimination learning in a T-maze.

Three of the experiments in the visual sensory modality varied only in task difficulty: low (Bright/Dark), moderate (Bright/Dim I), and high (Bright/Dim II). The X-irradiated rats showed a learning deficit only in the reversal phase of Bright/Dim I but both in the acquisition and reversal phases of Bright/Dim II. The remaining experiment in the visual sensory modality, Black/White, differed from the Bright/Dim I experiment only in that the Black/White cue was located in the walls of the T-maze (localized cue) whereas the arms of the T-maze were diffusely lit in the Bright/Dim I experiment (diffuse cue). The X-irradiated rats showed a learning deficit in both the acquisition and reversal phases of the Black/White experiment. The remaining three experiments were in the tactile sensory modality. As in the first three experiments, these three studies varied only in task difficulty: low (Rough I/Smooth), moderate (Rough II/Smooth), and high (Rough III/Rough IV). The X-irradiated rats showed no learning deficit in either the acquisition phase or the reversal phase of both the Rough I/Smooth and Rough II/Smooth experiments. The Rough III/Rough IV experiment is still in progress.

These results are discussed in light of current theories of hippocampal function.

907 EFFECTS OF NEONATAL DEPLETION OF HIPPOCAMPAL DENTATE GRANULE CELLS ON RADIAL-MAZE PERFORMANCE. Lauren Gerbrandt*, Gary Thomasson* and Joel L. Davis. Dept. Psych., Calif. State Univ., Northridge, CA 91330, and Psychobiol. Res. Lab., V.A. Hosp., Sepulveda, CA 91343.

About 90% of presumed hippocampal CA 1 pyramidal cells show feature-extraction responses selectively to only certain spatial positions on an 8-arm radial maze (Olton et al., 1978a). That these spatial codes contribute to spatial performance by rats is suggested by the finding that extrinsic lesions of fornix or entorrhinal inputs to hippocampus result in a loss of these codes (Best, pers. comm.) and inefficient attainment of the 8 rewards placed at the end of each maze arm (Olton et al., 1978b). The present study investigates whether selective disruption of intrinsic dentate granule-cell inputs to the cornu ammonis, induced by neonatal x-irradiation, also results in inefficient radial-maze performance. Specifically, 4 groups of rats (6/group) were x-irradiated on postnatal days 2,3 (200R), and 5,7,9,11 (150R), on days 2,3 (200R), on days 2 (200R),7 (150R), or sham-irradiated, in order to induce varying amounts of dentate granule-cell agenesis. At 150 days of age (± 60 days), animals were trained to retrieve the 8 food pellets placed on each arm of the maze. Preliminary results indicate that even x-irradiation schedules which deplete up to 82% of dentate granule cells result in normal radial-maze performance (>7/8 correct choices). However, with this amount of granule-cell agenesis, rats abnormally continue to run the maze after all rewards are obtained. Attempts are made to increase the spatial difficulty of the task by reducing the number of extra-maze cues available, and to eliminate sequential-response strategies by maze rotation and delays after each choice. Groups are also tested for differences in general activity and passive avoidance. Rats with fimbria/fornix ablations are compared in maze performance to granule-cell-depleted groups. The results are relevant to spatial versus response-inhibition hypotheses of hippocampal function (O'Keefe and Nadel, 1978) and to whether sparing or recovery of function (Bayer et al., 1973) occurs in dentate-granule-cell depleted rats.

908 SUBSTRATES OF INTRACRANIAL SELF-STIMULATION OF THE THALAMUS IN THE RAT. Charles R. Gerfen* and Ronald M. Clavier. Dept. Anat., Northwestern U. Med. Sch., Chicago, Ill 60611 and Dept. Anat., U. British Columbia, Vancouver, V6T 1W5, Canada.

Intracranial self-stimulation (ICSS) has been reported previously with electrode placements in midline and intralaminar thalamic nuclei of the rat thalamus (Cooper and Taylor, 1967). The recently described anatomical relations of these nuclei with ICSS regions in the dorsal agranular insular (sulcal prefrontal) cortex and the substantia nigra have led us to initiate a series of studies of the substrates of thalamic ICSS. First, ICSS probes were placed into those thalamic nuclei in which labeled cell bodies were seen (in a previous study) after injections of horseradish peroxidase into the sulcal prefrontal cortex. Specifically the parafascicular and ventromedial thalamic nuclei were examined for ICSS properties. To date, it appears that ICSS is obtained from these nuclei, but not from the nuclei in their immediate vicinity. Further mapping of the thalamus for ICSS-related areas is in progress. A second study examined catecholaminergic (CA) and non-CA fiber pathways related to these thalamic ICSS sites. After stable bar-pressing rates were obtained from thalamic electrodes, anodal electrolytic lesions (1 mA/5 sec.) were made via the ICSS probes. After a 4-day post-lesion recovery period, the animals were sacrificed and perfused with ice-cold 4% paraformaldehyde solution in phosphate buffer, via an intracardiac needle. The brains were removed and treated in 2 ways: 1) The region within 1-2 mm caudal to the lesions was blocked and sectioned on the Vibratome for histochemical fluorescence examination of possible build-up of CAs; 2) The remaining parts of the brain were sectioned after 1 week of immersion in 10% formalin for silver impregnation treatment with the method of Fink and Heimer (1967). The pattern of CA and non-CA fibers in the vicinity of the ICSS electrode tips was then compared with that seen after similar lesions made via non-ICSS electrodes in the thalamus. In several cases, lesions made via ICSS probes resulted in CA build-up in axons caudal and medial to the electrodes. These axons may be related to the dorsal periventricular system. In a third study, we are trying to determine which systems, whether or not they contain CAs, that originate in, pass through, or terminate in thalamic ICSS regions are related causally to that behavior. This is being studied with pharmacological and histochemical experiments, and with the use of electrolytic or (where appropriate) 6-hydroxydopamine-induced lesions of their cell bodies of origin.

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909 CHANGES IN THE DENTATE GRANULAR CELLS FOLLOWING TETANIC STIMULATION OF THE ENTORHINAL AREA. J. B. Glenn, Margherita Marzotto* and Eva Fifková. Dept. Psych., Univ. Colorado, Boulder, CO 80309.

Since tetanic stimulation of the entorhinal area has a pronounced effect on spine dimensions of the dentate molecular layer which might be caused by increased protein synthesis, the aim of this paper was to study the effect of tetanic stimulation of the entorhinal area on the dentate granular pericarya. In EXPERIMENT I 6 controls, 3 of them with a sham procedure and 5 stimulated mice (30/sec for 30 sec) which survived stimulation for 15-90 min were used. The parameters examined were: the nuclear cross section area, the number of nucleoli and the density of membrane-bound ribosomes. There was no significant difference in these parameters between the shams and controls. However, following stimulation the nucleus was significantly larger (by 40%, $p<.05$), the number of nucleoli was increased by 22% ($p<.05$), the density of ribosomes remaining unchanged. Because of the effect of ACTH on protein synthesis and electrical activity in the hippocampus, EXPERIMENT II combined tetanic stimulation with the administration of $ACTH_{4-10}$. Twelve controls were injected either with saline (group 1) or ACTH (group 2) 15 min before sacrifice. Six saline injected mice which survived the stimulation for 1.5 min were used as another control (group 3). In 12 experiments ACTH was administered 5 min prior to the tetanic stimulus and 6 mice survived the stimulation for 10 min (group 4) and 6 mice for 55 min (group 5). In another 6 mice stimulation preceded ACTH administration by 15 min and the survival time was 70 min (group 6). In group 3 the nucleus was larger by 28% ($p<.01$) than in group 1 without a change in the number of nucleoli or ribosomal density. In comparison with EXPERIMENT I where longer poststimulation intervals showed both the nuclear enlargement and increased number of nucleoli, this result suggests that nuclear enlargement develops very quickly after stimulation and is within 10-15 min followed by an increased number of nucleoli. Between groups 1 and 2 there was no difference in all the parameters studied indicating that ACTH per se had no effect within the interval examined. Combination of ACTH with tetanic stimulation induced in group 4 nuclear enlargement (by 31%; $p<.01$) and increased number of nucleoli (by 39%; $p<.01$). Furthermore, also the density of membrane bound ribosomes became higher by 15% ($p<.05$). In group 6, where ACTH was applied 15 min after stimulation, the results were similar to those of EXPERIMENT I: nuclear enlargement by 40% ($p<.01$), increased number of nucleoli by 24% ($p<.01$) and unchanged ribosomal density. Tetanic stimulation affects the protein synthetizing system of granular cells which can be further accentuated by ACTH. [Supported by NIMH Grant MH 27240-04.]

910 CHARACTERIZATION OF POSSIBLE NEURONAL CIRCUITS IN THE OLFACTORY SYSTEM BY ELECTROPHYSIOLOGICAL AND HISTOLOGICAL METHODS. R. Guevara-Aguilar, L.P. Solano-Flores* and H.U. Aguilar-Baturoni. Departamento de Fisiología, División de Investigación, Facultad de Medicina, U.N.A.M., México 20, D.F.

The goal of our work has been to characterize the neuronal circuits and their possible function in the olfactory system. On this paper we described the results obtained applied three different techniques: horseradish peroxidase, evoked potentials and unit activity.

Horseradish peroxidase (HRP, Sigma type VI, 25%) was applied unilaterally in the olfactory bulb (OB) and in the olfactory tubercle (OT), of 20 rats and 4 cats. The HRP was delivered by microiontophoretic ejection by positive DC current (2.0-2.5μA 10 min) from glass micropipettes with tips of 25-40 μm. After 24 hr of survival time the animals were fully anesthetized and their brain perfused. The histological sections 50μ were processed following the Nauta technique. Labeled neurons were identified in the OT, prepyriform cortex (PPC), nucleus of the diagonal band, preoptic region, hypothalamus, zona incerta, mesencephalic reticular formation and other mesencephalic structures. A few cells had been observed in PPC and hypothalamus of the contralateral side.

Locus coeruleus (LC) electrical stimulation produced homolateral and contralateral evoked potentials in OB, OT and PPC. The latency of the first component of the evoked potential in the OT was 3.6 ms. In 10 rats unitary activity of the OB, OT and PPC was recorded with a glass micropipetted of 3-10 MΩ. The stimulation of the LC or the hypothalamus induced changes of the single unit activity. This results evidence olfactory afferent projections to the different structures and the electrophysiological results allowed us to give further support to the existence of centrifugal influences upon the olfactory system.

911 SENSORY BASIS FOR FIRING OF SPATIAL UNITS IN DORSAL HIPPOCAMPUS OF RATS. A. J. Hill (SPONS: P. J. Best) Dept. Psych., Univ. Virginia, Charlottesville, VA 22901.

"Spatial" units have been identified in micro-electrode recordings fron dorsal hippocampus of rats. Such units fire at their maximum rates only when rats are in specific regions of the recording space, called the units' "fields", and seem to be independent of details of the animals' behavior.

Spatial units were recorded from rats which had been trained to perform a spatial alternation task in an enclosed automated T-maze. Observations were made during and after various changes made within the recording environment. Visual changes included covering parts of the maze with striped cloth, varying the level and location of light sources and covering the rats' eyes with a rubber mask. Olfactory tests included thorough cleaning of the apparatus, and introduction of strong odors (cologne, acetone) or other rats into the field region. Auditory stimuli were changed by placing a speaker which emitted sharp clicks at various locations around the maze, and by inserting baffles or opening the top of the maze to change its acoustical properties. The rat's running path was altered by introducing hurdles and baffles into the maze. Finally rats were spun at 70 rpm in the start box between trials to produce spurious vestibular cues.

Spatial firing was eliminated in 3 out of 12 cases by visual alterations and in 6 out of 12 cases by changes in running path. No other changes were found. Results indicate that hippocampal spatial firing is based upon multi-modal sensory cues. In order to explore this aspect of sptaial firing in more detail, groups of rats were selectively deprived of either vision (by masking or enucleation), hearing (by chronic administration of neomycin antibiotic), olfaction (by intranasal $ZnSO_4$), or vibrissal input (by shaving). Spatial firing was found reliably in rats from each of the four groups. (Criterion was at least 2 cases from 2 different rats in each group. Mortality and treatment effectiveness varied between groups, making comparisons using large numbers of subjects unreliable.) Preliminary observations indicate that there are no differences between spatial firing found in any of the sensory-deprived rats and that found in normal animals. Results show that spatial firing is not, in general, a purely sensory response via any one of the above modalities.

912 FREQUENCY POTENTIATION OF RESPONSES TO FIMBRIAL STIMULATION IN RABBIT MEDIAL PREOPTIC NUCLEUS. John I. Hubbard* and Jon F. DeFrance. Dept. of Neurobiology & Anatomy, University of Texas Medical School, Houston, TX 77025.

Field potentials recorded in the medial preoptic nucleus (MPO) of urethane anaesthetized rabbits following ipsilateral fimbrial stimulation consisted of an initial spike-like negativity (latency to peak 2 msec) followed by a negative wave (latency to peak 5 msec). This in turn declined into a positive wave (latency to peak 10 msec). The position of recording and stimulating sites was confirmed by passage of fast green dye from the electrodes and later examination of fixed tissue in 120 µm frozen sections. The field was maximal in the MPO about 1 mm below the anterior commissure and 100 µm lateral to the midline.

All components of the field had similar thresholds and recording of single units suggested that the field was explicable by an entering volley, the excitation of MPO cells and the recurrent inhibition of these cells.

The negative and positive waves increased 2-4 times in amplitude during 8 stimuli at 2-12 Hz, the maximum effects being seen at 6-8 Hz, the natural output frequency of the hippocampus. There was 1-3 sec of post-tetanic potentiation.

913 PERICALLOSAL LIMBIC FORMATIONS OF DOLPHIN BRAIN. M.S. Jacobs*, P.J. Morgane and W.L. McFarland. Dept. of Path., NYU Coll. Dent., New York, NY 10010; Worcester Found. Exp. Biology, Shrewsbury, MA 01545; NIH, Bethesda, MD 20014.

Our initial cytoarchitectonic studies of the limbic lobe (LoL) in the cetacean brain have concentrated on the pericallosal (superior) limbic formations which are sharply and constantly delineated on the medial surface of the hemisphere by the callosal sulcus (SCC) and by the cetacean homologue of the sulcus cinguli we term the limbic cleft (ClL). Several significant differences are evident as compared to terrestrial mammals including man.

The cell architecture of the limbic cortex nowhere shows sharp transitions but exhibits very gradual and subtle changes as it is followed both outward from the limbic bank of SCC (peritectal area, Pt) over the free surface of the LoL (limbic area proper, ArL) to the limbic bank of the ClL, and also anteroposteriorly from the subgenual region to the splenium of the corpus callosum. In the cetacean brain the internal granular layer (layer IV) is weakly developed throughout the neocortex and is totally absent from the posterior limbic region in sharp contrast to the terrestrial pattern. Anterior limbic sectors are, overall, paucicellular as compared to posterior limbic sectors. Many of the cells present, however, are larger than found posteriorly. Cell density increases very gradually towards the posterior limbic region, the cells that appear being predominantly small in size. This increased cellularity in supragranular layers posteriorly results in the boundary between layers II and III being less distinct than anteriorly. The presence of these small cells in layer V and the tendency for larger pyramidal cells in that layer to scatter into the deep levels of layer III also make the boundary between layers III and V less clear than anteriorly.

Layers I and VI are wide, and the latter contains polymorphic cells of about the same size as in layer III. There is only a weak tendency for cells to organize into columns, a pattern distinctly different from that of primates. The area Pt is marked by the transition from archicortex to neocortex with gradual organization of the layers to the pattern found in ArL. From SCC to ClL the cell patterns of anterior and posterior limbic sectors become more definitive.

In summary, significant cytoarchitectonic variations from terrestrial patterns indicate that the cetacean limbic cortex reflects a special organization not found in land mammals. However, despite such differences, the dolphin shows a well laminated limbic cortex and areal differentiation into subsectors.

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914 SINGLE NEURONAL ACTIVITY IN THE ANTEROMEDIAL CORTEX DURING MOTIVATED BEHAVIOR. John P. Kanki*, Terry M. Skinner*, Harry M. Sinnamon. Lab. Neuropsych., Wesleyan University, Middletown, CT 06457

The anteromedial cortex (AMC) supports intracranial self-stimulation (ICSS) and is a site of orthodromic and antidromic activation from stimulation of other ICSS sites. The purpose of this study was to determine, in the unrestrained rat, the activity patterns of AMC neurons during ICSS, motivated behavior related to drinking, and the response to various sensory stimuli.

Rats were tested in a cylindrical chamber, the walls of which contained three holes set 90 degrees apart. Contact with a bar presented through any of these holes produced 50Hz stimulation of the ventral tegmental area which continued until the animal contacted a 15x15 cm. plate on the wall opposite the middle hole. The water-deprived rat was provided .01% saccharine through a tube presented also via these holes. Tests for sensory responsiveness included the presentation of a moving black-white grid, single clicks sufficient to elicit a startle response, various odors including conspecific urine, and tactile stimulation of the dorsal body and face. Neural activity was differentially recorded through any pair of 25 micrometer stainless steel wires, twisted into a bundle of four wires. The bundle was lowered without rotation by means of a chronically implanted microdrive. Well isolated units could be typically held for more than one hour.

A total of 51 single units have been studied in three rats. The most common well-defined class of neurons was related to orienting behavior (n=14). They increased in activity when the rat actively investigated any of several classes of stimuli. These units lacked any consistent pattern of activity during ICSS trains or during drinking, but they tended to show an increase at the behavioral response which produced stimulation onset. Activity in another seven units was related to head movements (six units increased while one decreased). Activity in three units was associated with sniffing, and another two units increased activity when the rat oriented to, or was situated at the locus of the offset plate. The largest class of neurons (n=19) showed no discernible pattern consistently correlated with any of the stimuli or behaviors studied. A final class of neurons (n=6) seemed to code aversive and rewarding states by increases or decreases respectively in rates of activity. Increases were seen when the animal showed distress to tactile stimulation, noxious odors, or the experimenter's hand. Decreases were seen when the rat oriented to conspecific urine, and to the holes in the chamber wall. Removal of the experimenter's hand, drinking, and the onset of stimulation were also associated with decreases in activity. These patterns of activity support the tentative suggestion that the AMC might be a site at which motivational information influences systems which participate in the control of orienting behavior.

915 MATHEMATICAL MODEL OF NEURONAL FUNCTIONS IN VARIOUS SUBPOPULATIONS OF THE HIPPOCAMPUS. W. R. Klemm, N. W. Naugle*, and G. M. Barnwell.* Dept. Biology, Texas A&M University, College Station, TX 77843.

We have developed a mathematical model of hippocampal function that is intended to describe the interactions of input activity with the activity of various intrahippocampal populations of neurons. The pools of neurons that are assumed to be functionally homogeneous include the septal and entorhinal sources of input, and the granule cells, CA field pyramidal cells, and basket cells. The model is bilaterally symmetrical, with activity of each homogeneous population in each hemisphere described as a first order nonlinear differential equation. Parameters are simulated, with activity defined in relative units ranging from 0 to 1.0. The 26 equations (13 identified pools in each hemisphere) are solved simultaneously by computer to produce plots of the time course of activity changes in each of the populations.

Each neuronal pool has its own specific version of the equation that is based on its known functional connectivities, but the general form of the equation is:

$$dX_i/dt = (X_i/X_{max})(X_{imax}-X_i)(IE_{xi} + \Sigma_j EE_{ij}X_j + \Sigma\ NnE_{xi}) - (\Sigma_k EI_{ik}X_k + \Sigma\ NnI_{xi}) - D_{xi}$$

where X_i = relative activity in any given pool, X_{imax} = maximum possible activity in any given pool, IE = internal (recurrent) excitation within a pool, EE = external excitaton from each of the various inputs to the pool, NnE = nonneural sources of excitation, EI = external inhibition from inputs to the pool, NnI = nonneural inhibition, and D = a decay constant (i.e. rate at which activity would decay in a given population if input ceased).

The simulations performed thus far seem to be useful in evaluating the population dynamics that might underlie such hippocampal phenomena as the electrographic "theta" rhythm. Solutions to certain of the equations can be made by appropriate parameter assignment to oscillate at different frequencies or made to reach a non-oscillating steady state of activity.

916 THE HIPPOCAMPAL CA1 REGION IN THE CHRONIC IMPLANTED RAT: A STUDY OF ANESTHETIC ACTION AND BEHAVIORAL STATES. L. Stan Leung. Dept. Psych., U. Western Ontario, London, Ont., Canada, N6A 5C2.

Stimulation of varying intensity was delivered via electrodes implanted in the anterior alveus (AA), Schaffer collaterals (Sch) and posterior alveus (PA) and averaged evoked potentials (AEPs) were recorded at different depths in CA1 region. Previous reports (Leung, Neurosci. Abst. 4: 224, 1978) showed that at least three synapses are involved in the generation of the AEPs in sodium pentobarbital (NEM) anesthetized rats: (1) excitatory (E-) postsynaptic potentials (PSPs) in str. radiatum; (2) EPSPs in str. oriens; and (3) inhibitory (I-) PSPs in str. pyramidale and radiatum. Similar AEPs with different component amplitudes could be found in the waking, immobile rat.

AEPs and behaviors were studied following treatment with ether (ETH) or NEM. From the AEPs, it was inferred that (1) both ETH and NEM attenuated the population EPSPs at doses that immobilized the animal except for the str. radiatum EPSP which was resistant to NEM; (2) population spikes evoked by high stimulus intensities were suppressed under ETH or NEM; (3) the population IPSP was only slightly affected by ETH but its amplitude and duration increased greatly at low intensity stimulation under NEM (>10 mg/kg i.p.). The results showed a non-uniform action of ETH and NEM in the hippocampus.

During behaviors that are accompanied by a hippocampal theta EEG, e.g., walking, rapid-eye-movement sleep, the Sch-AEPs (and less apparently, the AA-AEPs) characteristically showed a decreased initial peak and an increased second (and possibly third) peak occurring at an earlier latency as compared to AEPs during behaviors that are not accompanied by theta, e.g., immobile awake and slow-wave sleep. This behavior-AEP relation however virtually disappeared after a 50 mg/kg i.p. dose of atropine sulfate while the behavior-EEG relation could still be seen. Extension of a reported model (Leung, Biol. Cybernetics 31: 219, 1978) suggests that theta related behaviors increase the negative feedback gain within an interactive population of excitatory and inhibitory neurons, possibly mediated by muscarinic cholinergic synapses.

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917 RELATIONSHIP BETWEEN THE LIMBIC THETA RHYTHM AND NEURAL ACTIVITY IN THE OLFACTORY BULB. Foteos Macrides, William M. Youngs and Barry J. Davis. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

The present study examined the temporal relationship between the limbic theta rhythm, monitored as rhythmic slow wave activity (RSA) in the dorsal hippocampus, and the slow wave and spontaneous single unit activity recorded simultaneously in the main olfactory bulb (MOB). Adult male hamsters were paralyzed with d-tubocurarine and artificially respired with a mixture of nitrous oxide and oxygen. Pulsatile nasal airflow was eliminated so as to remove this influence on slow wave and unit activity in the MOB. In some preparations, infusions of physostigmine salicylate were used to increase the incidence of hippocampal RSA. Antidromic stimulation of the lateral olfactory tract was employed to distinguish recordings from output neurons versus interneurons.

Retrospective computer analyses of tape recorded data revealed a strong relationship between hippocampal RSA and neural activity recorded in the MOB. When the principal frequency component of the hippocampal slow wave record was in the theta range (as determined by the Fast Fourier Transform), slow wave activity of the same frequency also was prominent in the MOB. The FFT analyses demonstrated that these frequency components of the MOB slow wave activity bore consistent phase relationships to the corresponding components of the hippocampal RSA. Under these conditions, zero crossing analyses demonstrated that single units in the MOB fired with consistent temporal relationships to individual cycles of the hippocampal RSA. Significant correlations were found both for output neurons and for interneurons of the MOB, and in some cases the temporal relationships were as striking as those reported for neurons in the hippocampus itself. Additional analyses ruled out the possibility of spurious correlations introduced by heart- or respiration-related pulsation artifacts.

These results are interesting in view of our previous findings that MOB units fire in odor-specific, inhalation-related patterns which can be selectively enhanced or suppressed by appropriately timed activation of centrifugal inputs to the MOB, and our demonstration that during investigation of behaviorally relevant odors hamsters and rats often time their sniffs with a consistent temporal relationship to individual cycles of the hippocampal RSA. Our HRP studies have revealed centrifugal projections to the MOB from the vertical limb of the diagonal band, which is thought to be part of the pacemaker system for the limbic theta rhythm. The present results suggest that these or related centrifugal projections may interact with local circuits in the MOB to modulate the temporal firing patterns of MOB output neurons.

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918 MORPHOLOGY AND ELECTROPHYSIOLOGY OF DENTATE GRANULE CELLS: INTRACELLULAR RECORDING AND STAINING IN THE HIPPOCAMPAL SLICE PREPARATION. Brian A. MacVicar and F. Edward Dudek. Dept. Zool. and Erindale College, Univ. Toronto, Mississauga, Ont. L5L 1C6.

The granule cells of the dentate gyrus form a major relay in the basic circuitry of the hippocampal formation and represent an unusually homogeneous population of short-axon neurons. Although these cells have been the subject of numerous studies with extracellular recording, their small size (~10 μm) has limited electrophysiological analyses with intracellular recording techniques. The main purpose of these experiments was to determine whether granule cells exhibit fast prepotentials (FPPs), which have been extensively analyzed in hippocampal pyramidal cells.

Conventional, high-resistance micropipettes were used to impale granule cells in slices of rat hippocampus maintained in vitro. FPPs occurred in 9 (53%) of the 17 cells (action potential amplitude 40-70 mV) so far examined. In this group of 9 cells, 3 (33%) had spontaneously occurring FPPs. Excitatory postsynaptic potentials (EPSPs) from perforant path (PP) stimulation evoked FPPs in 5 (55%) of these cells whereas in 7 (77%) of these cells antidromic mossy fiber stimulation evoked FPPs. The FPPs of granule cells had many characteristics similar to dendritic spikes of pyramidal cells. They were constant amplitude (range 4 to 15 mV in different cells), occurred in an all-or-none fashion and had fast rising and falling phases. During orthodromic activation by stimulation of PP, they were observed on the peak of the EPSP or were revealed by hyperpolarizing current injection which blocked the soma spike. Antidromic activation also elicited short-latency FPPs that had a lower threshold than the full spike.

FPPs could arise from several mechanisms which are not mutually exclusive: 1) dendritic spikes; 2) electrotonic coupling; 3) axonal spikes. We are presently using intracellular staining with Lucifer Yellow to examine dendritic anatomy and to investigate the possibility of dye-coupling. These anatomical observations, combined with further electrophysiological tests, should help delineate the underlying source(s) of the FPPs.

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919 CONVERGENCE OF LIMBIC PROJECTIONS UPON SINGLE NEURONES IN THE VENTROMEDIAL NUCLEUS OF THE HYPOTHALAMUS OF THE RABBIT. J.E. Marchand and J.F. DeFrance. Department of Neurobiology and Anatomy, The University of Texas Medical School at Houston, P.O. Box 20708, Houston, Texas.

Electrophysiological techniques were used to record extracellular unitary potentials of neurones within the ventromedial nucleus (VMH). Stimulating electrodes (glass micropipettes, filled with 2 M NaCl) were placed in the dorsal and ventral components of the stria terminalis (ST) and the lateral portions of the fimbria. Animals were anesthetized with urethane (1g/kg, i.p.).

Cells within the VMH were activated from all three stimulation sites. Central ST stimulation evoked action potentials with little variability in latency, 8-10 msec. Dorsal ST stimulation activated the same neurones with a considerable variability in latency, 25-35 msec. Lateral fimbria stimulation activated the same neurones, also with a considerable variability of latency, 15-20 msec.

In addition to the excitatory influence dorsal ST and lateral fimbria stimulation effected upon neurones in the VMH, both stimulation sites produced an inhibition of spontaneous activity. This inhibitory period coincided in onset with the positive field potential evoked by each of these stimulation sites when recording within the VMH. This suggests that the positive field potential represents IPSP's evoked intracellularly, as first suggested by Murphy and Renaud (1969).

These studies provide evidence for convergence of ST fibers, which arise largely from the amygdala (De Olmos & Ingram, 1972) and lateral fimbria fibers, which arise from the ventral subiculum (Swanson & Cowan, 1977), upon single neurones within the VMH. The excitatory responses to dorsal ST and lateral fimbria appear to relay through one or more synapses, as evidenced by their variable latency. These relay centers might be located in more anterior areas of the hypothalamus or basal forebrain to which these fiber pathways are known to project (Swanson & Cowan, 1977, and DeOlmos & Ingram, 1972).

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920 DELAYED DEFICIT IN NEST BUILDING AFTER LATERAL OLFACTORY TRACT CUTS IN THE HAMSTER. David M. Marques, Robert J. O'Connell, Nicholas Benimoff* and Foteos Macrides. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

The lateral olfactory tracts of male hamsters were severed between the posterior end of the olfactory peduncle and the posterior end of the olfactory tubercle to determine the involvement of bulbar efferents in nest building and sexual behavior. Animals were tested during the week preceding surgery and during the first and third weeks after surgery for 1) nest building in large clean cages, and 2) sexual behavior with cycling females. They also were rated daily for the quality and size of nests and food piles in their home cages. Before sacrifice, animals were given a food sniffing test. Although, as expected (Devor, BRAIN RES. 64: 437, 1973), sex behavior was eliminated immediately in animals with complete cuts, most animals continued to build good nests during the first week after surgery. Nest building disappeared completely in these animals by the end of the second week. This unusual delayed deficit was observed both in home cage nest building and in the more sensitive clean cage nest building tests. Concurrent activity in the LOT thus appears unnecessary for nest building, but an intact LOT seems to be important for the continued normal functioning of a neural system which supports nest building. Slow degenerative changes in that system may account for this interesting delay in the appearance of the behavior deficit.

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921 THE EFFECT OF KAINIC ACID ON CHOLINERGIC ACTIVITY IN THE AMYGDALA-PIRIFORM CORTEX OF THE RAT. James McCaughran, Jr.* and Nisson Schechter. (SPON: A. Orr). Departments of Psychology, Psychiatry and the Long Island Research Institute, SUNY at Stony Brook, Stony Brook, New York 11794.

The local intracerebral injection of kainic acid, an analogue of glutamate, reportedly destroys intrinsic neuronal cell bodies but not afferent fibers. In the present study, kainic acid or phosphate buffer alone were injected into the amygdala-piriform cortex area of the rat. The effects on the cholinergic enzymes, choline acetyltransferase (CAT) and acetylcholinesterase (AChE), and the binding of the muscarinic ligand, ^{3}H-quinuclidyl benzilate (QNB), and the nicotinic ligand, ^{125}I-alpha bungarotoxin (aBuTX), were investigated. Twenty-four hr following the injection of kainic acid, CAT activity in the amygdala-piriform cortex area was reduced to 85% of the control. The reduction in CAT activity was time-dependent with the maximal reduction occurring at 5 days post-injection of kainic acid. Kainic acid also reduced the AChE activity by 17% at 24 hr and remained unchanged. At 24 hr, the binding of QNB and aBuTX was also unchanged. By 72 hr, the number of QNB and aBuTX binding sites were reduced to approximately 65% of the control. The number of sites fell to approximately 45-50% of the control by 5 days and remained at this level. In addition, at 72 hr the binding of QNB and aBuTX in the amygdala-piriform cortex area contralateral to the kainic acid injection was reduced to 85% of that of the same area in the control rats. The binding of QNB in the area contralateral to the injection site remained at 85% of the control. However, binding of aBuTX in the area contralateral to the kainic acid injection site fell to 60% of the control by 5 days and remained unchanged. The results indicate that the loss of QNB and aBuTX sites after kainic acid injection approximates the loss of CAT activity. Whether these binding sites are located on the proposed intrinsic cholinergic neurons or on some, as yet undefined, post-synaptic structure is not clear. The rapid and differential loss of aBuTX sites in the contralateral amygdala-piriform cortex after 5 days suggests either a presynaptic locus of these sites (presumably lost due to anterograde degeneration) or a postsynaptic locus of these sites (presumably lost due to retrograde degeneration). Regardless, the differential loss of aBuTX sites in the contralateral structure may reflect a difference in the neuronal localization of the muscarinic and nicotinic receptor sites in the amygdala-piriform cortex area, and further suggests the existence of reciprocal monosynaptic connections between these two areas.

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922 LOCAL CIRCUIT NEURONS OF THE OPOSSUM BASOLATERAL AMYGDALA: A GOLGI STUDY. Alexander J. McDonald* (SPON: N. S. Shah). West Va. Univ. Med. Ctr., Morgantown, WV 26505.

In this study 46 adult opossum brains were stained with the zinc chromate and Kopsch modifications of the Golgi technique. The neuronal morphology in all nuclei of the basolateral amygdala was found to be similar and four major neuronal classes were recognized. Class I neurons are spiny projection neurons which resemble those described in the cat, except that the axon hillocks and initial segments of these cells bear spines in the opossum. Class II, III and IV neurons appear to be local circuit neurons. Class II neurons are characterized by a paucity of dendritic spines and a moderate to very dense local axonal arborization. In the lateral nucleus these cells have small, round somata with 4-6 primary dendrites, while in the basal nuclei they have medium-sized fusiform or multangular cell bodies with 2-4 primary dendrites. Another variety of Class II neuron resembles the "chandelier cell" of the cat neocortex (Szentagothai, '75). The axons of these amygdaloid neurons have several major branches which give rise to beaded collaterals. These collaterals coil and fold back on themselves within a restricted area to form "axonal nests" that are 3-5 μm wide and 10-25 μm long. One neuron may form as many as 40 such nests and these are often interconnected by collaterals to form an extensive local axonal network. Axonal nests are randomly-oriented and have been seen embedded in an amber-colored, glial-like sheath. Class III neurons have small, spherical somata with 6-9 short, tortuous dendrites which sometimes appear to contact spiny dendrites of class I neurons. Dendrites have a moderate covering of spines; some of these spines have two swellings in series and an elongated stalk. Axons are rarely observed. Class IV neurons have very small cell bodies (6-10 μm) with 2-4 thin processes which branch sparingly and never extend more than 100 μm. It is often difficult to determine which, if any, of the processes is the axon.

923 AFFERENT CONNECTIONS OF THE AMYGDALA IN THE CAT. William R. Mehler, Patrick W. Mantyh* and Kevin D. Phelan*. Ames Res. Ctr., Moffett Field, CA 94035 and Dept. Anat. Univ. Cal. San Francisco, CA 94143.

Previous horseradish peroxidase (HRP) studies of the subcortical amygdalar afferent connections in the monkey (Mehler, et al., Anat. Rec. 190:477, 1978) and in the rat (Pretorius, et al., Anat. Rec. 193:657, 1979) confirmed the presence of some amygdalopetal cells described in the nucleus of the tractus solitarius and a large number of cells from the parabrachial nuclei at pontine levels in the rat (Ricardo and Koh, Brain Res. 153:1-26, 1978). In addition to these cells, HRP injections into various parts of the amygdalas in monkeys and rats also labelled cells in the dorsal and medial (Bechterew and linearis) raphe nuclei in the midbrain.

HRP injections of amygdalae of 18 cats (3.0 - 0.5 ul, 50%$^{+}$ solution, 24-52 hr survival, 1.5% glutaraldehyde - 0.5% paraformaldehyde/ pH 7.4, DAB or BDHC substrate) demonstrate comparable patterns of HRP-positive (HRP+) cells at pontine and mesencephalic levels. Large numbers of HRP+ cells delineate from the medial geniculate body the feline homologue of the simian and rodent peripeduncular nuclei.

In the hypothalamus, varying populations of HRP+ cells appear most consistently in the dorsomedial nucleus and in the lateral hypothalamic area at tuberal and mammillary levels. Labelled cells appear throughout the ventromedial nucleus only in the most medial amygdala injections.

The most imposing pattern of amygdalopetal cell labelling appears in certain ipsilateral subdivisions of the midline nuclei of the dorsal thalamus, namely; the paraventricularis (PA) and a cell group interposed perpendicularly between PA and the nuc. centralis medialis (Cem), the latter of which may also send axons to the amygdala. Cem, however, appears to project chiefly to the claustrum or adjacent cortical areas (Mantyh and Mehler, In preparation). Small HRP injections involving only the dorsal part of the amygdala fail to label PA but uniquely label nuc. subparafascicularis, for example, and also selectively labels cells in the bed nucleus of the stria terminalis not seen even in large ventral or lateral quadrant amygdala injections.

Although amygdalopetal labelled cells have been found to exist in the medial part of the nuc. medialis dorsalis in the rat (Pretorius, et al., 1979) there is no evidence, to date, for such connections in either the cats of the present series or in the monkey previously studied (Mehler, 1979, In press).

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924 PLACE UNITS ARE DIFFERENTIALLY DISTRIBUTED WITHIN HIPPOCAMPAL FIELDS IN THE RAT. Virginia M. Miller and Phillip J. Best, Dept. of Psychol., Univ.of Va., Charlottesville, Va. 22901.

Correlation between hippocampal unit activity and an animal's location in space has been reported during a variety of spatial discrimination tasks. Units which show significant change in firing rate dependent only on an animal's location in space and independent of the animal's activity in that location are called place units. Manipulations such as maze rotation which do not disrupt the animal's spatial task performance, do not disrupt place unit integrity. Further, if spatial task performance is debilitated by lesions of hippocampal connections, then place unit integrity is also disrupted.

The flow of information through the hippocampus proceeds from the entorhinal cortex to the dentate gyrus and then to CA-3 and CA-1. The present study addresses the questions: What is the relative distribution of place units within the hippocampal fields and dentate gyrus, and do lesions of hippocampal connections differentially affect the integrity of place units within each field?

Rats were trained to continuously traverse a radial 8-arm maze for food reward. Hippocampal unit activity was recorded and compared among 3 groups: control (C), no lesion; fornix lesion (FL); and bilateral entorhinal lesion (EL).

All of the 18 units recorded in the regio-superior (RS) from the C group were place units. That is, firing rate significantly increased when the rat traversed 1 or 2 particular arms of the maze. Upon 90^{o} rotation of the maze, all units in the C group persisted with increased activity in the original directional orientation. Either lesion, FL or EL, significantly reduced the number and degree of persistence of place units in the RS: in the FL group, 22 of 29 units were place units, 10 of 22 persisted; in the EL group, 10 of 19 units were place units, 1 of 10 persisted.

Fewer place units were found in the regio-inferior (RI) than in the regio-superior in all groups. Only 3 of 8 in the C group, 1 of 7 in the FL group and 4 of 7 in the EL group were place units in the RI. The degree of persistence during maze rotation was not different between the regios-inferior and superior within groups.

The reduction in number of place units in both RS and RI following lesion of hippocampal connections suggests that convergence of input is necessary for the establishment of a unit's place field. The differential distribution of place units within the hippocampal fields has implications as to the sequence of processing of spatial information. (Supported by grants NSF #SER7618457 and NIMH #16478 to PJB).

925 COHERENCE SPECTRAL STUDIES OF THE ELECTRICAL ACTIVITY OF THE LIMBIC SYSTEM AND CEREBRAL CORTEX. R. J. Morgan, C. C. Turbes and G. T. Schneider*. Dept. Biophysics and Physiol., Colorado State Univ., Ft. Collins., CO 80721 and Dept. Anat., Sch. Med., Creighton University, Omaha, NE 68178.

Estimates of coherence functions are used to investigate the relationships between signals in two areas of the brain. Some of these signals have been recorded from frontal, occipital and temporal cortex, amygdala, septal and accumbens nuclei. Recordings are made with cats using hardwire and radiotelemetry. This permitted analysis of electrical activity of the brain during free moving and limited motor behavior states.

The coherence of a signal, as 40 Hz rhythm and sensorimotor rhythm (12-14 Hz) (SMR) is a function of frequency. At each frequency of the power spectrum the coherence function has a value between zero to one which gives a measure of correlation or phase locking of the signal in two areas in the brain.

The coherence estimates of 40 Hz, SMR and theta rhythm are made during certain behavioral states, and during the action of central nervous stimulants and depressants. During action of depressants there is a decrease in coherence in the 15 Hz to 40 Hz frequency range. There is a decrease of coherence first between areas of the cerebral cortex followed by cortex and amygdala. In the 1 Hz to 5 Hz range, signals between the cortex and amygdala showed coherence levels higher than those between two areas of the cortex.

Central nervous system stimulants, the amphetamines, increased coherence in the 30 Hz to 50 Hz range between cortical areas and cortex and subcortical nuclei. In the 1 Hz to 5 Hz range there was an increase in coherence whether the data came from two cortical areas or a cortical and subcortical area. There is a shift of increased coherence in signals in the 40 Hz to 50 Hz range and higher frequencies. These findings were more evident in the cerebral cortex and amygdala data.

926 HIPPOCAMPAL INFLUENCES ON AMYGDALA UNITS IN THE AWAKE MONKEY. Frederic Morrison and Charles Poletti*. Massachusetts General Hospital, Boston, MA 02114.

Amygdala extracellular unit activity in the awake, restrained squirrel monkey was studied in response to hippocampal electrical stimulation. Hippocampal volleys elicited responses in 20% (96) of 476 units tested in three monkeys. The spontaneous firing rate of responsive units was higher than that of nonresponsive ones. Ipsilateral anterior hippocampal stimulation was more effective than posterior or contralateral stimulation. Hippocampal influence was topographically organized. In the six areas with more than 25 tested units, the basomedial nucleus has the highest percentage of responsive units (39%), followed by the accessory basolateral (33%), central (22%), basolateral (21%), and lateral (5%) nuclei, and the anterior amygdala area (4%). Initial excitation (E) was more prevalent than initial inhibition (I) in the central (90% E vs. 10% I) and basomedial (82% E vs. 18% I) nuclei; but initial inhibition was more common in basolateral (37% E vs. 63% I) and accessory basolateral (33% E vs. 67% I) nuclei. The mean response latency was 30.8 msec, ranging from 12-130 msec.

The basomedial nucleus appears to receive the most potent hippocampal influence with the highest percentage of responses to single and multiple shocks and smallest mean latency. In this nucleus there was a preponderance of short latency (12-20 msec), brief (less than 20msec duration) excitatory responses with little variability in latency to single shock stimulation; 16% of tested units in basomedial nucleus had these characteristics compared to only 1% of units in other amygdala nuclei. These response characteristics are consistent with a hypothesized relay of nonfornix hippocampal influences on basal forebrain and hypothalamus via the basomedial nucleus (Poletti and Sujatanond, Soc. Neurosci. Abstr. 3: 203, 1977).

927 INSULAR CORTEX AND AMYGDALA HAVE RECIPROCAL CONNECTIONS IN THE RHESUS MONKEY. Elliot J. Mufson, M.-Marsel Mesulam, and Deepak N. Pandya. Bullard and Denny-Brown Laboratories of the Beth Israel Hospital and Harvard Medical School, Boston, MA 02215 and the V.A.M.C., Bedford, MA 01730.

Connections of the insular cortex are currently being investigated with ^{3}H amino acid (AA) autoradiography as well as with horseradish peroxidase (HRP) histochemistry. In this report we present initial observations regarding the connections between insula and amygdala. Following ^{3}H-AA injections in the rostral part of the insula, widespread labeling was observed in the amygdaloid nuclei rostral to the anterior commissure, as well as in the central, lateral, and basal medial nuclei. On the other hand, injections of ^{3}H-AA confined to the caudal parts of the insula showed a more restricted pattern of label predominantly concentrated in the central and lateral amygdaloid nuclei. Our observations also indicate that there is further topographical organization among certain projections from the insula to individual nuclei of the amygdala. As far as the lateral nucleus of the amygdala is concerned, ^{3}H-AA injections into the rostral insula showed distinct concentrations of labeling in its anterior part while injections of more caudal parts of the insula showed a discrete locus of labeling in the more caudal and dorsal parts of this nucleus. In the basal medial nucleus, injections within the rostral insula resulted in labeling restricted to its medial aspect while more caudal injections resulted in additional labeling more laterally in the same nucleus.

In other experiments, HRP was injected into insular cortex. When the injection remained confined to the anterior insula, HRP-positive neurons were observed in several amygdaloid nuclei rostral to the anterior commissure. On the other hand, injections which also involved more caudal parts of the insula resulted in additional HRP-positive neurons within the lateral and basal medial nuclei.

Even though the enzyme and amino acid injections in most cases spread into parts of the claustrum, the extent of this involvement was quite minor. In conclusion, the data would indicate that the insular cortex and the amygdala in the rhesus monkey have reciprocal connections that are topographically organized.

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928 KAINIC ACID NEUROTOXICITY TOWARD HIPPOCAMPUS: DEPENDENCE ON SPECIFIC EXCITATORY PATHWAYS. J. Victor Nadler and Gilbert J. Cuthbertson*. Dept. Pharmacology, Duke Univ. Med. Ctr., Durham, NC 27710.

The potent convulsant, kainic acid (KA), readily destroys rat hippocampal neurons when injected locally or intraventricularly. In several regions of brain the neurotoxic action of KA depends on the integrity of excitatory inputs which are thought to be glutamatergic. We have investigated this issue by making selective lesions of hippocampal afferents and 3 days later injecting KA either intraventricularly (3.75 nmol) or locally (2.34 nmol) over a 30-min period. At the doses used in this study, intraventricular KA destroys only the CA3-CA4 cells, whereas locally injected KA destroys the majority of each neuronal type in the hippocampal formation.

Both destruction of hippocampal mossy fibers with colchicine and transection of these fibers markedly attenuated or abolished the subsequent toxicity of intraventricular KA toward CA3 cells. These pretreatments conferred no protection against KA injected locally. Conversely, removal of projections from the entorhinal cortex protected dentate granule cells and, to a somewhat lesser extent, hippocampal pyramidal cells from destruction by locally injected KA, but little affected the hippocampal toxicity of intraventricular KA. A commissurotomy did not greatly change the hippocampal lesion made by either route of administration. The entorhinal projection to dentate granule cells is probably glutamatergic, and some of the commissural fibers may be also, but the mossy fibers probably are not. Intraventricularly and locally injected KA destroyed somewhat different populations of neurons outside the hippocampal formation. All three types of deafferentation appeared to reduce this extrahippocampal damage.

These results emphasize the dependence of KA neurotoxicity on excitatory circuitry, in accordance with the idea that generalized and sustained seizure activity plays an important role in the etiology of KA-induced neuronal degeneration. The critical excitatory pathways probably need not be glutamatergic. Finally, the neurotoxicity of intraventricularly and locally injected KA involves somewhat different mechanisms. (Supported by NSF grant BNS 73-13051).

929 CONDITIONING OF HIPPOCAMPAL THETA ACTIVITY IN AWAKE, FREE-RANGING CATS. Arden V. Nelson*, William J. Jackson and June Kearns*
It has been shown that theta conditioning of Flaxedilized animals can be readily accomplished (Black, Am. Sc.,1971, 59:236-245; Glazer, JCPP, 1974, 86:267-273) but little work has been done with animals in their normal awake state. In the present study an attempt was made to condition the unique 4-7 Hz slow wave activity (theta waves) of the dorsal hippocampus in awake free-ranging cats. We used two different methods to detect the occurence of theta activity. One method calculated the ration of 4-7 Hz filtered activity to broad-band filtered (0.1-40 Hz) activity, and if the ratio was greater than the target value, a reward was given. The other method measured the wavelength and amplitude of the hippocampal activity and when a fixed number of waves met the wavelength and amplitude criterion, a reward was given. The rewards for both groups was electronic stimulation to a pleasure center in the lateral hypothalamus.

Two groups (one for each detection method) of three cats each were first trained to lever press for a reward to establish that the electronic stimulation had rewarding properties. Once this had been confirmed, the cats were conditioned for at least 30 days using a differential training method. At the end of the training period, each animal was also tested during an extinction trial. Initial examination of the learning curves indicated that only one cat learned the task, but closer examination of its learned activity showed that it had not learned to increase theta activity. Power spectrial analysis coupled with step-wise discriminate analysis did show that changes in the hippocampal theta activity did occur in some animals.
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930 SELECTIVE DISTRIBUTION OF PROJECTIONS FROM AMYGDALA TO PREFRONTAL CORTEX IN RHESUS MONKEY. Linda J. Porrino* and Patricia S. Goldman. Sec. Devel. Neurobiol., NIMH, Bethesda, MD 20205

The projections of the amygdala to prefrontal association cortex in rhesus monkey were studied using horseradish peroxidase histochemistry and autoradiography for tracing neural connections. Microquantities of HRP were injected into one of the five following subdivisions which together comprised virtually the entire prefrontal association cortex: (1) the superior prefrontal gyrus on the dorsomedial wall of the hemisphere; (2) the dorsal bank of the principal sulcus and adjacent dorsal convexity; (3) the ventral bank of the principal sulcus; (4) the lateral inferior convexity; and (5) the medial orbital and rectus gyri. After two days, the monkeys were perfused with mixed aldehydes and frozen sections were reacted with benzidene dihydrochloride.

Following HRP injections in the superior prefrontal, medial orbital and rectus gyri, labeled neurons were located in the lateral and basolateral nuclei of the amygdala. However, no reaction product could be located in neurons in any part of the amygdala following HRP injections into the cortex of either the dorsal or ventral banks of the principal sulcus or the cortex of the lateral inferior convexity.

To expose the full extent of amygdalo-prefrontal projections directly, all nuclear components of the amygdala were injected with tritiated amino acids. In serial sections processed for autoradiography, radioactivity was distributed in sharply circumscribed territories of the prefrontal cortex. Supporting our HRP data, silver grains were found only over the orbital cortex and gyrus rectus on the ventral and ventromedial aspects of the lobe and also over the superior prefrontal gyrus on the medial surface of the hemisphere. In marked contrast, label did not exceed background levels over the territories of the entire dorsolateral convexity that extend from the edge of the hemisphere to the longitudinal fissure, including the principal sulcus.

Thus, on the basis of amygdalofugal projections, the primate prefrontal cortex of rhesus monkeys may be divided into two sharply segregated systems: i) a ventromedial system which constitutes the entire prefrontal projection of the amygdala and ii) a dorsolateral system which is essentially devoid of amygdalofugal projections. The ventromedial subdivision seems to correspond to the prefrontal cortex of the rat in which projections from the amygdala, the mediodorsal nucleus of the thalamus and the ventral tegmentum overlap (Krettek and Price, 1977; Divac, et al., 1978). However, there appears to be no obvious homologue in the rodent comparable to the extensive amygdala-free dorsolateral convexity so prominent in primates.

931 BACLOFEN DOES NOT BLOCK THE SYNAPTIC ACTION OF THE PERFORANT PATH ON HIPPOCAMPAL CELLS. W. Reinhardt*† and Y. Ben-Ari*+(SPON: B. Milner). Dept. Research in Anaesthesia, McGill University, 3655 Drummond St., Montreal PQ Canada. H3G 1Y6

The β-(4-chlorophenyl) derivative of GABA (approved name, baclofen; proprietary name, Lioresal) depresses the release of endogenous excitatory transmitters - in particular glutamic acid - evoked in cortical slices by electrical stimulation (Potashner, J. Neurochem. 32 (1979) 103). Furthermore, Fox *et al.* (Neuroscience, 3 (1978) 495) have suggested that systemic baclofen blocks primary afferent synapses by a presynaptic action, perhaps due to a depression of glutamate release. We have investigated the effects of baclofen on transmission from the perforant path (originating in the entorhinal cortex and projecting to the fascia dentata, and CA3 area). The excitatory transmitter released from the perforant fibers is believed to be glutamic acid (Nadler Nature, 360 (1976) 538). Baclofen (CIBA-Geigy) was administered systemically (i.v. or i.p., 0.2-20 mg/kg) or *in situ* (see below) in male Sprague-Dawley rats. The animals were kept under nembutal or dial anaesthesia and bipolar concentric metal electrodes were placed in the entorhinal cortex, fascia dentata and CA3. The field potentials (negative in the dendritic layer, positive in the cell body layer, latency 5-8 msec) were similar to those previously described by Lømo (Exp. Brain Res. 12 (1971) 18). They also manifested the typical frequency potentiation, and post tetanic potential which are characteristic of the perforant path response (ibid). Systemic baclofen, in doses which abolished the withdrawal reflex in response to electrical stimulation of the paws, had no effect on the hippocampal field potentials. In another experimental series, hippocampal responses were recorded using a micropipette (filled with NaCl 2M) and baclofen (5 μg/μℓ applied *in situ* via an adjacent pipette (50 μ tip diameter, 100 μ distance from the recording pipette). Baclofen (up to 10 μℓ) did not provoke the prolonged depression previously described in the spinal cord (Fox *et al., ibid*). In contrast, local applications (via another pipette) of Mn (250-1000 mM, 1 μℓ) completely blocked the responses.

These results suggest that if the perforant path indeed releases glutamate, then one must conclude that this system differs from other glutaminergic terminals. One cannot exclude the possibility that baclofen blocks transmission by another mechanism.

†Fellow of the Deutsche Forschungsgemeinschaft.
+Fellow of the C.N.R.S. (France).

932 DIENCEPHALIC AND BRAINSTEM AFFERENTS TO THE HIPPOCAMPAL FORMATION OF THE RAT. J.N. Riley, E.R. Marchand, and R.Y. Moore: Dept. Neurosciences, Univ. California San Diego, La Jolla, CA 92093

The afferents to the hippocampal formation (HF) of the rat were studied with the horseradish peroxidase (HRP) retrograde tracing method. Our results indicate that the HF receives a projection from virtually the entire hypothalamus, several thalamic nuclei, and portions of the brainstem that have not been reported previously to project to the HF.

HRP was injected or deposited microelectrophoretically in the dorsal or ventral HF of 25 adult female Sprague-Dawley rats. Following survival times of 16-28 hrs., animals were perfused and frozen sections were processed by a modified benzidine dihydrochloride procedure.

In the thalamus, labeled neurons were found in the nucleus reuniens, paraventricular nucleus, ventromedial nucleus, and zona incerta. The projection from nucleus reuniens appears to be topographically organized: dorsal HF injections label cells predominantly in the dorsomedial part of the nucleus; ventral HF injections label cells predominantly in the ventrolateral region.

In the hypothalamus, scattered neurons were observed in virtually all hypothalamic nuclei and areas. Hypothalamic regions containing labeled neurons are too numerous to list here. The supramammillary region contained the largest number of labeled cells. This projection appears to be topographically organized: dorsal HF injections label cells in the anterior-lateral region; ventral HF injections label cells in posterior-medial region.

In the brainstem, labeled neurons were observed in the mesencephalic raphe (both raphe dorsalis and central superior) and nucleus locus coeruleus, as reported previously. Labeled neurons were also observed in a restricted portion of the interpeduncular nucleus (pars dorsalis magnocellularis) and surrounding ventral tegmental area, the region corresponding to the location of group B9, the so-called dorsal tegmental nucleus pars lateralis, and the region caudal to the nucleus raphe dorsalis apparently corresponding to the nucleus paramedianus dorsalis of Ziehen.

These results indicate that the HF receives a larger number of diencephalic and brainstem afferents than has been reported previously and provide additional anatomical support for Nauta's (1958) formulation of a "limbic midbrain" region.

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933 EFFECTS OF MORPHINE AND OPIOID PEPTIDES ON CELLULAR ACTIVITY IN THE HIPPOCAMPAL SLICE. J.H. Robinson* and S.A. Deadwyler* (SPON: J.G. McCormick). Dept. of Physiology and Pharmacology, Bowman Gray Med. Sch., Winston-Salem, NC 27103.

Morphine, methionine enkephalin and other opioid peptides were investigated with respect to their influence on hippocampal neural activity. Field potentials and spontaneous cellular activity were studied following the addition of morphine sulfate, methionine enkephalin or [D-Ala,^{2} Met]-enkephalin to the bathing media of rat hippocampal slices maintained in vitro. Recordings from the CA1 molecular and cell layers showed that morphine sulfate (5.0×10^{-5} -1×10^{-3} M) produced a dose-dependent increase in the amplitude and number of population spikes elicited by Schaffer collateral stimulation. These changes were not accompanied by increases in the dendritically located synaptic current or the presynaptic fiber volley. Input-output curves revealed that morphine sulfate (concentrations > 1.0×10^{-4} M), 1) decreased the threshold for initiation of the CA1 population spike 2) increased the amplitude of the population spike at lower stimulus intensities, and 3) increased the number of population spikes per stimulus pulse at higher stimulus intensities (creating a multiple discharge effect). The effects of morphine at the lower stimulus intensities could be partially reversed by the addition of naloxone hydrochloride (1.0×10^{-4} M) to the bathing media, but the multiple population spikes elicited at higher stimulus voltages were not affected by naloxone. However, pentobarbital sodium (2.0×10^{-4} M) was effective in reducing the number of population spikes elicited by orthodromic stimulation in morphine treated slices at all stimulus intensities, without significantly changing the amplitude of the primary population spike.

Intracellular analyses verified the morphine induced hyperresponsiveness in CA1 cells. Increases in the rate of spontaneous discharges, decreases in the amplitude of stimulus induced IPSPs and the appearance of depolarizing after potentials (DAPs) were observed within 5-20 min following localized opiate application to the dendritic and somatic regions by microperfusion technique. In most cases, these treatments lead to spontaneous after discharges in CA1 cells which persisted well beyond the time of drug application. Other opioid compounds mimicked these results. These effects are similar to those reported by other investigators following the application of penicillin to the hippocampus (1). Our results indicate that specific as well as nonspecific influences of opioids may be responsible for the excitatory effects of these agents on hippocampal tissue

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934 ACCUMBENS CONNECTIONS: INPUTS FROM THE TELENCEPHALON AND DIENCEPHALON. R.W. Sikes, R.B. Chronister, J.E.Marchand, and J.F. DeFrance. Department of Neurobiology and Anatomy, The University of Texas Medical School at Houston,P.O. Box 20708, Houston, Texas and The University of South Alabama, Mobile, Alabama.

Iontophoretic and micropressure injections of horseradish peroxidase through glass cappillary electrodes were made into the nucleus accumbens of fifteen adult rabbits. The size of the injections varied considerably but were usually restricted to the anterior part of the nucleus with minimal diffusion into adjacent regions. The animals were allowed to survive for one to two days. The tissue was processed with either the diaminobenzidine(LaVail,1973) or the tetranitrobenzidine technique (Mesulam,1976) The later method proved to be quantitatively superior to the diaminobenzidine method, and the following results were derived from animals processed with this method.

Marked concentrations of labeled neurons were found in the ventral subiculum. The cells were restricted to the part of the subiculum adjacent to the CA1 field of the hippocampus. The numbers of labeled cells decreased dorsally, but the location of the neurons was constant. Many labeled neurons were also found in the medial and lateral entorhinal cortex. Scattered neurons were labeled in layer five of the cingulate gyrus beneath the lateral sulcus and in the midline frontal cortex. Amygdaloid projections originate in the caudal central,basal,and cortical nuclei, and septal projections originate in the caudal dorsal septal nuclei.

The dorsal and ventral periventricular nuclei of the thalamus contained many labeled neurons. Scattered neurons were also seen in the dorsal medial and centromedial nuclei. Labeled neurons in the hypothalamus were restricted to the lateral anterior, lateral preoptic, and paraventricular nuclei.

Finally, neurons were located in the region surrounding the habenulopeduncular tract, the area just lateral to mammilary peduncule, and the ventral striatal region.

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935 EFFECT OF RAPHÉ STIMULATION ON GRANULE CELL ACTIVITY IN THE HIPPOCAMPAL DENTATE GYRUS. Robert S. Sloviter* and John D. Connor. Department of Pharmacology, Pennsylvania State University College of Medicine, Hershey PA 17033

Neurons in the entorhinal cortex innervate (via the perforant path) the dendrites of granule cells in the hippocampal dentate gyrus. Stimulation of this pathway evokes a field potential which reflects EPSPs in granule cell dendrites. Superimposed on this potential is a "population spike" which represents action potentials generated simultaneously in many granule cells. The amplitude of the spike is proportional to the number of granule cells firing (Lømo, 1971).

During stimulation of the perforant path, coincident stimuli to the median raphé nucleus cause a rapid increase in the granule cell spike amplitude (often 7-10 fold). This response is highly reproducible and site specific (n = 40). Injection of 5-methoxy-N,N-dimethyltryptamine (5-MeODMT), a serotonin (5-HT) receptor agonist, mimicked the effect of raphé stimuli, but only at parameters of entorhinal stimulation where raphé stimuli were effective (n = 8).

Anatomical evidence (Moore & Halaris, 1975) suggests that 5-HT neurons innervate basket cells which lie in the hilus of the dentate gyrus. These basket cells are believed to be responsible for a γ-amino butyric acid (GABA) mediated recurrent inhibition of granule cells. Intravenous injection of bicuculline and picrotoxin (GABA antagonists) also increased the spike amplitude at all perforant path stimulus parameters (n = 6). Strychnine was without effect (n = 6). A number of cells with firing characteristics expected of these inhibitory interneurons i.e., high frequency and long duration, have been recorded in the hilar region. Efforts are underway to determine the effects of raphé stimulation on the firing characteristics of these cells and of granule cells.

While single unit studies show mainly inhibitory effects of raphé stimulation (e.g., Segal, 1975), our experiments suggest that the net effect of 5-HT release in the dentate gyrus, under these design conditions, is excitatory. Although not proven, the available evidence favors an inhibitory action of 5-HT on inhibitory interneurons.

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936 THE EFFECT OF MORPHINE ON THE EXCITABILITY OF HIPPOCAMPAL PYRAMIDAL CELLS. Stanley, J.C., DeFrance, J.F., Taber, K. and Dafny, N. Dept. of Neurobiology and Anatomy, Univ. Texas Medical School, Houston, TX 77025.

The hippocampal formation (HF), a major component of the brain's limbic system, is thought to mediate part of the opiate response (Simon and Hiller, 1978). Therefore, the effect of morphine with respect to the excitability of hippocampal pyramidal cells was studied in rabbits acutely prepared under Urethane anesthesia.

Glass microelectrodes configured in either a 3-barrel or a 4-barrel array were used for recording and the iontophoresis of drugs. The drug ejection barrels contained either morphine sulfate (0.1M, pH 6.5) or naloxone hydrochloride (0.1M, pH 6.3) mixed in de-ionized H_2O.

The analysis of the morphine effect was based upon HF field potential changes. Monosynaptically activated field potentials were recorded in CA1 following microstimulation of the contralateral field CA3.

The results indicate that: (1) morphine has a potent excitatory effect upon hippocampal pyramidal cells, (2) the most effective layer is within the pyramidal cell body stratum, (3) the excitatory effect is readily reversed by naloxone, and (4) there appears to be a "supersensitivity" phenomenon.

The fact that at subthreshold stimulus intensities for the population spikes, the positive component is diminished by morphine with a subsequent appearance of the population spike is consistent with the conclusion of Siggins et al. (1978) that morphine enhanced pyramidal cell excitability by the inhibition of interneurons.

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937 EVIDENCE FOR THE ACTION OF DIAZEPAM ON BOTH HIPPOCAMPAL PYRAMIDAL CELLS AND INHIBITORY INTERNEURONS. K.H. Taber, J.F. DeFrance and J.C. Stanley. Dept. of Neurobiology & Anatomy, Univ. of Texas Medical School, Houston, TX 77025.

Diazepam is known to alter the excitability of hippocampal neurons. We have found that diazepam, administered iontophoretically, suppressed the post-tetanic potentiation (PTP) of hippocampal pyramidal cells, while at the same time enhancing tetanic potentiation (TP). This study was undertaken to clarify the mechanism of the enhanced TP following diazepam administration.

Rabbits were acutely prepared under Urethane anesthesia. The cortex and corpus callosum were removed to allow for the placement of electrodes under visual control. Glass microelectrodes configured in a 3-barrel array were used for recording and the iontophoretic delivery of diazepam (0.05-0.1M, pH 3.5). Analysis of the diazepam effects were based upon field potential changes in various layers of hippocampal field CA1. Monosynaptically activated field potentials were recorded following microstimulation of the contralateral field CA3.

A paradigm was used which included 7 control stimuli at 0.5Hz, 8 tetanic stimuli at 6Hz, and up to 15 post-tetanic stimuli at 0.5Hz. With the recording electrode in the pyramidal cell layer, and utilizing a stimulus intensity subthreshold for the appearance of the population spike, the response is dominated by a large positivity. This component appears to be the current flow associated with IPSPs within the pyramidal cells. The positivity undergoes marked TP. Diazepam, however, has the effect of suppressing the TP. When using stimulus intensities sufficient to generate population spikes, diazepam has the effect of enhancing spike TP. It is suggested that diazepam has an effect upon the interneuronal population hereby enhancing pyramidal cell excitability at high rates of stimulation.

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938 PRESUBICULAR, PARASUBICULAR, AND POSTEROMEDIAL TEGMENTAL PROJECTIONS TO THE RAT HIPPOCAMPAL FORMATION. Konrad Talbot (1) and Glenn J. Giesler, Jr. (2). Department of Psychology, UCLA, Los Angeles, CA 90024(1) and Marine Biological Institute, Galveston, TX 77550(2)

Hippocampal formation (HF: hippocampus + dentate area) afferents have been repeatedly studied in the rat with diaminobenzedine (DAB) horseradish peroxidase (HRP) histochemistry (e.g., Pasquier & Reinoso-Suarez, Br. Res. Bull. 3:373, 1978).Recently, however, Giesler et al.(J. comp. Neurol. 184: 107, 1979) have demonstrated that the de Olmos o-dianisidine (OD) HRP technique (Exp. Br. Res. 29: 541, 1977) can reveal far more completely the number and morphology of retrogradely labelled neurons than does the standard DAB method of Graham and Karnovsky (J. Histochem. Cytochem. 14: 291, 1966). We have thus reexamined HF afferents utilizing OD HRP histochemistry.

Albino rats were injected with 0.04-0.08ul of 30-50% Type VI HRP solutions into (1) medial (N=6) or lateral (N=4) dorsal HF, (2) ventral HF (N=4), or, for control material, neocortex adjacent to the dorsal (N=3) or ventral (N=2) HF. Animals were sacrificed 1-2 days postoperatively and brain tissue reacted for HRP according to the de Olmos (1977, loc. cit.) OD method.

While all HF afferents reported in previous HRP studies were confirmed, several additional input sources were discovered: (1) many nerve cells in the ipsi- and contra-lateral principal external lamina of the pre- and para-subiculum following enzyme injection into the dorsal HF, (2) an appreciable number of neurons in the ipsi- and contra-lateral principal external lamina of the parasubiculum after HRP infusions into the ventral HF, and (3) a small group of neurons in the posteromedial tegmental nucleus of Morest (J. Anat. [Lond.] 95: 229, 1961) after enzyme injections into the dorsal and, to a lesser extent, ventral HF, unilateral in the former case and bilateral in the latter.

Two other findings were not predicted by earlier HRP work. First, nerve cells in the principal internal layer of the entorhinal cortex are labelled only when a high enzyme concentration has been delivered to the posterior HF (crus and/or ventral HF). Second, HF commissural neurons linking strictly homolateral HF areas constitute most, if not all, of the nerve cells in the dentate hilus and stratum pyramidal of CA3, as well as an appreciable number in stratum pyramidal of CA2.

939 ELECTRICAL ACTIVITY OF THE CEREBRAL CORTEX AND LIMBIC NUCLEI DURING REPEATED DRUG INDUCED DESYNCHRONIZATION. C. C. Turbes, G. T. Schneider* and R. J. Morgan. Dept. Anat., Sch. Med., Creighton University, Omaha, NE 68178 and Dept. Biophysics and Physiol., Colorado State Univ., Ft. Collins, CO 80721.

Recordings were made on cats using radiotelemetry. This made it possible to study the electrical activity of the brain during free movement behavior. Early spectral analysis studies, after repeated doses of dextro and levo amphetamine, had shown changes in power in the theta, sensorimotor rhythm (SMR) and the 40 Hz rhythms. The loss of power in these signal rhythms seen in the initial treatment with amphetamine showed an increase in power in the second and third doses with a persistence of specific drug related symptoms.

In this paper we are looking at the coherence functions of these rhythms in the same areas of the brain and on the same data used in frequency spectral studies. Technically, we estimate coherence functions since all of our experimental data are time limited. It is important in relating signals, in recordings of brain electrical activity, to behavioral or drug induced events that averaging of spectrograms is very important. A sufficient number of data samples must be averaged to satisfy normal statistical criteria. During action of amphetamines coherence spectral estimates show an enhancement of the 30 Hz to 50 Hz frequency range between cortical areas and cortical and subcortical nuclei. There is also an increase in power and coherence in frequencies above 100 Hz. These findings were apparent between the amygdala and nucleus accumbens, amygdala and sigmoid gyrus, and sigmoid gyrus and nucleus accumbens of the same side. Also, there is an increase in power and choerence in the 1 Hz to 5 Hz frequency range. This is associated with increased respiration and heart rate and stereotypy.

940 EFFECT OF REGIONAL MICRO-INJECTION OF MORPHINE ON SOMATOSENSORY, MOTOR, AND ANALGESIC ASPECTS OF SEPTAL HYPERREACTIVITY: A MULTIVARIATE ASSESSMENT. James J. Valdes*, Fred H. Gage, III, and William R. Cameron. Chemistry of Behavior Program, Texas Christian University, Fort Worth, Texas 76129.

Rats with septal lesions demonstrate a quantitative increase in reactivity to noxious stimuli as well as a qualitative change in their responsiveness to non-noxious stimuli, responding as if both were noxious. Morphine analgesia is characterized by a diminished reactivity to noxious stimuli without a change in the sensory threshold for such stimuli. Morphine has thus been said to alter the affective component of behavioral responsiveness to stimulation. Many of the same brain systems involved in aspects of septal hyperreactivity are also involved in morphine analgesia, and the endogenous opiate system may provide a common substrate for these effects. The fact that the endogenous opiate system more closely parallels limbic systems involved with affective components of behavioral responsivity rather than primary sensory pathways supports the contention that morphine may act by altering behavioral responsivity to, rather than sensation of, noxious stimulation.

Rats given either septal or sham lesions were injected with either morphine (10ug) or vehicle, followed by either naloxone (10mg/kg i.p.) or vehicle. Morphine injections were into either the corticomedial amygdala (CMA), posterior hypothalamus (PHYPO), or the ventral hippocampus (VHIP). The rats were tested after each injection on a battery of behavioral tests designed to measure reactivity to both noxious (hotplate and footshock) and non-noxious (somatosensory) stimuli, as well as gross motor abilities. Discriminant function analyses revealed that the greatest morphine effect was to attenuate septal hyperreactivity to non-noxious somatosensory stimuli, although morphine did show a significant, though less pronounced, attenuation of reactivity to noxious stimuli. These effects were site-dependent, with morphine achieving complete reversal of the lesion-induced hyperreactivity when injected into the CMA and VHIP, and only partial reversal when injected into the PHYPO. Naloxone showed specific reversal of the morphine effect in rats with morphine injections in the CMA, exhibited partial reversal of the morphine effect in the VHIP, and in addition had unspecified effects which were independent of both the lesion and morphine effects in rats with morphine injections in the VHIP and PHYPO.

The data suggest that the primary mechanism by which morphine alters behavioral responsivity is by modulating limbic systems involved with reactivity to somatosensory stimulation. In addition, the naloxone data suggest the existence of multiple opiate receptors in the VHIP and PHYPO.

941 HIPPOCAMPAL SYNCHRONIZATION AND DESYNCHRONIZATION ELICITED BY STIMULATION OF SPECIFIC BRAINSTEM LOCI. Robert P. Vertes. Dept. Physiol., Univ. of Mich., Med. Sch., Ann Arbor, MI 48109.

We have previously shown that cells of the medial magnocellular reticular formation discharge at high rates of activity during both waking-movement and REM sleep and at low rates during quiet waking and slow wave sleep (Vertes, Brain Res. 128:146, 1977 and J. Neurophysiol. 42:214, 1979). We suggested that these reticular cells may be involved in the generation of hippocampal theta rhythm since they fired maximally during the identical states (general movement and REM sleep) in which theta is selectively present in the hippocampus of the rat.

In the present investigation the involvement of the brainstem in hippocampal theta generation was tested by systematically mapping the entire brainstem with stimulation and determining its effect on hippocampal activity. Under sodium pentobarbital anesthesia rats were fitted bilaterally with bipolar hippocampal electrodes, indifferent and ground leads. Only rats showing distinct high amplitude theta (1 mV or more) in a free moving situation in at least one pair of the bipolar hippocampal electrodes were used in the stimulation phase of the experiment. Stimulation was done under either methoxyflurane or urethane anesthesia. Stimulation was delivered through bipolar electrodes as they were lowered in 0.5 mm steps through tracts (4-6/rat) in the brainstem. Rectangular pulses (0.2 ms, 300 Hz, 50-150 μA) were applied for a duration of 4-6 sec at approximately 1 min intervals. The results of stimulating the entire width, depth and length of the brainstem from the bulbo-spinal junction to the caudal midbrain were as follows. (1) Unexpectedly, the most effective sites for eliciting theta were located in medial longitudinal fasciculus (MLF) at the pontine and caudal midbrain level. (2) The only nuclei from which theta could clearly be elicited belonged to the magnocellular reticular chain. The nucleus pontis oralis was most effective in driving theta followed by pontis caudalis and rostral gigantocellularis, respectively. (3) Brainstem nuclei such as locus coeruleus, dorsal raphe, and raphe magnus as well as the lateral reticular areas and cranial nerve nuclei were ineffective in eliciting theta. (4) The only electrode locations that produced desynchronization were located in the median raphe. Desynchronization at this site was accompanied by a dramatic decrease in the amplitude of hippocampal activity. The results suggest that there are two separate brainstem systems controlling hippocampal activity--one originating in the medial raphe and producing desynchronization and the other in the rostral magnocellular reticular field whose fibers primarily course in the MLF producing synchronization.

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942 ORIGIN OF HIPPOCAMPAL COMMISSURAL PROJECTIONS IN THE RAT. Theodore J. Voneida, Stephen E. Fish* and Richard M. Vardaris. Department of Neurobiology, Northeastern Ohio Universities College of Medicine, St. Rt. 44, Rootstown, Ohio 44272 and Department of Psychology, Kent State University, Kent, Ohio 44240.

Pressure (.005-.02μl) and iontophoretic injections of horseradish peroxidase were employed to study the origin of the hippocampal commissures in the rat. Infusion of discrete amounts of HRP into hippocampal fields CA1, CA2, CA3 or into the hilus fasciae dentatae (CA4) invariably resulted in the labeling of cells within the hilus of the contralateral dentate gyrus. In addition, a small number of labeled cells was also identified in areas homotopic to each injection site. Thus, injection of area CA1 resulted in labeled perikarya in the contralateral homotopic area CA1. Injections restricted to CA3 gave rise to labeled cells in contralateral CA3, etc. The number of homotopically labeled cells resulting from the injection of specific hippocampal fields was always very much samller than labeled hilar cells.

It appears, therefore, that cells within the hilus of the dentate gyrus (CA4) may influence widespread areas within the contralateral hippocampus.

943 EARLY POSTNATAL DEVELOPMENT OF THE MEDIAL AND LATERAL NUCLEI OF THE AMYGDALA. Caroline L. Wakefield. Dept. Anat., Sch. Med. Sci., UNR, Reno, NV 89557.

The postnatal development of the amygdaloid complex was studied in kittens, age newborn to 34 days, using Golgi and EM techniques. The development of the medial nucleus, a phylogenetically older nucleus, and the lateral nucleus, a phylogenetically newer nucleus of the amygdala will be compared in this report.

At birth, all the nuclei of the amygdala which have been described in the adult cat can be identified in the kitten. The neurons in the medial nucleus are stellate shaped and have long fine processes with few spines. The synaptic boutons contained round vesicles and a small population of dark-core vesicles at all ages studied. Many of the boutons were filled with vesicles, others were larger and contained few vesicles in proportion to their size. There was little myelin formation until 23 days of age.

The cells of the lateral nucleus are pyramidal in shape, with thick primary dedritic branches. Long spines with club endings were seen to increase in number up to 14 days of age. The axons of both the medial and lateral nuclei were oriented toward the stria terminalis and a few collateral branches within the nucleus were observed. The myelin in the lateral nucleus is very sparse compared to the medial nucleus. Boutons in the lateral nucleus are similar to those in the medial nucleus and are less numerous.

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944 TAIL PINCH INDUCES SEXUAL BEHAVIOR IN OLFACTORY BULBECTOMIZED MALE RATS. Lolin Wang and Elaine M. Hull. Dept. Psych., SUNY, Buffalo, NY 14226.

Bilateral olfactory bulbectomy disrupts copulatory behavior in some inexperienced male rats. Tail pinch has been shown to facilitate copulatory as well as feeding, maternal, and aggressive behavior. The present experiments were conducted to determine whether tail pinch would induce male sexual behavior in noncopulating bulbectomized rats.

Bilateral olfactory bulbectomies (BOB) were performed on 34 male Long Evans rats, 90 days of age; 4 animals underwent sham surgery (SC), while 3 served as unoperated controls (UC). In tests with a primed, ovariectomized female 24 BOB animals failed to ejaculate on two separate 40 min tests. All control animals exhibited normal sexual behavior, and 10 BOB animals ejaculated at least once during the two trials. Later histological examination revealed a correlation between size of lesion and behavioral deficits.

After a third test to verify the continued disruption of sexual behavior, 16 of the nonejaculatory animals received approximately 6 applications of a clip to the tail. Similar 60 sec applications to normal animals had induced eating and/or drinking within 5 to 6 trials. Mating tests were conducted as before, and were repeated four days later. The remaining 8 nonejaculatory animals were tested similarly, but without application of the clip. Ten of the 16 tail pinch (TP) animals began to mount, intromit, and ejaculate on the first test following the TP procedure, and an additional 2 began to exhibit complete sexual behavior on the second test. Only 1 of the 8 animals not receiving TP ejaculated on these trials. Thus, TP applied shortly before sexual behavior tests can induce copulation in some males whose behavior had been disrupted by olfactory bulbectomy.

945 FUNCTIONAL CORRELATES OF THE HIPPOCAMPAL FORMATION IN THE RAT: A 14-C-2DG ANALYSIS. Robert E. Watson, Jr*., Allan Siegel, Jennifer Poulakos*, and Raymond Troiano*. Departments of Neuroscience and Physiology, N.J. Medical School, Newark, N.J. 07103.

In an attempt to develop a better understanding of the functional organization of the hippocampal formation, we employed 14-C-2-deoxyglucose (2DG) radioautography following electrical stimulation of the various regions along its longitudinal axis in both awake and anesthetized rats.

The experimental paradigm consisted of electrical brain stimulation delivered continuously over 30 sec on and 30 sec off for periods of 45 minutes following injection of 2DG. Brains were then removed and processed for radioautography.

The most significant observations were as follows: (1) in unstimulated (control) animals the molecular layer of the hippocampal formation was normally densely labeled; (2) stimulation of the anterior dorsal hippocampus rostral to the level of the subiculum resulted in bilateral activation of all pyramidal cell fields with a marked reduction of label in the hippocampal molecular layer. The dorsomedial septum was only poorly labeled and no evidence of label was seen in the hypothalamus; (3) stimulation of the dorsal hippocampal formation at the level of prosubiculum and subiculum proper also resulted in bilateral activation of all pyramidal cell fields and a reduction in label over the molecular layer. In contrast, label was clearly noted throughout the extent of the dorsomedial septum and medial mammillary nucleus. There appeared to be no activation of either the ventral hippocampus or other portions of hypothalamus; (4) stimulation of the presubiculum at the level of the posterior hippocampus resulted in activation of only the medial mammillary and anteroventral thalamic nuclei. Stimulation of this region produced no diminution in label over the molecular layer nor did it result in activation of either the hippocampal pyramidal cell layer or other regions of hypothalamus; (5) stimulation of the ventral hippocampus at the level of the subiculum proper resulted in activation of the entire extent of the lateral part of the lateral septal nucleus and the medial corticohypothalamic tract (and hypophysiotrophic zone of hypothalamus). No evidence was noted for the presence of bilateral activation of the pyramidal cell layer of the ventral hippocampus, or of any part of dorsal hippocampus; nor did the stimulation alter the pattern of label present over the molecular layer.

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946 CIRCADIAN MODULATION OF GRANULE CELL SENSITIVITY TO PERFORANT PATH SYNAPTIC INPUT IN THE RAT. M.O. West and S.A. Deadwyler.* Dept. of Physiol. and Pharmacol., Bowman Gray Sch. of Med., Winston-Salem, NC 27103.

Circadian variations in dentate gyrus monosynaptic field potentials elicited by stimulation of the perforant path were recorded from chronically implanted rats entrained to a 12:12 light-dark cycle (0700 ON, 1900 OFF). Localization of the recording electrode to either the granule cell somal layer or the dendritic region of perforant path synapses was accomplished through the use of a miniature microdrive system which allowed maximization of field potentials prior to each data collection period. Input-output curves for both the population spike and synaptic potential were constructed daily at five 2 hr intervals (1400, 1600, 1800, 2000, 2200). Population spike I-O curves for eight rats showed a 22-28% reduction in mean amplitude at all stimulus voltages at 2000 and 2200 relative to 1600. Population spike laminar profiles also showed a 22-28% reduction in amplitude at 2000 and 2200; the decreases depicted in the I-O curves therefore were not due to differences in location of the recording electrode. Similar reductions in population spike amplitude at 2000 were observed even on selected days when the light period was extended to 2100, indicating that the population spike decreases were related to the entrained circadian cycle and not to the presence or absence of light. In further experiments, comparisons were made at 1600 and at 2000 among different behavioral states previously shown (1) to produce changes in granule cell population spike amplitude. Neither the magnitude nor the direction of the circadian related decrease in population spike amplitude could be accounted for by alterations in behavioral arousal.

The hourly alterations in amplitude of the granule cell layer population spike were not accompanied by significant variations in the dendritically located perforant path synaptic current, suggesting a change in granule cell sensitivity to this input. To test whether this change might be influenced by cycling levels of corticosterone, a glucocorticoid actively taken up by hippocampal tissue, four animals were tested before and after adrenalectomy. Circadian cyclicity in granule cell population spike amplitude was unchanged by the elimination of corticosterone via adrenalectomy. The circadian controlled oscillation of dentate granule sensitivity to perforant path synaptic input appears to be independent of alterations in corticosterone levels and must therefore be controlled by some other as yet unknown modulatory influence.

1. Winson, J. and Abzug, C., J. Neurophysiol. 41:716-732 (1978).

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947 SENSORY AFFERENT RESPONSE OF HUMAN LIMBIC NEURONS. Charles L. Wilson, Thomas L. Babb, Eric Halgren and Paul H. Crandall. Brain Res. Inst., Reed Neurol. Res. Cntr., and Dept. of Surg. (Neurol.) Sch. Med., UCLA, Los Angeles, CA 90024.

Considerable interest exists in sources of sensory afferents to the hippocampal formation and adjoining temporal lobe structures, largely because of this area's role in memory function. Anatomical evidencefrom the monkey indicates that the parahippocampal region receives multisynaptic projections from all major sensory modalities, although visual projections are most prominent(e.g. Seltzer and Pandya, Exper. Neurol. 50: 146-150, 1976). Electrophysiological investigation substantiates the visual responsiveness of neurons in monkey posterior hippocampal gyrus and adjacent areas(MacLean et al. J. Neurophysiol. 31: 870-833, 1968). Recently, the response properties of human limbic neurons to diffuse retinal illumination have been described(Babb et al. Neurosci. Abstr., 1976, 380; Halgren et al. Electroenceph. clin. Neurophysiol. 45: 585-601, 1978). In the present investigation, action and field potentials were recorded from microwires implanted chronically in temporal lobe epilepsy patients (Babb and Crandall, Electroenceph.clin. Neurophysiol 40: 225-243, 1976) in order to 1) compare relative responsiveness of different limbic regions to simple visual, auditory and somesthetic stimuli, 2) determine the relationship between unit and field potential measures of sensory response, and 3) search for receptive field properties during presentation of complex patterned stimuli.

Analysis of over 200 hippocampal gyrus, pes hippocampi, and amygdala units indicated that 1)responses were substantially more common to visual than to auditory or somesthetic stimuli, 2) convergence was unusual, 3) responses showed rate increases, rate decreases, or sequences of both, and 4) the greatest proportion of responsive units was encountered in the posterior hippocampal gyrus. Field potentials evoked by sensory stimuli were of largest amplitude in posterior regions of hippocampal formation, but local responses in anterior hippocampus and amygdala were more prominent than might be predicted from the smaller number of responsive units. Photically evoked potentials were of larger amplitude than those evoked by stimuli of other modalities. Onset latencies ranged from 40 to 120 msec, depending upon the structure from which the recording was derived, with shortest latencies in posterior hippocampal gyrus. Preliminary results of tests with patterned stimuli such as reversing checkerboards suggest that response to such stimuli may not occur in isolation from response to diffuse photic stimulation.

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948 NEURONAL TRANSMISSION FROM PERFORANT PATHWAY THROUGH DENTATE GYRUS: DUAL BRAINSTEM ACTIVATING SYSTEM. Jonathan Winson, Rockefeller University, New York, NY 10021.

Previous work in this laboratory has shown that the population action potential evoked in granule cells by perforant path (pp) stimulation (the EAP) is greater during slow-wave sleep (SWS) than during the alert state (Winson & Abzug, J. Neurophysiol. 41, 1978). Further, a stimulus applied to the median raphe nucleus (MR) prior to the activation of the pp produces a marked augmentation of the already elevated EAP during SWS but not during the alert state (Winson, Neurosci. Abstr. 3, 1977). In studies reported here, prestimulation (1 or 3 pulses) has been applied at various brainstem locations followed by a test pulse applied to the pp. Extracellular responses were recorded at the granule cell level.

In freely moving rats with prestimulation applied to MR, the minimum delay time (time from MR to pp stimulus) at which the augmented EAP appeared during SWS was 4 msec. It was also found that a fast field potential (FFP) was elicited consistently at the granule cells, due solely to MR stimulation. The FFP latency was approximately 4 msec and its magnitude depended on behavior. The FFP was largest during SWS, smaller during alert, and was suppressed during REM sleep.

In rats anesthetized with Urethane, midline dorso-ventral penetrations were made at rostro-caudal levels encompassing the entire pons and medulla to ascertain areas in which prestimulation was effective in producing EAP augmentation and the FFP. EAP augmentation was obtained at low stimulus currents ($\leq$ 30 μA) from raphe nuclei (dorsalis, MR, magnus, and pallidus) but low currents were ineffective at other midline locations and along laterally placed penetrations. FFP responses could not be elicited by low currents at any location. Further investigation showed that the FFP produced by MR stimulation in freely moving animals was eliminated by Urethane. FFPs could be re-elicited by a prior conditioning pulse applied to MR.

FFPs could be elicited at low stimulus currents in animals anesthetized with Chloropent. Studies in such preparations confirmed the midline loci at which the EAP was enhanced by low stimulus currents and delineated a closely apposed but distinct series of midline loci at which low currents ($\leq$ 30 μA) elicited the FFP. FFPs could also be evoked at low currents from reticular formation locations. Further revealed was a second and slower potential (latency about 15 msec) evoked by brainstem stimulation. These results suggest a dual brainstem system activated by midline stimulation which elicits behaviorally dependent responses in the dentate gyrus.

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949 MEDIAN RAPHE LESIONS IMPAIR THE ACQUISITION AND PERFORMANCE OF AN 8-ARM MAZE TASK. David Wirtshafter, Karen E. Asin and Ernest W. Kent. Dept. Psychology, University of Illinois at Chicago Circle, Chicago, Il 60680.

Several authors have demonstrated impaired performance of an 8-arm maze task following damage to the hippocampus or a number of closely related structures including the septum and the entorhinal cortex. Since a number of similarities between the behavioral effects of median raphe and hippocampal lesions have been reported (Asin et al., this meeting), we investigated the effects of median raphe lesions on the acquisition of a food reinforced 8-arm maze task.

Rats with electrolytic lesions of the median raphe were profoundly impaired on the acquisition of an 8-arm maze task and showed virtually no improvement across 16 days of training. Median raphe lesioned animals showed pronounced response perseveration such that a given animal would often repeatedly enter a particular sequence of arms. Seventy one percent of the errors made by median raphe lesioned animals could be accounted for by such responding, in contrast to only 18 percent of control errors.

We next examined the effects of median raphe lesions in animals who were overtrained on the 8-arm maze task. Despite the near perfect performance of these animals prior to surgery, median raphe lesions produced an immediate disruption of maze performance which was similar in magnitude to that seen in animals lesioned before training.

The current results demonstrate that the median raphe, like the hippocampus, contains elements essential to the normal performance of an 8-arm maze task in the rat. Although the impairment may reflect a deficit in the utilization of spatial information, other explanations (e.g. alterations in attention or memory) cannot be excluded at this time.

950 THETA CELLS IN DORSAL CA1, CA3 AND DENTATE FIRE MAXIMALLY ON THE POSITIVE PHASE OF DENTATE THETA RHYTHM IN WALKING RATS. S. Wolfson*, S.E. Fox and J.B. Ranck, Jr., Dept. of Physiology, Downstate Med. Ctr., SUNY, Brooklyn, NY 11203.

Any theory of the cellular basis of theta rhythm must account for the fact that the rhythm is phase-reversed between the CA1 and dentate cell layers, yet the phase-locked variations in the amplitude of the monosynaptic population-spikes wax and wane simultaneously in the two cell layers (Fox, Rudell and Ranck, Fed. Proc. 38:1310, 1979). Such a theory must also account for the present result, namely that theta cells in all areas of dorsal hippocampus achieve their maximal firing rates simultaneously. Theta cells, which double their rate of firing during hippocampal slow wave theta rhythm, are presumed to be interneurons. These theta cells are generally phase-locked to theta rhythm and may participate in generation of theta by producing PSPs in projection cells.

Fixed reference electrodes in dorsal hippocampal formation and neocortex, and moveable microelectrodes aimed at hippocampus were stereotaxically implanted for chronic recording in 11 rats. The rats walked on a motor-driven treadmill. Slow wave theta rhythm was recorded from the fixed electrodes and both slow waves and units were recorded from the moveable microelectrode. Multiple slow wave recording sites allowed many internal checks on reference theta rhythm. Phase histograms were made by measuring the interval between two positive peaks of dentate theta rhythm and dividing this into 36 bins.

To describe the results, the first bin of the histograms was assigned the value of 90° (the positive peak). The mode of the phase histogram was measured for each unit. Seven theta cells in CA1 had their modes at 50 $\pm$ 30° ($\bar{X}$ $\pm$ s.d.). Four of five theta cells in CA3 had modes at 100 $\pm$ 30° (one was not phase related). Eight theta cells in the dentate area had modes at 60 $\pm$ 30°. Eight other theta cells (not histologically verified) had modes at 80 $\pm$ 40°. Firing during REM sleep was recorded in six cells and was the same as during walking. Complex-spike cells were also recorded in these areas. Twelve of 14 seemed to have phase relations similar to those of theta cells. (Two had no phase relation). The number of complex-spike cells recorded thus far is too small to make strong conclusions.

Thus both monosynaptic evoked potentials and the firing of theta cells covary with the theta rhythm simultaneously, independent of hippocampal subfield. Furthermore, CA3 theta cells appear to have the same phase relation as those in CA1 and dentate, even though CA3 does not generate a theta rhythm.

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951 EVIDENCE FOR A HYPOTHALAMIC INPUT TO THE DENTATE GYRUS IN THE RAT AND CAT. J.M. Wyss. Dept. Anat. & Neurobiol., Wash. Univ. Sch. Med., St. Louis, MO 63110.

Although it has been known for some time that there are cells in the supramammillary region that project to the hippocampal formation (Segal and Landis, 1974; Pasquier and Reinoso-Suarez, 1976), the site of termination of these axons has been difficult to establish. We have examined this issue by placing relatively large injections of tritiated amino acids in the posterior hypothalamus (including the supramammillary region) in a series of rats and cats. In every case in which a significant proportion of the cells in the supramammillary region was labeled, there was a clear indication of axonally transported label in the outer one-half of the stratum granulosum of the dentate gyrus, and over the adjacent 20 µm of the stratum moleculare immediately deep to the zone of termination of the commissural and associational afferents. The projection is to both blades of the dentate gyrus, and extends over the entire septo-temporal extent of the dentate gyrus. However, for reasons that are not yet apparent, the labeling is always appreciably heavier over the suprapyramidal rather than the infrapyramidal blade. In experiments in which the injections were strictly unilateral, labeling in the dentate gyrus was always bilateral; but in every case, the labeling on the side ipsilateral to the injection was significantly heavier than that on the contralateral side.

The supramammillary fibers reach the dentate gyrus by way of the medial forebrain bundle, the septal complex and the dorsal fornix and fimbria The supramammillary region also projects to the anterior hippocampal rudiment. This projection is similar to that seen in the dentate gyrus, in being both bilateral (but heavier on the ipsilateral side) and limited to the outer part of the cellular layer and the adjoining part of the overlying molecular layer.

References:
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952 SEPTAL CORRELATES OF CONDITIONED INHIBITION AND EXCITATION. Elna Yadin* and Earl Thomas. Dept. Psych., Bryn Mawr College, Bryn Mawr, PA 19010.

The recording of multiple units was introduced into the study of the role of the septum in Pavlovian conditioned inhibition. This technique enabled the monitoring of septal activity in the freely moving rat throughout a long-term acquisition period of classical conditioning.

In Experiment 1, a Pavlovian aversive discrimination paradigm was used in which one CS was followed by a shock US and another CS was presented unpaired with shock. Another group of animals received a truly random control procedure in which the shock USs were presented randomly with regard to the CSs. Septal activity was recorded during CS+ and CS-, during a preCS period and during a post-shock period.

The results may be summarized as follows: (1) septal unit activity was found to increase during presentations of CS- only in the conditioning group. (2) Presentations of CS+ caused a marked suppression of septal unit activity in the conditioning animals. (3) The baseline unit activity during the preCS period remained unchanged throughout the experiment in the conditioning animals while decreasing in the truly random controls. (4) Termination of the shock US was marked by a large burst of firing in the conditioning group but no such rebound was found in the controls.

The results are interpreted in terms of a fear-relief hypothesis which suggests that the septal area is importantly involved in the mediation of relief from fear. The differential patterns of firing between the experimental and control groups suggest the importance of the predictability of shock to the expression of relief.

In Experiment 2, a Pavlovian appetitive discrimination paradigm was used in which the CS+ was followed by a food US. The results were virtually the opposite from those seen in the aversive case: (1) Septal activity to CS- was suppressed. (2) Unit responding to CS+ was markedly enhanced.

The findings from the appetitive experiment were interpreted as indicating that increased septal activity during CS- seems to be quite specific to the nonoccurrence of a noxious stimulus and does not represent the mechanism underlying all types of conditioned inhibition.

MEMBRANE BIOPHYSICS

953 CALCIUM CHANNELS PERMIT THE PASSAGE OF Mn^{++} IONS: A POSSIBLE EXPLANATION. Margaret Anderson. Dept. of Biol. Sci., Smith College, Northampton, MA 01063.

The myoepithelial cells that make up the proventriculus of the marine polychaete worm Syllis spongiphila undergo calcium spikes which are associated with contractions. (Spikes elicited by direct intracellular stimulation are (a) reversibly abolished in Ca-free artificial sea water (ASW), (b) not abolished in low-sodium ASW or in ASW containing 10^{-6} - 10^{-5} M TTX, (c) abolished reversibly by Co^{++} ions, D-600 (1 mM) and verapamil (1 mM) and essentially irreversibly by La^{+++} ions (1 - 10 mM), and (d) supported in Ca-free ASW containing either 1 mM Ba^{++} or 10 mM Sr^{++} ions). Mn^{++} ions appear to pass through the calcium channels. ((a) Regenerative responses not associated with contractions can be elicited in Ca-free ASW containing 5 - 50 mM Mn^{++}. (b) In Ca-free ASW containing Mn^{++}ions, spikes are abolished by Co^{++} and La^{+++} ions, D-600 and verapamil at concentrations similar to those effective in abolishing Ca-spikes. (c) In Ca-free ASW containing varying $[Mn]_o$'s, amplitudes of spike overshoots increase about 27 mV for a 10-fold change in $[Mn]_o$ (this value approaches that predicted by the Nernst equation for a membrane permeable to a divalent cation); in ASW containing 10 mM Ca^{++} and varying $[Mn]_o$'s, the amplitudes of overshoots increase about 15 mV for a 10-fold change in $[Mn]_o$ (these data suggest a competition between Ca^{++} and Mn^{++} ions for the same channels)). Of the divalent first transition series metals, Mn^{++} exhibits the lowest energy of hydration. It seems probable that Mn^{++} ions can relatively easily shed their waters of hydration and pass through the Ca-channel. On the basis of this hypothesis, divalent cations of the first transition series with higher energies of hydration should block the Ca-channel. The effectiveness of blocking should increase as the energy of hydration increases; the prediction of effectiveness would be

$$Fe^{++} < Co^{++} < Ni^{++}.$$

The relative effectiveness of Fe^{++}, Co^{++} and Ni^{++} ions was tested by applying a series of concentrations of each ion to the preparation in Ca-containing solutions and determining the concentration required to abolish Ca-spikes elicited by direct stimulation. (During the application of Fe^{++} ions the preparation was bathed in nitrogen-bubbled, low-oxygen solutions). Ni^{++} (1 - 10 mM) consistently abolished the Ca-spike at lower concentrations than Co^{++}; Co^{++} (5 - 20 mM) abolished the Ca-spike at similar or lower concentrations than Fe^{++} (10 - 20 mM). These data support the idea that the capability of an ion to block the calcium channel increases as the energy of hydration increases. Supported by USPHS Grant # NS12196.

954 MEMBRANE CONDUCTANCE INCREASE IN THE GIANT ABDOMINAL NEURON (R_2) OF APLYSIA INDUCED BY LOW-SODIUM SOLUTIONS. J. P. Apland and D. R. Livengood. Department of Neurobiology, Armed Forces Radiobiology Research Institute, Bethesda MD 20014.

The effects of low-sodium (low-Na) solutions on membrane conductance (G_m) in the giant abdominal neuron (R_2) of Aplysia were investigated with electrophysiological methods. We have previously shown (Apland and Livengood, Soc. Neurosci. Abstr. 3: 659, 1977) that perfusion with low-Na solutions, using choline, glucosamine, mannitol, tetraethanolammonium, and tetramethylammonium as sodium substitutes, caused an increase in G_m in the majority of experiments with all substitutes. This increase in G_m was consistently blocked by addition of cobalt to the perfusion solution. In those cells that did not show an increase in G_m in low-Na solutions, application of cobalt caused a decrease in G_m during perfusion with low-Na solutions. This finding was attributed to a high resting G_{Na} in the latter cells. We also observed that cobalt blocked anomalous rectification in all cells studied.

Low-Na solutions characteristically caused a transient hyperpolerization followed by a persistent depolarization. After treatment with ouabain, the cell exhibits a persistent hyperpolarization in low-Na solutions, indicating that the depolarization seen without ouabain present is due to inactivation of the electrogenic sodium pump as intracellular sodium becomes depleted. When barium was substituted for calcium, results similar to those with cobalt were produced. Anomalous rectification was blocked by barium, and a decrease in G_m in low-Na perfusion solution was produced where no change in G_m had previously been shown in low-Na solution. Deletion of potassium from the perfusion solution, or replacement of potassium with rubidium, blocked the increase in G_m previously observed in low-Na solutions and also blocked anomalous rectification. Cold temperatures, which block anomalous rectification, also block the G_m increase in low-Na solutions. Finally, a lowered extracellular pH blocks the G_m increase in low-Na solutions. Meech (Ann. Rev. Biophys. Bioeng. 7: 1, 1978) has shown that lowered pH blocks G_K.

The observations with cobalt and barium suggest that calcium is involved in the G_m increase in low-Na solutions. The results using potassium-free solutions, rubidium, cold, and reduced pH suggest that the increased G_m is to potassium. This conclusion is reinforced by the fact that all treatments abolishing anomalous rectification, which is presumed to involve the potassium-conductance system (Marmor, Progr. Neurobiol. 5: 169, 1975) also block the G_m increase in low-Na solutions. Reduction of the extracellular sodium probably inactivates the sodium-calcium exchange mechanism, causing intracellular calcium to accumulate and increase G_K. The fact that cobalt and barium, which interact with calcium channels, abolish anomalous rectification, suggests that calcium also may be involved in that phenomenon.

955 MEMBRANE CURRENTS IN INSECT MUSCLE FIBRES. Frances M. Ashcroft* and P.R. Stanfield*. Dept. of Physiology, University of Leicester, Leicester, U.K. (SPON. G.F. Gwilliam).

Membrane currents in the ventral longitudinal muscle fibres of the stick insect, Carausius morosus, were investigated with a 3-electrode voltage clamp. Experiments were carried out at 2-5°C, in hypertonic saline to block contraction. The membrane current consisted of an early inward current, an early outward current and a delayed outward current. The presence of the early outward current is probably responsible for the graded (as opposed to all-or-none) action potentials which are characteristic of insect muscle. The inward current was studied in Ringer containing 120mM TEACl to reduce outward currents. In normal Ringer (20mM $CaCl_2$) the maximum inward current was -77.6± 9.5 $\mu A./cm^2$ (n=10) at a membrane potential of 0mV. Peak inward currents were increased in 50mM Ca^{2+}-Ringer (Mg^{2+}-substitution) and reduced in 5mM Ca^{2+}-Ringer, and there was a 31.0mV shift in the potential at which the membrane current reversed in sign for a 10-fold change in external calcium. At high $[Ca^{2+}]_o$ the peak inward current showed saturation. Inward currents were blocked by 1mM La^{3+} and halved by 10mM Ni^{2+}. These results indicate that the inward current is carried by calcium ions. The inward current (I_{Ca}) shows voltage and time-dependent inactivation. Steady-state inactivation was described by $h_\infty = \{1+\exp(V_1-V_h)/k_h\}^{-1}$ where V_h= -21.0±1.8mV (n=5) and k_h=5. The time constant of inactivation depended on the experimental protocol. Inactivation followed a single exponential with a time constant of 55msecs at -10mV when determined with 2-pulse experiments; however the decay of I_{Ca} during a single voltage step was best fitted by the sum of 2 exponentials. The activation kinetics of the Ca channel are currently under study. As insect muscle is readily voltage-clamped it provides a favourable preparation for the study of muscle calcium channels. Supported by the M.R.C.

956 LOCUS COERULEUS AXONS EXHIBIT PRONOUNCED ACTIVITY-DEPENDENT VARIABILITY IN IMPULSE CONDUCTION LATENCY. G. Aston-Jones[1,2], M. Segal[3] and F. Bloom[1]. [1]A.V. Davis Ctr. for Behav. Neurobiol., Salk Inst., La Jolla, CA 92037.

It is generally conceived that axons carry impulses with constant velocity and act as simple transmission lines between soma and synapse. Recent studies, however, have suggested that certain axons can modulate their own impulse flow; indeed, we now report that this ability is especially pronounced in the axons of noradrenaline-containing locus coeruleus (LC) neurons.

Chloral hydrate-anesthetized rats were implanted with stimulating electrodes in anterior cingulate cortex and olfactory bulb. Glass micropipettes recorded isolated impulses from single LC neurons, subsequently identified histologically. While studying antidromic (AD) conduction properties of these neurons, we observed striking alterations in AD latency not previously reported. Each of 12 antidromically activated LC neurons tested in 8 rats exhibited a pronounced gradual and continuous increase in latency (in some cases > 20 msec) during 2 to 10 Hz stimulation, and a gradual and continuous recovery to basal AD latency (mean=55.3 ± 4.2 msec) over 1-2 minutes when stimulus frequency was reduced. During trains of constant frequency stimulation, the AD latency increased somewhat asymptotically to a new, fairly stable value after 50 to 400 stimuli. In addition to these AD latency increases, closer examination revealed that the second and closely succeeding stimuli in a 10 Hz train yielded decreased latencies, followed by the larger increases in AD latency during subsequent stimuli in the train. In neurons tested with pulse pairs, a decrease in AD latency of .5 to 3.5 msec for the second stimulus was consistently observed. The magnitude of variation in AD latency was positively correlated with three factors: 1) the initial AD latency of the neuron, 2) the frequency of stimulation and 3) the number of pulses in a train of stimuli. Preliminary experiments have revealed marked AD latency alterations in the raphe and substantia nigra systems as well.

Data are presented which demonstrate similar conduction time variability as a consequence of non-stimulated activity and suggest that changes in conduction velocity along the axon itself underlie these AD latency fluctuations. These fluctuations may allow long, unmyelinated axon systems to transfer information most effectively with short bursts of activity, and suggest that large axon diameters and myelination may be necessary for high fidelity as well as high velocity of impulse flow in nervous tissue. (Supported by USPHS Grant #AA 03504 and NIH Training Grant #GM 02031. [2]California Inst. of Tech., Pasadena, CA. [3]Weizmann Inst., Rehovot, Israel.

957 STIMULATION OF EXCITABLE TISSUE BY RAPIDLY CHANGING MAGNETIC FIELDS. Charles P. Bean, General Electric Corporate Research and Development, Schenectady, N.Y. 12301

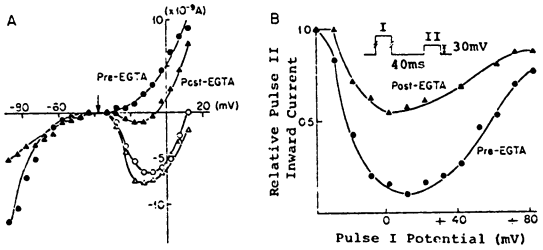
Recent interest in whole-body exposure to time varying magnetic fields has been stimulated by the proposal and development of imaging techniques using nuclear resonance. Some methods require rapidly ramped magnetic fields. Theoretical insight can be gained into the circumstances under which such exposure will stimulate excitability of such tissue. The mechanism of stimulation is taken to arise from the electric fields created by exposure to time varying magnetic fields. The assumptions of the present treatment are standard. A cell of nervous tissue is presumed to have a threshold value of transmembrane depolarization, ΔV_c. The cell is taken to be in a medium of uniform electrical properties and all calculations are made for the limit of steady excitation, i.e. the rheobase limit. Two cases are considered: the direct excitation of a cell body and excitation of an extended process--an axon or muscle fiber. Consider a spherical cell body of radius a in a uniform electric field E, the potential difference between inside and outside, ΔV, is $\Delta V = (3aE/2)\cos\theta'$ where θ' is the angle that an internal radius vector makes with the field direction. In the incorrect approximation that the cell is uniformly excitable, the critical field for excitation, E_c, is expressed by $E_c = 2\Delta V_c/3a$. Owing to the small size of most cell bodies this excitation limit is much higher than that for fibrous structures. For an extended process we assume a defined electronic coupling distance, λ, for small signals. Further, it is taken that the electric field, E, makes an angle θ with the fiber axis and sensibly exists over a length L of the long fiber. A remarkable early paper by W.A.H. Rushton (J. Physiol. 63, 357-377 (1927)) treats this case to obtain for the critical field, $E_c = (2\Delta V_c/\lambda)[1/(1-\exp(-L/\lambda))]$. The factor of the second parenthesis approaches unity as $L/\lambda >> 1$. To connect this criterion to changing magnetic fields, I presume a uniform flux rate of change, $\dot{B}$, to exist within a circle of outer radius R. By Faraday's law of induction, the maximum field is along the periphery and is given by the formula $E = 10^{-8}\dot{B}R/2$. Equating this to the earlier expression for a long axon gives for a critical rate of change, $\dot{B}_c$; $\dot{B}_c = 4\cdot10^{-8}\Delta V_c\cos\theta/\lambda R$. Even for the most easily excited fast fibers where $\lambda \sim 0.5$ cm and $\Delta V_c \sim 10$ mV well over one million gauss/sec would be expected to be required for excitation in cases most liable to such excitation. Further such rates would have to be maintained for a time on the order of a millisecond. These theoretical expectations will be compared to experiment.

958 ELEVATION OF INTERNAL FREE Ca^{++} IS REQUIRED FOR INACTIVATION OF Ca CONDUCTANCE IN PARAMECIUM. P. Brehm* and R. Eckert (SPON: D. Junge) Dept. of Biology, UCLA, Los Angeles, CA.

Inactivation of the Ca conductance in Paramecium does not exhibit the voltage dependency characteristic of Na channels. Instead, the inactivation requires the entry of Ca (Brehm and Eckert, Science 202:1203-1206, 1978). To further investigate the role of Ca, we injected the Ca chelator EGTA to limit the elevation of free Ca^{2+} within the cell during Ca entry.

Specimens were clamped with holding voltage equal to V_{rest} in 1 mM $CaCl_2$ + 4 mM KCl+1 mM HEPES at pH 7.2. EGTA was iontophoresed from a separate intracellular electrode filled with 100 mM K-EGTA while under steady voltage clamp. Inactivation of Ca current was measured before and after EGTA iontophoresis using the following regime. Two 20 msec depolarizations, P_I and P_{II}, were delivered with a 40 msec interval. P_{II} was fixed at 30 mV, a level at which little late outward current was observed. P_I was presented over a wide range of amplitudes. The amount of inactivation remaining after the 40 msec interval was determined from the reduction in peak P_{II} current resulting from presentation of P_I.

Prior to EGTA injection the P_{II} Ca current showed prominent inactivation for the midrange of P_I potentials (Fig. B). Little inactivation was seen at low or high P_I potentials, for which Ca entry should be minimal. Separate evidence has indicated that this behavior does not result from altered potassium currents (Brehm and Eckert, 1978; Eckert and Brehm, Ann. Rev. Biophys. Bioengin., 1979). Following EGTA injection, P_{II} inactivation for the P_I midrange was significantly diminished (Fig. B), and as a result of the reduced inactivation a late inward current developed (Fig. A). These actions of injected EGTA provide further evidence that it is the rise in intracellular ionized Ca that leads to inactivation of the Ca channel. Supported by NSF BNS 77-19161.



959 CESIUM CARRIES LARGE OUTWARD CURRENTS IN INTERNALLY-DIALYZED SNAIL NEURONS. Lou Byerly, Susumu Hagiwara, Masako O. Masuda*, and Mitsunobu Yoshii*. Dept. of Physiology, UCLA Medical School, Los Angeles, CA 90024.

We have applied a modified version of the internal-dialysis, voltage-clamp method developed by Lee, Akaike and Brown (J. Gen. Physiol. 71:489, 1978) to ganglion nerve cell bodies of the freshwater snail, Limnea stagnalis. The ganglia are treated with trypsin to facilitate isolation of the cell bodies and to increase the shunt resistance (typically > 100 MΩ) between the suction electrode and the neuronal membrane. Cells from the visceral and right parietal ganglia with diameters between 70 and 120 microns have been studied.

When both internal and external K^+ are replaced with Cs^+, voltage and time dependent outward currents are still observed when the cell is clamped to positive potentials. The I-V plot measured at 60 msec has a slope at potentials greater than +50 mV that is 20-100 times greater than the slope at potentials negative to the holding potential (-70 mV). This outward current, which has both fast and slow components, causes the net current measured to reverse at potentials less than +50. The slow component, which is still increasing at the end of a 70 msec voltage pulse, may account for the apparent inactivation of the inward current (carried by Ca^{++}) observed at potentials above -30 mV. When 10 mM EGTA is added to the internal Cs^+Aspartate$^-$ solution, the inward currents become more prolonged; even with 10 mM EGTA the slow component accounts for about half of the total outward current at potentials greater than +50 mV. Under no conditions have we seen the calcium current decay with a time constant less than 100 msec.

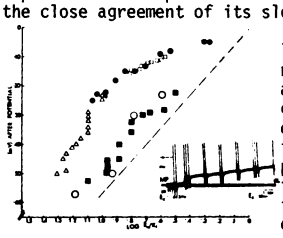
The outward currents are not affected by replacing external Cl^- with methanesulfonate$^-$, but are greatly reduced by replacing internal Cs^+ with Tris$^+$, TEA$^+$, or Arginine$^+$. Internal Na^+ only slightly reduces the outward currents observed with internal Cs^+. There is a striking correlation between the amplitude of the calcium current and the Cs-mediated outward current. Any treatment that reduces the calcium current (Ca-free external solution, external verapamil or La^{+++}, aging) also reduces this outward current. In converse, replacing Cs^+ with Tris$^+$, which greatly reduces the outward current, reduces the calcium current. We doubt that this Cs-mediated current is carried by the Ca-dependent K channels because there is no N-shaped region, it is not reduced when Ca^{++} is replaced by Ba^{++}, and there is 10 mM EGTA in the dialyzing solution.

Supported by U.S.P.H.S. grant NS09012, an MDA fellowship to Dr. Byerly, and a CNPq fellowship (Brazil) to Dr. Masuda.

960 A COMPARISON OF OUABAIN-SENSITIVITY AND HYPOXIA ON THE INTRACELLULAR POTASSIUM ION ACTIVITIES IN IDENTIFIABLE NEURONS OF Aplysia californica. Philip E. Coyer. Dept. of Neurol. and Neurosciences Program, Univ. of Ala. Medical Center, Birmingham, Alabama 35294.

In different types of neuronal activity, either regenerative action potentials or decremental generator signals, hypoxia often causes membrane depolarization and concomitant alterations in the action potential frequency or the amplitude of the potential. Three independent explanations or possibly a combination thereof may account for these phenomenae: (i) inhibition of the existing electrogenic pump resulting in (ii) a change in the equilibrium potentials of the principal ions contributing to membrane potentials or (iii) a membrane conductance change.

To test these hypotheses, identifiable neurons from the abdominal ganglion were impaled with double-barrel, potassium ion-selective microelectrodes capable of measuring intracellular potassium ion activities and membrane potential. Neurons were maintained in a constantly-suffused chamber supplied by normal bathing solution held at 18°C. After nitrogen bubbling, gaseous equilibration of the normal saline was detected by pO_2 microelectrodes placed in the recording chamber. While the relative potassium ion activities on either side of the neuron's membrane were determined with the ion-selective electrodes, external sodium, calcium, and potassium ion concentrations were altered. Upon calculating the potassium equilibrium potential at the point of the after-potential reflecting the maximum potassium conductance (see figure), the effects of hypoxia were compared to that of 4×10^{-4} M ouabain exposure (open triangles). Control data were assembled by substituting choline for sodium and subsequently rearranging the external potassium ion concentration (10-40 mMK). The effects of hypoxia were initially expected to mimic pump inhibition resulting in alterations of the equilibrium potential of potassium and sodium. However, not all cells responded in this way. As expected from control points (open circles), a neuron depolarized in a predictable Nerstian manner, which is shown by the close agreement of its slope and that of the expected result (solid squares fit slope of dashed line). Several other neurons did not follow this prediction; it was apparent that a change in membrane conductance to potassium had occurred. Removal of the inactivation factor by a hyperpolarizing current pulse resulted in spiking indicating that a change in conductance and not the equilibrium potentials had occurred.



961 A MOLECULAR ORBITAL APPROACH TO SELECTIVE ION BINDING BY MACROCYCLIC ANTIBIOTICS. C. Zuazaga de Ortiz. Lab. of Neurobiol., U.P.R. Med. Sci. Campus, San Juan, P.R. 00901.

A qualitative hypothesis, based on molecular orbital theory is proposed to account for the alkali metal ion selectivities exhibited by the macrocyclic antibiotics valinomycin and enniatin B. Even though the complexes formed between the metal ions and the macromolecules are of low molecular symmetry, the bonding parts of the complex have relatively high local symmetries. Spectroscopic studies have shown that in both valinomycin and enniatin B, the six oxygen atoms which bind the central cation form a trigonal antiprism (Shemyakin, M. et al., J. Membrane Biol. 1:402 (1969). Therefore, the bonding parts of the complex are assumed to belong to the D_{3d} point group. Binding is explained by the sharing of electrons from the oxygen atoms of the macromolecule and the vacant valence orbitals of the cation. Group theoretical methods are used to determine the allowed orbital interactions. The observed specificity is explained in terms of the bonds that are symmetry-allowed and qualitative considerations of orbital overlap and steric effects. On this base, the observed selectivity sequences of the macrocyclic antibiotics can be accounted for. Experimental ways of verifying some of the bonding features proposed to explain the selectivities are also suggested.

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962 NEUROTRANSMITTER MODULATION OF VOLTAGE-SENSITIVE CALCIUM CURRENTS IN SENSORY NEURONS. K.Dunlap* and G.D.Fischbach (SPON: C.L.Weill). Dept. Pharmacology, Harvard Medical School, Boston, Ma. 02115

The action potential recorded from the soma membrane of embryonic chick sensory neurons in culture has both a fast Na^+ component and a slower Ca^{++} component, responsible in part for a plateau on the falling phase of the spike. GABA, nor-epinephrine and serotonin (10^{-7}-10^{-4}M) decrease the duration of this plateau without affecting the resting membrane potential or input conductance, Fig A (Dunlap and Fischbach, Nature, 276:837, 1978). This could result from either a decrease in inward current or an increase in outward current. The voltage clamp experiments reported here show that this decrease in action potential duration results from a direct interaction of these neurotransmitters with a voltage-sensitive Ca^{++} conductance in the soma membrane. If the action of the neurotransmitters on the soma Ca^{++} current parallels their action at the nerve terminal, these results suggest a novel mechanism for presynaptic modulation of transmitter release.

The calcium current (I_{Ca}) is defined as a tetrodotoxin-resistant inward current which is dependent upon the extracellular Ca^{++} concentration and is blocked by Co^{++}. It follows a slower time course than the inward sodium current, reaching an average peak of about 250μA/cm^2 in 4ms. Application of low concentrations of the neurotransmitters decreases the peak inward current, I_{Ca}, without affecting the net outward K^+ current (I_K) measured at the end of the depolarizing voltage step (Fig B).

The effects of these drugs on I_{Ca} isolated from I_K was studied in two ways. First, depolarization to activate membrane conductance was followed by repolarization to the K^+ equilibrium potential (defined as the potential at which the tail current reverses in a solution containing TTX and Co^{++}). Application of the neurotransmitters decreases the magnitude of the inward tail current at E_K. Second, the outward K^+ current was completely eliminated in 125mM tetraethylammonium chloride (substituted for NaCl). The transmitters decrease I_{Ca} without affecting the time course or the voltage-dependence of current activation. Neither is the Ca^{++} equilibrium potential affected by drug application. It is concluded that these neurotransmitters decrease I_{Ca} through a reduction in the maximum, or limiting, Ca^{++} conductance.

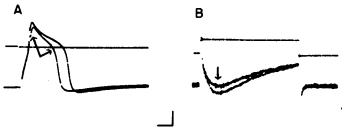


Figure: Effect of 10^{-5}M norepinephrine (arrows) on spike duration (A) and inward Ca^{++} current (B) in TTX. Cal: A-20mV,5ms; B-50mV/5nA,2ms.

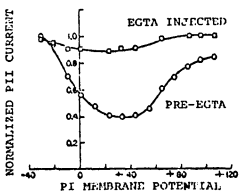
963 NEW THEORY OF KINETICS DESCRIBES EXCITABLE MEMBRANE BEHAVIOR. Dexter M. Easton. Dept. Biol. Sci., The Florida State University, Tallahassee, Florida, 32306.

According to conventional kinetics, the rate coefficient of a primary process is constant during the progress of the process. When this assumption is found to be incorrect, ad hoc adjustment is made. Thus, in Hodgkin-Huxley type equations, rate constants, implicit in initial assumptions, generate m,n,h processes, but their changing relation to V_M is subsequently found empirically. A more realistic initial assumption is that the rate coefficient of a primary process changes systematically during the course of the event, because conditions change as the system adjusts to a new equilibrium or steady-state. Rate constants then specify the rate of change of the rate coefficients of the primary process. This assumption, yielding simple equations of great power, has been used to model the conductance data of Hodgkin and Huxley, as well as oxyhemoglobin dissociation curves (Easton, Biophys. J. 22, 1978, J. Theoret. Biol. 76, 1979). The assumption that the rate coefficient changes exponentially with the independent variable is particularly well suited to such systems, involving asymmetric sigmoid relations between dependent and independent variables. Current-voltage-time records of voltage clamp data can now be modelled directly (rather than via conductance) from the following assumptions of the new (expo-exponential) kinetics: following a voltage step, current increases exponentially, but the rate coefficient of that growth decreases exponentially with time and increases exponentially with voltage. From this assumption, prediction of the V clamp results may be made by use of a single equation (with appropriate constants) derived to describe either specific ion current I_K or I_{Na}. (Aided by Psychobiology Center and Computer Center, Florida State University)

964 INTRACELLULAR EGTA INTERFERES WITH INACTIVATION OF THE Ca CURRENT IN APLYSIA NEURONS. R. Eckert and D. Tillotson.* Dept. of Biology UCLA, Los Angeles, CA 90024

Ca current, largely uncontaminated by other currents, can be examined in neurons in which most of the intracellular K is replaced with Cs through the use of the ionophore nystatin (Russell et al, J. Memb. Biol. 37:136-156, 1977; Tillotson and Horn, Nature 273:312-314, 1978). Inactivation of the Ca current is seen both as a relaxation of the current and as a decrease, following a prior depolarization, in the magnitude of the early inward current evoked by a given depolarization (Tillotson and Horn, 1978). The process of inactivation has been ascribed to Ca entry as opposed to membrane depolarization (Tillotson, 1979 PNAS 76:1497-1500; Brehm and Eckert, 1978 Science 202:1203-1206). Thus the Ca entering during the first depolarizing pulse (P_I) seems to cause inactivation during that pulse as well as a residual inactivation of the Ca current evoked by a subsequent pulse (P_{II}).

We further tested the role of Ca by interfering with the accumulation of intracellular free Ca^{2+} during Ca entry. The Ca chelator EGTA was iontophoresed into Cs-loaded neurons of the Aplysia abdominal ganglion while they were held at -40 mV under voltage clamp. The bath contained 100 mM $CaCl_2$+ 50 mM $MgCl_2$+410 mM Tris-HCl. Membrane currents in response to depolarizing steps were tested at intervals, and iontophoresis was continued until no further EGTA-related changes occurred. Following injection the relaxation of the inward current was greatly curtailed so that the inward current remained relatively strong under steady depolarizations lasting hundreds of milliseconds and longer. In the Figure it can be seen that the strong depression of the P_{II} inward current normally associated with P_I potentials that elicit large Ca entry (Tillotson, 1979) was largely abolished by the injection of EGTA. These results provide further evidence that inactivation of the Ca current depends on intracellular accumulation of free Ca^{2+} during depolarization. Supported by USPHS NS08364.



965 MEMBRANE EXCITATION PROPERTIES FROM A STATISTICAL EVALUATION OF IMPULSE TRAINS. Jürgen F. Fohlmeister, William J. Adelman Jr. and Richard E. Poppele. Lab of Neurophysiol., Univ. of Minn. Minneapolis, MN 55455 and Lab of Biophys. NINCDS, NIH, Woods Hole, MA 02543.

Impulse trains (fig. 1) generated by the space clamped squid giant axon in response to, and phase locked to the current $I(t) = I_o(1+\sin 2\pi f_o t)$ were modulated by a second, small amplitude sinusoidal current. The resulting impulse density was estimated for a complete period of the modulation sine wave from an impulse train with a duration of many modulation cycles (fig 2). For sufficiently small modulation amplitudes this impulse density

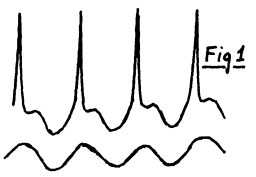


Fig 1

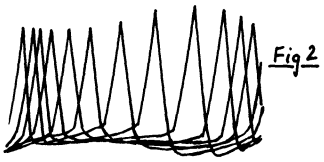
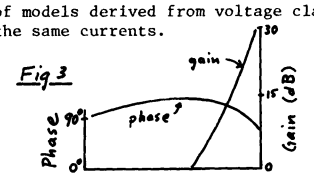
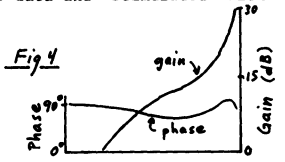


Fig 2

function is itself a sinusoid with the same modulation period. Gain and Phase (shift relative to stimulus modulation cycle) of the impulse density are determined as a function of the modulation frequency (Bode plots, figs. 3,4). Model studies have shown that a corner frequency and a gain resonance determine the magnitude of membrane conductance. The phase curve is a function of the conductance time course. Additional structure in the Bode plots may suggest the presence of "summing" phenomena (variables which are not reset by an impulse), and specify the relaxation times of those variables. The Bode plots of the squid axon membrane (fig. 3, for example) and of several models including the Hodgkin-Huxley model (fig. 4, for example) are qualitatively different. The necessary stimulus current and the measured conductance level are considerably higher for the axon. More importantly, the relaxation times of "recovery variables" (K-conductance and/or Na-inactivation) appear to be considerably longer for the axon when stimulated with sinusoidal currents than those of models derived from voltage clamp data and "stimulated" with the same currents.





966 ANOMALOUS RECTIFICATION IN FROG MUSCLE FIBERS: KINETICS AND VOLTAGE-DEPENDENT BLOCK BY CESIUM IONS. Shaul Hestrin* (SPON: Ann Kammer). Dept. of Physiology, UCLA Medical School, Los Angeles, CA 90024.

The time-course of anomalous rectification potassium current of frog twitch fibers was analyzed using Hille and Campbell type voltage clamp, which gives a time resolution of 1-5 msec. When muscle fibers are hyperpolarized an "instantaneous" inward current is observed, which is followed by a time dependent increase of the current. Both "instantaneous" and steady-state I-V relations have the property of an inward rectifier. The time-dependent current can be described by first-order kinetics. The "instantaneous" I-V relation may reflect the inward rectification of a single channel. Alternatively, the "instantaneous" I-V relation might reflect a change in the number of open channels which is complete within a few msec, and thus cannot be resolved with the present technique.

When Cs^+ ions are present in the external solution a decrease, or blockage of inward rectification is observed. As the membrane is hyperpolarized to a more negative potential a reduction of the current is observed. This voltage-dependent blockage causes a negative slope region in the steady-state I-V relation. When the membrane potential is stepped between two levels, the ratio of blocked to unblocked channels is changed in a time-dependent process.

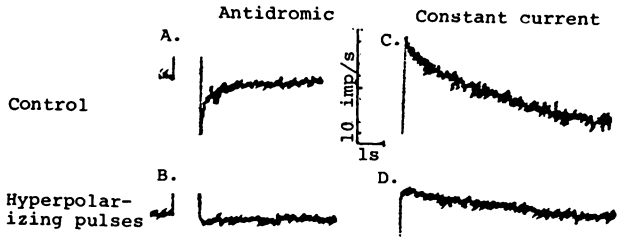
Inward rectification channels of starfish egg cells (Hagiwara et al., J. Gen. Physiol. 67:621, 1976) have remarkably similar properties. However, the time course of the conductance increase is more than ten times faster in muscle fibers, but the kinetics of blockage by Cs^+ ions is on the same order of magnitude. This might indicate that Cs^+ blocking and channel gating are separate mechanisms.

Supported by USPHS grant NS09012 to Dr. Hagiwara and NIH Department Training Grant 5 T01 GM 00448.

967 SENSITIVITY OF A Na^+ PUMP TO MEMBRANE POTENTIAL. S. F. Holloway* (SPON: G. W. King). Lab. of Neurophysiology, University of Minnesota, Minneapolis, MN 55455.

In the crayfish stretch receptor neuron, a train of antidromically evoked action potentials is followed by a post tetanic hyperpolarization that has been shown to be due to the action of an electrogenic Na^+ pump. The same phenomena also underlies a post tetanic depression of firing in an active neuron (A below;) and also the adaptation of firing to a constant current (C below). It has been proposed that the small sodium influx associated with each action potential is sufficient to trigger the electrogenic pump. Alternatively, the following evidence suggests that electrogenic pumping is activated by the changing electric field associated with the action potential.

The application of a hyperpolarizing pulse shortly after an action potential will not effect the sodium influx but will alter the electric field across the membrane. As shown below, a hyperpolarizing pulse (100 mV in amplitude, 5 ms in duration; applied intracellulary to the neuron soma 10 ms after each antidromic action potential) abolishes the post tetanic depression normally observed after a 1 s antidromic train (50/s) (B). The adaptation to a step change in current can also be abolished when the hyperpolarizing pulse is applied after each action potential (D).



Supported by grants from NSF, BMS 77-22532 and PCM 78-25168.

968 VOLTAGE-CLAMP ANALYSIS OF CA3 NEURONS IN HIPPOCAMPAL SLICES. Daniel Johnston and John Hablitz. Sect. Neurophysiol., Dept. Neurol., Baylor Coll. Med., Houston, TX 77030.

CA3 pyramidal neurons in the hippocampus fire in spontaneous bursts of action potentials (APs) at fairly regular intervals (Kandel and Spencer, 1961). These neurons are reputed to act as the presynaptic elements in the production of epileptic-type discharges in CA1 neurons after treatment with convulsant drugs. Moreover, long-term posttetanic potentiation of the mossy-fiber input to CA3 may have a postsynaptic locus. In an attempt to better understand these and other phenomena, a voltage-clamp study was initiated in order to identify some of the membrane currents in these neurons. Guinea-pig hippocampal slices, 400-500 μm thick, were maintained in vitro, using standard techniques. Neurons were impaled with 30-50 MΩ electrodes filled with either 3M KCl or 4M KAcetate. Voltage-clamping was performed, using a single microelectrode, based on the technique described by Wilson and Goldner (1975). Only cells meeting stringent criteria for viability were used for clamping. The cable properties of these neurons were determined by analyzing the current and voltage transients, as outlined by Rall. The equivalent electrotonic length of the CA3 cells was found to be, on the average, less than one space constant; therefore, a reasonable space clamp could be maintained for some distance from the soma. The average dendritic to soma conductance ratio was less than 2. Hyperpolarizing voltage-clamp commands of 100 msec duration elicited an inward-going current or anomalous rectification in most cells. This also was quite evident in current clamp and was seen as a "drooping" voltage transient. With depolarizing commands, large, fast inward currents were observed which could not be resolved by our clamping techniques. These were tetrodotoxin-sensitive and presumably were AP currents. In some cells, a much slower inward current also was seen with depolarizing commands. This current was increased by Ba^{++}, blocked by Co^{++}, and met the usual criteria for a voltage-dependent Ca^{++} current. In these same cells, a slow outward current was observed which persisted following the depolarizing command, decayed with a time constant of 1 sec, and was blocked by Co^{++}. These data would suggest a Ca^{++} activated K^+ current. Although difficult to resolve with our clamping techniques, a fast transient outward current observed with depolarization also may be present in these neurons. The results thus far indicate that CA3 neurons have a large repertoire of membrane currents similar in many respects to those described in other preparations, especially invertebrates. The role these currents may play in the behavior of CA3 neurons will be discussed. Supported by NIH Grant NS 11535.

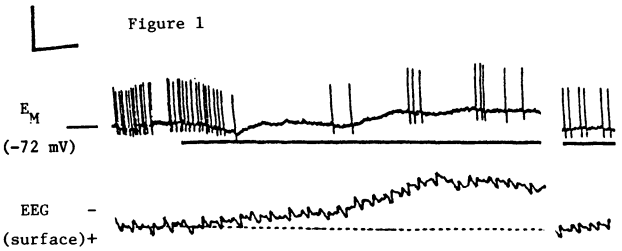
969 MEDIATED Cl^- TRANSPORT IN PRIMARY RAT ASTROGLIAL CULTURES. H.K. Kimelberg *, S. Biddlecome*, and R.S. Bourke, Albany Medical College, Albany, N.Y. 12208.

Astroglia are known to readily swell in vivo when the central nervous system is subjected to a variety of insults. Their perivascular location is also well suited to elaboration of brain extracellular fluid and pH control. These effects are clearly based on the ion transport characteristics of these cells and we have previously emphasized the role of Cl^- in astroglial swelling. Since ion transport in glia is difficult to define precisely in intact tissue, we are using a primary culture started from the cerebral hemispheres of 1-2 day old rats as a suitable in vitro model of astroglial cells. We have found that 70-90% of the cells in these cultures stain positively for glial fibrillary acidic protein (Stieg et al. this volume).

The rate of the steady state undirectional efflux or influx of Cl^-, measured with $^{36}Cl^-$, was reduced by up to 80% by 0.1 to 1.0 mM of the anion inhibitor SITS at 37°C pH 7.4. Furosemide and derivatives of ethacrynic acid were also found to inhibit the rate of influx, and the final steady state levels of Cl^-. Measurements of the initial steady state rate of uptake of Cl^- at varying external chloride concentrations gave saturation kinetics with an apparent Km for Cl^- of 40 to 60mM. These data suggest that a significant amount of Cl^- transport in these cells occurs by a mediated process, possibly involving an exchange carrier similar to that found in red blood cells. The intracellular chloride concentration, based on an estimated internal volume of 4.8µl/mg protein from K^+ distribution studies, and a Cl^- content of 0.145µmoles/mg protein from steady state $^{36}Cl^-$ levels, was calculated to be 30mM. This was 3 times greater than would be in equilibrium with a measured average membrane potential of -70 mV. Other indications that $[Cl^-]_i$ was not at Donnan equilibrium was that steady state $[Cl^-]_i$ was the same with $[Cl^-]_o$ reduced at either constant $[K^+]_o$, or with $[K^+]_o$ increased to maintain a constant external K x Cl product. Furthermore, pretreatment of the cells with ouabain, which resulted in a reversal of the normally high intracellular K^+:Na^+ ratio, had no effect on Cl^- uptake or final steady state levels as measured with $^{36}Cl^-$. At present it is unclear whether the high $[Cl^-]_i$ is due to operation of a Cl^- pump, a Na^+-Cl^- symport or increased uptake of Cl^- on a SITS-sensitive carrier in exchange for another anion, such as HCO_3^-. In brain slices, however, the occurrence of HCO_3^--dependent and SITS-sensitive swelling involving increased NaCl uptake and swelling of astroglial cells, suggests exchange of intracellular HCO_3^- for Cl^-. Since addition of Na^+ to cells incubated in Na^+-free medium led to an increased rate of acidification of the medium, increased uptake of Na^+ could be due to Na^+-H^+ exchange. Supported by NINCDS grant 13042.

970 SLOW MEMBRANE POTENTIAL SHIFTS IN CORTICAL NEURONS OF THE CONSCIOUS CAT IN RESPONSE TO MEANINGFUL STIMULI. Gregory L. King* and James E. Skinner. Neurophysiol. Sect., Neurology Dept., Baylor College of Medicine, Houston, Texas 77030.

When a conscious animal or human is presented with a tone that forewarns electric shock, a novel object, or a strong stimulus, an extracellular slow potential (SP) is evoked in the frontal and parietal association cortices. In different situations in which these same stimuli have no meaning, they do not evoke SPs, and are therefore evoked by the context of the stimulus, not its physical attributes. Current evidence suggests that these cerebral event-related SPs may be generated by the same cellular mechanism that is thought to produce SPs in the sympathetic ganglion neurons. In the isolated ganglion the SP is accompanied by slow postsynaptic potentials and is correlated with shifts in the intracellular levels of cyclic AMP (cyclic 3',5'-adenosine monophosphate). Our laboratory has shown that the amplitude of an event-related SP in the rat parietal cortex is negatively correlated with the local tissue level of cyclic AMP (Skinner et al., J. Neurochem., 1978). Our most recent observations show that slow 20-30 sec membrane depolarizations occur in some neurons in the frontal cortex of the conscious cat during a surface-negative event-related SP that is recorded from the same local area (Figure 1). These large depolarizations are associated with a reduction in the spontaneous action potential discharges, and, in some cells, are accompanied by a conductance increase. Other cells do not manifest detectable changes in the current-voltage relationship during the evoked slow membrane potential shift. By employing a "floating" micropipette method of recording, we have been able to perform several replications of the observations in the same cell.



Cal: 20mv (upper), 1 mV (lower), 167 msec

971 THE EFFECT OF THE INSECTICIDE TETRAMETHRIN ON THE SODIUM CHANNEL OF CRAYFISH GIANT AXONS. A. E. Lund* and T. Narahashi, Dept. Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611.

A variety of natural and synthetic toxins have proved useful in studying ionic conductance mechanisms in nerve membranes. They synthetic pyrethroid insecticides are a relatively new group of toxins with potent and unique actions on axonal membrane. Using the sucrose-gap, voltage clamp technique, we have studied the effect of one of these, tetramethrin, on crayfish giant axons. Median giant axons were isolated from crayfish and internally perfused with a solution containing 220 mM K^+, 15 mM Na^+, 170 mM glutamate, 50 mM F^-, 15 mM Cl^-, and 96 mM sucrose with a pH of 7.3. The holding membrane potential was -100 mV for all experiments.

Tetramethrin, applied at a concentration of as high as 300 µM internally or externally, had no effect on the time course or the steady-state amplitude of the potassium current measured in the presence of 300 nM tetrodotoxin. However, the (+) optical isomers of tetramethrin had a specific effect on the sodium current as measured with an internal perfusate in which Cs^+ was substituted for K^+. A concentration of 10 µM applied to either side of the axonal membrane caused the inward sodium currents to become biphasic, a normal initial transient sodium current being followed by a secondary inward current of much slower time course. This slow inward current was observed without the initial sodium transient at membrane potentials between -70 mV and -50 mV. Thus the voltage dependence of the slow current is shifted in the direction of hyperpolarization with respect to the initial transient sodium current. The time course of the sodium tail current, which followed the first order kinetics in the control axon, became the second order in the tetramethrin-treated axon. The first time constant (< 1 msec) coincided with the control tail current time constant, and the second time constant was on the order of hundreds of millseconds. Increasing the concentration of tetramethrin had no effect on the tail time course, but increased the amplitude of the tail current. Experiments in which depolarizing pulses of varying duration were applied showed that the development of the slow phase of the tail current closely followed the development of the slow inward current. These observations suggest that a population of sodium channels is modified by tetramethrin to give rise to drastically slowed activation and inactivation. The "slow channels" activate with a time course of 50-500 msec and inactivate with a time course of several seconds. Supported by NIH grant NS14143.

972 LONG-TERM AFTEREFFECTS OF SUBTHRESHOLD ELECTRICAL STIMULATION OF PERIPHERAL NERVE AXONS. Kenneth McLeod* and Stephen A. Raymond. Research Laboratory of Electronics, MIT, Cambridge, MA 02139.

In cat dorsal horn, Merrill, et al, reported aftereffects that linger for 20 msec or more following single subthreshold electrical pulses (J. Physiol. 284:127-145, 1979). Gasser (Am.J.Physiol. 121:193-202, 1938) showed indirect evidence for such long-term aftereffects in his study of "recruitment" of nerve fibers in whole nerve given near threshold tetanic stimulation. We find that when an excised frog sciatic nerve is bathed in Boyle-Conway Ringers at constant pH 7.0-7.7 and constant temperature, stimulation of the nerve with current pulses that are near but below threshold results in brief transient increases in excitability that decay within 2 msec. The last stimulus in a short burst of such subthreshold stimulation is followed by the same transient superexcitability that follows a single pulse or the last stimulation of a subthreshold tetanus. Under these conditions there are no detectable long-term aftereffects of subthreshold stimulation as assayed either by threshold hunting or by raising the amplitude of subthreshold stimuli gradually.

The pH of the Ringers solution was set by adjusting the mixture of 5% CO_2 in air, 30% CO_2 in O_2, and O_2 bubbled through reservoirs of Ringers. After several hours at pH 9.0, rapid restoration to pH 7.5 produced prolonged rises in threshold lasting for more than an hour. During such depressed excitability, individual fibers given repeated stimulation showed a superexcitability es having a minimum threshold about 10% below resting level that was detected for more than 50 msec following the last pulse in a train. This superexcitability appears to account for recruitment:

1) It appears after acid shifts (as does recruitment).

2) It lasts about as long as the maximum period between stimuli in trains that produce some recruitment in gross sciatic nerve.

3) The amplitude of the threshold change diminishes (as does the rate of recruitment) as the period between stimuli gets longer.

We are investigating relations of pH-induced depression to depression induced by activity, which is thought to depend on electrogenic Na^+ pumping.

973 COMPARTMENTAL ANALYSIS OF ELECTRICAL CONSTANTS IN CULTURED MOUSE DORSAL ROOT GANGLION NEURONS. Jane C. Norris*, Thomas H. Brown and Donald H. Perkel. Depts. of Neurology and Biological Sciences, Stanford University, Stanford, California 94305

Cultured mouse dorsal root ganglion (DRG) neurons offer both experimental and theoretical advantages for evaluating the electrical constants in a mammalian neuron. Their simple geometry makes it possible to represent the cell by an isopotential soma compartment (consisting of a capacitance C_S in parallel with a conductance G_S) which is attached to a single dendritic cylinder (represented by a resistive-capacitive network in parallel with the soma compartment) having an electrotonic length L. We have developed and applied to these cells a convenient method for obtaining specific membrane properties and electrotonic structure.

The DRG cell soma was penetrated with two independent microelectrodes, one for delivering current steps and the other for recording voltage responses. Current-voltage curves were obtained and subsequent analysis was restricted to the voltage range in which the cell membrane gave an ohmic or linear response to hyperpolarizing current steps. The slope of the hyperpolarizing charging curve was fitted by the function

$$-\frac{dV}{dt}(t) = \Sigma a_i e^{-t/\tau_i} ,$$

where t is the time from the onset of the current step. The dendritic to somatic input conductance ratio ρ was obtained from the relation

$$\rho = \frac{G_N \tau_0}{I} \Sigma a_i - 1 ,$$

where G_N is the input conductance of the whole cell, τ_0 is the membrane time constant, and I is the amplitude of the current step. The remaining parameters could then be obtained as follows

$$G_S = G_N/(\rho + 1) \quad , \quad C_S = G_S \tau_0 \quad , \quad L = \pi \left(\frac{\rho/(\rho + 1)}{\tau_0/\tau_1 - 1}\right)^{1/2} ,$$

where τ_1 is an "equalizing" time constant. The cell soma area A_S was determined and, from A_S and the values of C_S and G_S, we calculated the specific membrane resistivity R_m and specific membrane capacitance C_m.

All of the electrical constants were within the usual range for vertebrate neurons except ρ, which fell between 0 and 2. The assumptions underlying this analysis were assessed with computer simulations, using the compartmental analysis of Perkel and Mulloney (Amer. J. Physiol. 235,R93,1978), and were found to be reasonable for cells with the general shape of a DRG neuron. The applicability of these analytical methods to some neurons with very different cellular geometries is currently under investigation. (This research was supported by NS 06161, NS 12151 and NS 09744).

974 PRESSURE INCREASES MEMBRANE CONDUCTANCES IN VOLTAGE CLAMPED MOLLUSCAN NEURONS. James L. Parmentier, Brij B. Shrivastav and Peter B. Bennett* Dept. of Anesthesiology, F.G. Hall Laboratory, Duke University Medical Center, Durham, North Carolina, 27710.

The narcosis induced by general anesthetics and excitability produced by raised hydrostatic pressure are known to be mutually antagonistic in whole animals, including man. The cellular mechanisms involved in these responses remain unclarified. The "critical volume hypothesis" (Miller, Fed. Proc. 36:1663-1667, 1977) states that anesthesia will occur when the volume of a hydrophobic region of the membrane is caused to expand beyond a critical value by the absorption of an inert substance. An applied pressure will then oppose this expansion and reverse the narcosis by restoring the membrane region to its former functional position. This hypothesis assumes that pressure acts directly at the site of anesthesia to alter its effect. We have tested this assumption in three seperate single neuron preparations using voltage clamp techniques to study membrane currents associated with 1) the action potential in squid giant axons, 2) the slow inward current controlling burst formation in R15 of Aplysia, and 3) the slow outward current underlying adaptation responses in R2 and LP1 of Aplysia.

Following exposure to the volatile anesthetic halothane all three preparations showed a reduction of excitability and an increase in membrane permeability. Compression to 150 ATA caused an increase in potassium conductance in the squid axon and an increase in the ultra-slow outward current, thought to be carried by potassium in the giant cells of Aplysia. The rate of adaptation of the Aplysia neurons was increased by pressure in a manner similar to that caused by the anesthetic. When pressure and the anesthetic were applied simultaneously both effects were noted, and no indication of pressure reversal was apparent. In each case the anesthetic appeared to act in a manner functionally distinct from the applied pressure. For the squid axon it was possible to calculate changes in the membrane time constants associated with the pressure enhancement. It is concluded that pressure reversal is a function of multicellular homeostasis in central nervous organization rather than direct interaction at the level of the unit membrane and that the "critical volume hypothesis" may have to be refined to account for pressure reversal observations in whole animals.

975 ANION CHANNEL SIZE IN CEREBELLUM DURING SPREADING DEPRESSION. J.M. Phillips* and C. Nicholson. Dept. Physiol. & Biophys., New York Univ. Med. Ctr., 550 First Ave., New York, NY 10016.

Extracellular Cl^- (diameter 3.6 Å) falls transiently during spreading depression (SD) in the cerebellum (Kraig & Nicholson, Neuroscience 3: 1045, 1978), presumably due to entry into cells (Van Harreveld & Schadé, Am. J. Physiol. 189: 159, 1957). Using ion-selective micropipettes (ISMs) we have studied the behavior of several "probe anions" i.e. exogenous anions larger than Cl^-, during SD to determine characteristics of anion permeability.

ISMs were made from theta-glass capillaries and contained, as anionic exchanger, either Aliquat 336 or Crystal Violet dissolved in 3-nitro-O-xylene (Senkyr & Petr, in Ion Selective Electrodes, ed. by E. Pungor, Elsevier 1978). They had Nernstian responses to Na-salts of the following anions: thiocyanate (SCN), hexafluorophosphate (PF_6), hexafluoroarsenate (AsF_6), hexafluoroantimonate (SbF_6), salicylate (SAL) and α-naphthalene sulfonate (α-NS). The selectivities over Cl were: SCN, 500:1; PF_6, 7000:1; AsF_6, 15,000:1; SbF_6, 600:1; SAL, 800:1; α-NS, 1500:1. Rats were anesthetized with urethane and the exposed cerebellum superfused with warm saline. Anion probes (1-5 mM) were introduced into the extracellular space by adding them to the superfusate, except for SAL which was introduced by local iontophoresis. Extracellular anion concentration was measured with an ISM at a depth of 50-100 μm. Local potential change was measured from the reference barrel of the ISM. SD was induced by switching the superfusate to a hypotonic, low NaCl (25 mM) Ringer and then locally stimulating briefly at 50 Hz.

SD was defined by the characteristic slow negative transient in extracellular potential. SCN^- (diameter 4.2 Å), PF_6^- (5.6 Å) and AsF_6^- (6.35 Å) all decreased in extracellular concentration by about 40% of baseline concentration during SD. SbF_6 (7.30 Å), SAL (8.9 Å) and α-NS (11.2 Å) all increased in concentration by about 50% baseline value, during SD.

These findings suggest that during SD a channel of size 6.35 - 7.30 Å diameter becomes active. Anions larger than 7.30 Å are concentrated in the extracellular space by the entry of smaller ions (residual Cl^- and Na^+) into cells together with water (3.0 Å) and consequent cellular swelling at the expense of extracellular volume. While this anion channel size exceeds that postulated for several IPSPs, it is in excellent agreement with the Cl channel of skeletal muscle and erythrocytes. Our results thus show the existence of widespread anionic channels of defined size which can be demonstrated during SD. (Supported by USPHS Grants NS-13742 and GM-07308 (JMP).

976 IONIC CURRENTS UNDERLYING THE ACTION POTENTIAL OF NEUROBLASTOMA CELLS F. N. Quandt* and T. Narahashi (Spon: A. I. Farbman) Dept. Pharmacol., Northwestern Univ. Med. Schl., Chicago, IL 60611.

The action potential recorded from N1E-115 neuroblastoma cells exhibits a fast depolarizing phase followed by a transient repolarization, and finally a depolarizing after-potential that decays to the resting potential level in 0.1 to 10 sec. Through voltage-clamp experiments, Moolenaar and Spector (J. Physiol. 278, 265, 1978) found voltage-dependent Na, K and Ca channels in these cells. We have performed experiments to clarify the ionic basis of the components of the action potential and to examine the pharmacology of the channels in more detail. A two microelectrode voltage-clamp technique was utilized. The cell body was found to be isopotential during an action potential and series resistance was minimal. Early inward currents were recorded in response to step depolarizations to potentials less negative than -40 mV from a holding potential of -80 mV. The inward currents became maximal (-90 to -240 nA) at a depolarization to 0 mV, exhibited a time-to-peak of 2 msec (16 -17°C) at this potential, and rapidly inactivated. This current has a reversal potential of 45 mV and was completely blocked by 1 μM tetrodotoxin. Delayed, steady-state outward currents became prominent for depolarizations beyond -20 mV. The reversal potential for these outward currents measured from tail currents following step repolarizations was -70 mV. This current was reduced 90% by 15 mM tetraethylammonium and 30% by 1 mM 3, 4-diaminopyridine. While the fast depolarization and repolarization of the action potential appear to be primarily due to the early inward and delayed outward currents, respectively, current through the Ca channel is responsible for the depolarizing after-potential. The after-potential was found to be associated with an increase in membrane conductance, measured with constant current pulses. Application of 1 μM tetrodotoxin with 15 mM tetraethylammonium enhanced the slow potential. Substitution of 62 mM Sr^{2+} for Na^+ in this solution resulted in a prolonged action potential. Under these conditions, slow inward currents could be measured in response to step depolarizations to potentials less negative than -40 mV. These currents exhibited a maximum (-10 nA) at a depolarization to 0 mV with a time-to-peak of 20 msec. Inward current lasted for more than 150 msec at this potential. Cd^{2+} (1 mM) reversibly blocked this inward current. This study was supported by NIH grant NS 14144.

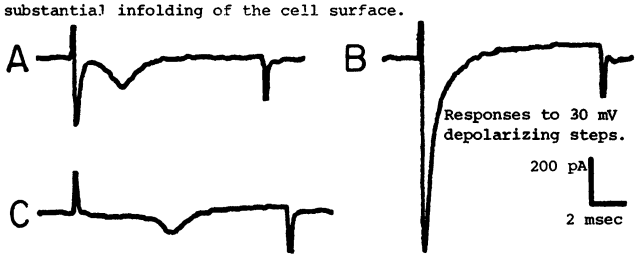
977 CRAYFISH STRETCH RECEPTOR NEURONS HAVE DIFFERENT SODIUM CHANNELS IN AXONS AND DENDRITES. William M. Roberts* (Spon: William B. Kristan). Dept. of Neurosciences, UCSD, La Jolla, CA 92093.

A modification of the technique of Neher and Lux (Pflugers Arch. 311: 272, 1969) was used to measure current through small (5-9 μ diameter) membrane patches in tonic abdominal stretch receptor neurons of the crayfish *Pacifasticus leniusculus*. Two intracellular electrodes were used to voltage clamp the cell. The extracellular patch electrode was positioned close to the intracellular voltage electrode; the membrane was not well clamped at distances greater than 50 μ.

Three major components of the active spike currents in the dendrites, soma, and proximal axon were found: a non-inactivating outward K^+ current and two transient inward Na^+ currents. Both of the inward components are present in the soma (A), while the axon (B) shows only the fast component and the dendrites (C) show only the slow component. The outward current was present at all locations studied. Both inward components were blocked by 2 nM-TTX and showed similar dose-response characteristics. Both were abolished in Na^+-free (choline substituted) saline and were unaffected by Li^+ substitution for Na^+, by low Ca^{++}, or by addition of Ba^{++} or Co^{++}. The two inward components observed in the soma could be separated by applying a 4 msec duration depolarizing step (to inactivate both components) with a test step 2 msec later. By this time the fast component had recovered while the slow component was still inactivated.

The slower inward current in the soma and dendrites causes a broadening of the dendritic spike and often produces a notched waveform of the soma spike. This current is probably involved in double-spiking seen at elevated temperature (Calvin and Hartline, J. Neurophys. 40:106, 1977). A similar mechanism could be present in other neurons which show notched soma spikes and double-spiking (Calvin and Sypert, J. Neurophys. 39:420, 1976).

Capacitance measurements give values of 2-5 $\mu F/cm^2$ (compared to 1 $\mu F/cm^2$ found in most lipid bilayer membranes), suggesting a substantial infolding of the cell surface.



978 A MUTATION AFFECTING MEMBRANE STABILITY IN PARAMECIUM TETRAURELIA. Youko Satow. Lab. Molecular Biology, Univ. Wisconsin, Madison, WI 53706.

It is well known that an increase in external Ca concentration changes the surface-charge pattern and stabilizes the membrane as shown by a shift in the voltage sensitivity of Na or Ca channels. teaB, a mutant P. tetraurelia, appears to have a more stabilized membrane. The curve of voltage sensitivity of the Ca channels (G_{Ca}-V relation) in teaB is about 10 mV more positive than that of wild type. The inactivation curve (max G_{Ca}-V_h relation) of the mutant is about 8 mV more positive than that of wild type. However, the mutant and the wild type have the same maximal G_{Ca} of 40 nmho/cell. The K conductance responsible for the anomalous rectification also appears more stabilized in the mutant.

Wild-type paramecia grown at a high temperature (34°C), but tested at room temperature (22°C), differ from those that never experienced the high temperature. The G_{Ca}-V relation shifted toward less sensitivity and both the activation and inactivation of the Ca channel are slowed down in the 34°C-grown wild type. teaB mutant, however, shows little change after incubations at 34°C. The maximal G_{Ca} is not affected by the growth temperature in both the wild type and the mutant. It is known that ciliated protozoa can detect temperature and redirect their phospholipid fluidity. The teaB mutation may damage this mechanism and cause a loss in thermo-responsiveness (corresponds with the behavioral observation by T. Hennessey). This would lead to perturbation in membrane lipid compositions, surface-charge pattern and membrane stability.

The experiments were done under a voltage clamp. The Ca currents were given by the subtraction method using non-leaky pawnA, pwA_{500}, since pwA locus has a defective site on a number of functional Ca channels (Satow & Kung, submitted to J. Exp. Biol.) and +115 mV for E_{Ca} was used for the calculation of G_{Ca} as a chord conductance. The external Ca concentration was 0.91 mM and the external K concentration was 4 mM through the experiments. This work was supported by NSF grant BNS77-20440 to C. Kung.

979 THE ACTION OF POTASSIUM CURRENT ANTAGONISTS ON CAT SPINAL MOTONEURONS STUDIED BY VOLTAGE CLAMP. P.C. Schwindt* and W.E. Crill. Dept. Physiol. & Biophysics, and Medicine, Univ. Washington, Seattle, WA 98195.

Depolarizing voltage steps from resting potential activate two kinetically distinct, persistent, outward current components in cat motoneurons, I_K^f, a fast component, and I_K^s, a component having time constants about 10 times slower. Since these are likely to be potassium (K^+) currents, we have examined their alteration during voltage clamp while extracellularly iontophoresing K^+ itself, and while iontophoresing extra- or intracellularly various agents known to depress K^+ currents in invertebrate neurons. Extracellular K^+ depressed both I_K^f and I_K^s, presumably by raising K^+ equilibrium potential. Agents depressing I_K^f without significant effect on I_K^s included intra- or extracellular tetraethylammonium (TEA) or barium (Ba^{++}) and intracellular 4-aminopyridine (extracellular not tried). Extracellular TEA and intracellular Ba^{++} produced the most powerful and rapid effects. Extracellular Ba^{++} apparently had to enter the cell before exerting its blocking effect. In cells where a persistent inward current (I_1) had deteriorated, only a relatively small widening of the action potential occurred after all I_K^f appeared to be blocked, suggesting that the large leak conductance and a fast transient outward current existing in motoneurons are sufficient to rapidly repolarize the spike in the absence of I_1. Slow K^+ currents in several invertebrate and vertebrate neurons appear to be calcium (Ca^{++}) mediated, but the Ca^{++} blockers, cobalt and manganese, depressed both I_K^f and I_K^s in cat motoneurons. Either these divalent cations have non-specific K^+ blocking effects in cat motoneurons, or both fast and slow K^+ currents are Ca^{++} mediated. Supported by VA Research Grant MRIS 1610.

980 VOLTAGE CLAMP STUDIES OF A-CURRENT VARIABILITY IN NEURONES OF ARCHIDORIS MONTEREYNSIS. Elba E. Serrano* and Peter A. Getting. (SPON: P. Lennard). Department of Biological Sciences, Stanford University, Stanford, CA 94305.

Voltage clamp studies of molluscan neurones demonstrate the existence of several ionic currents with voltage and time dependent conductance changes. Presumably the characteristic firing patterns of cells require the integration of these ionic currents in space and time across the membrane. Differences in the kinds of currents, their relative contribution to membrane excitability and/or variations in the nature of the specific voltage and time dependent conductance changes of the individual currents could all contribute to the observed variation in cell repetitive firing. This study focuses on characterizing the intracell and intercell variability of the A-current system under voltage clamp. The transient outward potassium current, I_A, is particularly suitable for these measurements since using the appropriate voltage paradigms it can be activated in isolation from other membrane currents without need for pharmacological blocks.

Isolated neurons from the cerebral, pleural and pedal ganglia of Archidoris montereynsis were voltage clamped at 10°C. The population of cell types is comprised of five neurons. Cells were chosen on the basis of constancy of location from prep to prep, ease of removal from the ganglion, reproducible firing patterns. Between cell types, however, there is a variation in the f-i responses under current clamp.

Variability has been examined in the following parameters: a) magnitudes of currents b) steady state activation and inactivation c) peak current activation and inactivation d) time constant for decay of I_A (τ_B) e) time constant for activation (τ_A) f) time constant for removal of inactivation. In this group of cells, the variation in the activation and inactivation curves within cells was of the same order as the variation between cells. The most striking variability between cells seems to be in the kinetics of their response to changes in potential. In particular, the time constants for decay of inactivation show constancy within a cell and variability between cells. Cells segregate into two populations: in one, the τ_B is of the order of 60-90 ms, in the other, it is between 260 and 330 ms.

981 EXCITATION OF SQUID AXON MEMBRANE INTERNALLY AND EXTERNALLY EXPOSED TO SINGLE-SALT SOLUTIONS. Susumu Terakawa* (SPON: S. Judge). NIH, Bethesda, Md. 20205

Squid axons were perfused both internally and externally with a solution containing $CoCl_2$ or $MnCl_2$ only (tonicity was adjusted using 12% (v/v) glycerol). At a divalent-cation salt concentration 1-10 mM, the potential difference across the membrane was close to zero. Upon application of a constant inwardly-directed current through the membrane, periodic variations of the voltage across the membrane were observed. These periodic variations of the voltage were very similar to repetitively-fired action potentials. They appeared even in the virtual absence of the concentration gradient across the membrane. The amplitude of these responses was commonly found to be 100 mV. The periodic responses obtained were accompanied by changes in the membrane conductance. The anions tested as cobalt salt included fluoride, chloride, acetate, citrate, and gluconate. There was no qualitative difference in the electrophysiological responses obtained with these anions. Nickel chloride and barium chloride were also able to elicit the periodic responses. The internal and external salt concentration could be varied independently without losing excitability. When the $MnCl_2$ concentration in the external medium was reduced from 10 mM to 2 mM, the duration of the response obtained by passing an inward current was prolonged. Reducing the $MnCl_2$ concentration of the internal medium from 5 mM to 1 mM shortened the duration of the response and enhanced its amplitude. An effect similar to that of reducing the internal salt concentration could be obtained by increasing the inward current. Tetrodotoxin (2 μM) added to the external medium did not suppress the excitability. This simplest ionic condition achieved may help advance the physico-chemical theory of nerve excitation.

982 QUANTIFICATION OF ELECTROMAGNETIC FIELD STRENGTHS IN MEDICAL LINEAR ELECTRON ACCELERATORS AND THEIR IMPLICATIONS FOR PATIENTS WITH ARTIFICIAL CARDIAC PACEMAKERS; P. J. Tetenes* and P. A. Anninos, Department of Radiology Albert Einstein College of Medicine, Bronx, New York (10461) and Department of Physics Concordia University, Montreal, P.Q.

Behavioral changes in the neurophysiological responses of living organisms exposed to radiofrequency and microwave radiations have been well documented in the scientific literature. The character and intensity of these changes are dictated, among others, by the high sensitivity of many compartments of the CNS to such radiation exposures, the variability of electrophysical properties of biological media, and the inherent characteristics of the ambient electromagnetic fields. Consequently, this vast array of interactive variables hampers the development of generalized but exact mathematical expressions to describe the many significant and novel bioeffects resulting from exposures to such wide energy spectrum. The specificity with which all pertinent parameters can be described may decrease the uncertainty in explaining the phenomenology of the effects both experimentally and theoretically. A unique biophysical problem with clinical implications involves individuals with artificial cardiac pacemakers exposed to pulse-modulated, 3000MHz, radiation emissions from medical linear electron accelerators. This work will describe the experimental methodology employed in the quantification of the field gradients present in these facilities. A theoretical analysis will follow of the extraneous and induced electric field intensities in the chest walls of humans. The level of susceptibility and functional disturbances of representative models of pacemakers will be investigated for " free-field" and "simulated-implant" conditions. Preliminary test results will be discussed in connection with their clinical implications.

983 CONDUCTANCE CHANGES ACCOMPANYING THE MATURATION OF MYOTUBES IN CULTURE. C.M. Thomson* and W.F. Dryden. Department of Pharmacology, University of Alberta, Edmonton, Alberta, T6G 2H7, Canada

During maturation, the resting membrane potential of myotubes in culture increases over a period of several days from a low level (-10 mV) to that found in mature muscle fibres. Ionic conductances have been investigated during this time using a variety of supposedly specific blocking agents. Breast muscle was obtained from 11 day chick embryos and grown in monolayer culture. The membrane potential and conductances were measured at daily intervals from the third day of culture, just after myoblast fusion. Standard current-voltage graphs were prepared directly from steady state membrane potential shifts in response to the application of small currents through the recording glass microelectrode. Input resistances were correlated with measured fibre diameters and values for specific membrane conductance were calculated. Measurements were made on identified fibres both before and after the application of ion conductance blocking agents.

Previously communicated results (Thomson and Dryden, Proc. Physiol. Soc., Dec. 1978) indicated that chloride conductance (G_{Cl}) declined with development and rising membrane potential, but that the decline in G_{Cl} was less than that observed for the unblocked membrane (G_m). In the present work tetraethylammonium (TEA) and Cs^+ have been used to block potassium conductance. Both agents gave similar results and revealed that potassium conductance declined between the third and fourth day of culture (from 0.2 mS cm^{-2} to 0.1 mS cm^{-2}) and thereafter remained constant. Replacement of Na^+, K^+ and Cl^- in the bathing solution by choline iodide revealed a considerable conductance (0.7 mS cm^{-2}) in young fibres which was attributable to Na^+ and was TTX insensitive. This conductance declined rapidly between the third and fifth days of culture and disappeared by the sixth day. The use of calcium channel blocking agents such as verapamil revealed that this Na^+ conductance could be further reduced suggesting that the Na^+ ions were permeating through channels capable of allowing the passage of a calcium current.

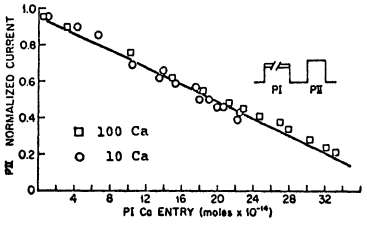
It is concluded that the low resting membrane potential of young myoblasts and myotubes is attributable to this substantial Ca^{++} and Na^+ conductance; and that the rise in membrane potential associated with development is a result of the loss of such steady state cation channels.

(Supported by the MRC of Canada)

984 Ca INACTIVATION IN APLYSIA NEURONS IS QUANTITATIVELY RELATED TO PRIOR Ca ENTRY. D. Tillotson* and R. Eckert. (SPON: A.L.F. Gorman) Dept. of Biology, UCLA, Los Angeles, CA 90024

Neurons of the abdominal ganglion of A. californica were loaded with Cs^+ by means of the nystatin method (Russell et al, J. Memb. Biol. 37:137-156, 1977), replacing internal K^+. Sodium and K were omitted from all solutions so that Ca current was relatively free from contamination by other currents. With this approach it was found that the Ca current exhibits inactivation (Tillotson and Horn, Nature 273:312-314, 1978), and that the inactivation depends on prior Ca entry (Tillotson PNAS 76:1497-1500, 1979).

The role of Ca was further tested by comparing the degree of inactivation (i.e., depression of peak early Ca current) seen during a non-varying pulse (P_{II}) with the amount of Ca having entered the cell during a prior pulse (P_I) in Cs-loaded cells R2 and R14. The bath solution contained 100 mM $CaCl_2$, 50 mM $MgCl_2$, 410 mM Tris-HCl. To produce a wide range of Ca entry, the amplitude and duration of P_I were varied, with extracellular Ca concentrations of both 10 mM and 100 mM. The difference in $[Ca]_o$ was made up with Mg in the 10 mM Ca solution. The time-integral of the P_I inward current was taken to approximate the number of Ca ions entering the cell during P_I, since the current is nearly eliminated by cobalt substitution for Ca. The Figure shows a plot of relative P_{II} Ca current (i.e., relative to the full current recorded during P_{II} under the prevailing conditions in the absence of a prior pulse I) plotted against moles of Ca having entered during the corresponding P_I. Inactivation of the P_{II} Ca current was a linear function of the quantity of Ca entering during P_I irrespective of P_I potential or duration. This provides further evidence that inactivation of the Ca channel following depolarization is a consequence of Ca entry rather than depolarization. Supported by USPHS NS 08364.



985 EFFECTS OF LOWERED INTRACELLULAR pH ON EXCITABILITY IN PARAMECIUM. Joy A. Umbach* (SPON: Peter M. Narins). Dept. Biology and Brain Research Institute, UCLA, Los Angeles, CA 90024.

Ammonium rebound acidification (Boron and deWeer, J. gen. Physiol. 67: 91-112, 1976; Aickin and Thomas, J. Physiol. 273: 295-316, 1977) was used to lower intracellular pH of P. caudatum. Paramecium, which normally exhibits regenerative but graded calcium responses (Naitoh, Eckert and Friedman, J. exp. Biol. 56: 667-681, 1972), produces all-or-none overshooting calcium action potentials under conditions of internal acidification.

The control bath solution consisted of 1mM $CaCl_2$, 3.5mM KCl, 0.5mM KOH, 0.1mM EGTA and 1mM Hepes (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) buffer at pH 7.1. The ammonium solution consisted of the control saline to which NH_4Cl was added to a concentration of 50mM. Specimens were bathed in the NH_4Cl solution for two 15-minute periods separated by a 15-minute interval in the control saline. The paramecia were then returned to the control solution for intracellular current clamp or voltage clamp experiments. All-or-none activity in response to depolarizing current developed within 15 minutes after the final return to the control solution.

Hyperpolarizing and depolarizing current pulses of 200msec duration were passed to determine steady-state current-voltage relations. These relations were not altered from control values in those cells that were acidified and expressed all-or-none activity. The resting potentials of the acidified cells were also normal. In preliminary voltage clamp measurements the conversion to all-or-none behavior was not seen to be accompanied by any changes in the late currents. In contrast, conversion from graded to all-or-none activity as a result of lowered internal pH has been found in arthropod muscle to be associated with a reduction in delayed rectification (Moody, Soc. for Neuroscience Abst. 4: 236, 1978). The present experiments suggest that in Paramecium, lowered intracellular pH leads to all-or-none activity through an action other than an interference with late outward current. Supported by USPHS1 T32M MH15345 and NSF BNS 77-19161.

986 MECHANISMS UNDERLYING DEPOLARIZING AFTERPOTENTIALS IN HIPPOCAMPAL CA3 PYRAMIDAL CELLS. R.K.S. Wong and D.A. Prince. Dept. of Neurology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

We have previously reported that the depolarizing afterpotential (DAP) is important in sustaining burst firing in hippocampal CA3 pyramidal cells (Wong & Prince, Brain Research 159: 385, 1978). In the present study we have examined the mechanism of DAP generation by using the in vitro slice preparation which enabled us to perform various ionic manipulations. Afterpotentials which followed directly or antidromically-evoked spikes could be arbitrarily divided into two events. These consisted of 1) the DAP, and 2) the afterhyperpolarization. Maximum spike repolarization in these neurons typically reached a level depolarized to the resting potential (i.e., there was no undershoot of the baseline). Spike repolarization was followed by a DAP which had two components. One was a depolarizing hump which reached a maximum amplitude of 5-10 mV in about 5 msec and usually crossed the threshold for spike generation, thus functioning to sustain burst firing. When the membrane potential was manipulated with intracellular current pulses, this depolarizing hump occurred at a threshold level of depolarization and could be blocked by hyperpolarizing pulses which were insufficient to block the antidromic spike. Perfusion with Ca^{++}-free solution containing 2 mM Mn^{++} blocked the depolarizing hump (and the succeeding afterhyperpolarization; see Hotson et al., Neurosci. Abstr. 3: 218, 1977). A second component of the DAP was obvious in the 0Ca^{++}, 2 mM Mn^{++} solution. This consisted of a slow depolarization which fell exponentially from the foot of the fast spike repolarization to the baseline with a time constant similar to the membrane time constant. This portion of the DAP had no threshold per se; its amplitude varied as the membrane potential level was manipulated in relation to the constant point of maximum spike repolarization. Focal application of 5 mM Ba^{++} caused almost immediate reappearance of the depolarizing hump of the DAP and the development of burst firing.

These data show that the DAP consists of two components. One is an active event evoked by the spike, and presumably mediated by a slowly decaying Ca^{++} conductance. The DAP also has a passive compoennt which results from the fact that active repolarization of the spike terminates at some level depolarized to the resting potential. During the burst the combined depolarization produced by these two components reaches threshold for spike generation and functions to sustain repetitive firing. This same mechanism may contribute to burst generation in hippocampal pyramidal neurons during epileptogenesis. (Supported by NS 06477 from the NINCDS).

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Withdrawn by Author

MEMBRANE STRUCTURE AND FUNCTION

988 BENZODIAZEPINE AND GABA RECEPTORS IN NB_{2a} NEUROBLASTOMA: ACTION ON Cl^- FLUXES. M. Baraldi*, A. Guidotti and E. Costa. Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hosp., Washington, D.C. 20032

In NB_{2a} neuroblastoma cells as in brain the "GABA receptor unit" includes high affinity ^{3}H-GABA and ^{3}H-diazepam, ^{3}H-clonazepam and ^{3}H-flunitrazepam receptors. The thermostable endogenous inhibitor of protein kinase (GABA-modulin) which inhibits both GABA and benzodiazepine binding is also present.

In these cells, as in brain, diazepam and GABA receptors appear to be functional. An expression of this function is that GABA (0.1 mM) increases the binding of ^{3}H-diazepam and ^{3}H-clonazepam. Since in brain GABA receptors are thought to be coupled with Cl^- ionophores we have tested whether also in NB_{2a} cells the activation of GABA receptors with GABA or muscimol causes an increase in the inward flux of $^{36}Cl^-$. In presence of GABA receptor agonist a new steady state in the Cl^- distribution across NB_{2a} membranes is obtained. The extent of this changes is related to GABA (10^{-10} to 10^{-4}M) or muscimol (10^{-10} to 10^{-5}M) concentrations. The increase in Cl^- flux caused by GABA or muscimol is blocked by 10^{-4}M bicuculline. In addition a dose of diazepam (10^{-6}M) which produces a release of GABA-modulin from the cells but by itself fails to change the Cl^- flux, is capable of facilitating the increase in Cl^- flux elicited by threshold doses of GABA or muscimol.

These results suggest that NB_{2a} cells are a suitable model to study the interaction of benzodiazepine and GABA receptors at the molecular level.

989 SOLUBILIZATION AND PURIFICATION OF THE SODIUM CHANNEL STX BINDING COMPONENT FROM RAT SARCOLEMMA. R.L. Barchi. University of Pennsylvania, Philadelphia, PA 19104.

Sodium channel STX binding sites can be solubilized from rat skeletal muscle sarcolemma using medium chain-length nonionic detergents (NP-40, Lubrol-PX, Brij-96). Solubilized binding sites could be identified by specific binding of ^{3}H-STX. These sites were unstable in detergent alone; stability was markedly improved by the addition of exogenous phospholipids (PC, PE but not cholesterol) in mole ratios of 1:4 to 1:6 with detergent molecules. Solubilized binding sites were very temperature sensitive with $T_{\frac{1}{2}}$ for loss of binding in excess of 24 hours at 0°C but less than 20 minutes at 30°C. Binding site stability to changes in pH below 7.0 or above 8.0 was considerably lower after solubilization than in the intact membrane. Specific STX binding in the solubilized material could be eliminated by reaction with the carboxyl modifying reagents TMO or carbodiimide by a mechanism which was blocked by the presence of excess toxin in a manner analogous to that seen in intact membranes.

Initial purification of sarcolemma from muscle resulted in a 30-40 fold increase in concentration of STX binding sites per mg. of protein. An ion-exchange column was synthesized having guanidinium groups immobilized by 20 atom spacers to agarose beads. Application of solubilized membrane protein and elution with 100 mM choline Cl^- elutes 90-95% of total protein and virtually no specific STX binding material. Subsequent elution with 400 mM choline Cl^- yielded >75% of initial binding sites with 10 to 20 fold increase in purification. Subsequent chromatography on Sepharose 6-B or Sephacryl 300 yielded a further 5 to 10 fold increase in specific STX binding activity. The purified STX binding protein had an apparent $S_{w,20}$ by the Ames method of about 9, comparable to that seen in solubilized but unpurified material. Further biochemical studies on the purified protein will be presented.

990 CHARACTERIZATION OF THE GLUTAMATE RECEPTOR-IONOPHORE INTERACTION OF BRAIN SYNAPTOSOMES AND SYNAPTIC MEMBRANES. H. H. Chang* and E. K. Michaelis. Neurobiology Section, Dept. of Human Development, Univ. of Kansas, Lawrence, KS, 66045.

The excitatory amino acid L-glutamic acid (Glu) is known to produce its depolarizing effects through increases in Na^+ conductance of neuronal membranes. Rat brain synaptosomes prepared in Ficoll-sucrose gradients exhibited a rapid and a slow phase of ^{22}Na uptake. This uptake process was enhanced by 0.5mM ouabain, 10μM gramicidin D, and 1μM L-Glu. Whereas both ouabain and gramicidin D inhibited the synaptosomal Na^+-K^+ ATPase (100 and 86% respectively), Glu did not inhibit at all this enzyme. The L-Glu-stimulated ^{22}Na uptake process exhibited dose response characteristics with maximal stimulation obtained at 10μM L-Glu.

Synaptic membrane ghosts prepared according to the method of Kanner (1978) and sealed with an internal K-phosphate concentration of 135mM were found to have primarily one phase of ^{22}Na uptake, a rapid phase. This ^{22}Na uptake was once again stimulated by L-Glu in a dose-dependent manner with maximal stimulation seen at 1μM L-Glu. This Glu-stimulated ^{22}Na uptake was insensitive to tetrodotoxin treatment and partially resistant to preexposure to organomercurials, treatments which are known to inhibit the voltage-dependent Na^+ channels and the Na^+-dependent Glu transport activity. In addition, this ^{22}Na uptake was stimulated by L-aspartate, but was not affected by D-Glu, or glutamine. In addition, concentrations of L-Glu in the 10^{-5}-10^{-4}M range were less effective in stimulating ^{22}Na flux than lower concentrations of L-Glu, possibly a sign of receptor desensitization. These observations are indicative of the similarity of the properties of the Glu-stimulated ^{22}Na uptake in these preparations to the Glu-receptor-activated Na^+ conductance in nerve cell membranes.

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991 IMMUNOCYTOCHEMICAL LOCALIZATION OF COATED VESICLE PROTEIN IN RODENT NERVOUS SYSTEM. Toni Po-on Cheng*, Frances I. Byrd* and John G. Wood. Dept. Anat., Univ. Tenn. Center Health Sci., Memphis, Tenn. 38163

Antibody to the major coat protein (clathrin) of coated vesicles has been used to study the distribution of this protein in the nervous system. The coat protein was purified by SDS polyacrylamide electrophoresis, extracted from the gel and used to immunize rabbits. Immunodiffusion assays and immunofixation of polyacrylamide gels indicated the presence of specific IgG in the immune serum against the gel extract protein. This antiserum was used to study the distribution of coat protein in mouse cerebellum. Mice were perfused through the aorta with a fixative containing 4.0% paraformaldehyde and 0.1% glutaraldehyde in 0.12M phosphate buffer. The cerebellum was stored overnight in phosphate buffered 4.0% paraformaldehyde and 40-50 u slices were obtained for immunocytochemistry using a vibratome. The slices were incubated in rabbit preimmune or immune serum (1:2000 dilution) followed by peroxidase conjugated goat anti-rabbit IgG. After processing the slices for electron microscopic peroxidase cytochemistry, ultrathin sections were obtained and identified synaptic contacts on Purkinje cells and in cerebellar glomeruli were studied. In these regions the label was highly concentrated in presynaptic terminals of basket cell axons, mossy fiber axons and Golgi II axons. The antibody present in immune serum reacted with coated vesicles within these three types of presynaptic terminals, but in addition, label was observed within the terminals in areas devoid of coated vesicles. These data suggest that either the coat protein contributes to structures within presynaptic terminals other than coated vesicles or that these presynaptic terminals contain a pool of coat protein which takes part in the assembly and disassembly of coated vesicles. Supported by USPHS Grant NS-12590 (JGW), USPHS Grant GM00202, and the Sloan Foundation (JGW)

992 INSERTION OF NEWLY-SYNTHESIZED ($Na^+ + K^+$)-ADENOSINE TRIPHOSPHATASE SUBUNITS INTO EEL ELECTROPLAX MEMBRANES. Lynn Churchill and Lowell E. Hokin*. Dept. Pharmacology, Univ. of WI Med. Sch., Madison, WI 53706.

Newly-synthesized ($Na^+ + K^+$)-adenosine triphosphatase (Na,K-ATPase) has been investigated by purifying the holoenzyme from eel electroplax membranes after incorporation of L-[3,4(n)-^{3}H] valine in vivo and in vitro. The purity of Na,K-ATPase is sufficient for accurate analysis of the [^{3}H] valine incorporated into the large and small subunits of the holoenzyme. The large and small subunits have similar rates of [^{3}H] valine incorporation in vitro and in vivo. Since the valine concentration is similar in both subunits and the mole ratio of the large and small subunits in the holoenzyme is probably one, the similar rates of [^{3}H] valine incorporation indicate a coordinated synthesis and/or assembly of the subunits into the holoenzyme.

Previously we reported a delay in incorporation of [^{3}H] valine into the subunits purified from eel electroplax membranes (Churchill and Hokin, Soc. for Neurosci. abst., 1977). The total cellular protein as analyzed by trichloracetic acid precipitation did not show a 2.5 to 3 h delay in incorporation. Therefore, the delay could not be explained by slow equilibration of the amino acid precursor. Further evidence that a lag in appearance of newly-synthesized Na,K-ATPase exists is presented. After exposure to the protein synthesis inhibitor, cycloheximide, the total cellular protein ceased to incorporate [^{3}H] valine in vitro; whereas, both the large and small subunits continued to increase in specific radioactivity for 1.5 to 2 h. These two lines of evidence demonstrate that a lag in the appearance of newly-synthesized Na,K-ATPase exists and that both subunits show the same lag. This lag probably represents post-translational intracellular processing prior to entry of the subunits into the plasma membrane. Moreover, the newly-synthesized Na,K-ATPase subunits prior to entry into the plasma membrane must have different physical properties from the holoenzyme in the plasma membrane, as they do not copurify with it.

The fact that the large, non-glycosylated subunit has a delay similar to the small, glycosylated subunit suggests that either (1) assembly of the holoenzyme depends on the availability of the small subunit as a rate-limiting step, or (2) assembly occurs prior to glycosylation of the small subunit so that the large subunit is delayed in the Golgi complex as part of the lipoprotein complex.

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993 CHEMICAL DISSECTION OF THE NERVE ENDING MEMBRANE. Garrett D. Crawford*, H. David Potter* (SPON: P. M. Salvaterra). Division of Neurosciences, City of Hope Nat. Med. Ctr., Duarte, CA 91010

A synaptic plasma membrane preparation was exposed to a sequential protocol designed to interrupt only "peripheral" membrane protein interactions, and the resulting treatment products were prepared for electron microscopy. Treatments included isotonic salt with the reducing agent dithiothreitol, EGTA, a low level of nonionic detergent, and EDTA. The scope of this study was limited to Gray's type I profiles, and a classification scheme based upon the components of the junctions was developed to analyse the results. After every treatment, profiles were scored for the following: (a) synaptic vesicles (SV), either normal or enlarged; (b) the presence of a polymorphous material abutting upon the SV and the synaptic membrane that often appears filamentous (ivfs); (c) the presynaptic membrane, and (d) a postsynaptic specialization with a continuously attached postjunctional membrane. The last attribute served as a morphological marker for the modified profiles. In addition the continuity of extrajunctional membranes and cleft structures was monitored.

All modified forms of the profiles were present in each preparation including the starting material; only the distribution changed. This suggests that the effect of the treatments is primarily upon restricted membrane loci, and supports the use of defined chemical media for the dissection of complex microscopic structures. Specifically, the data confirms the structural integrity of the ivfs and indicates the dependence of the SV organization upon it rather than upon the enclosing presynaptic membrane. Major differences in the structure of junctional and nonjunctional membranes are also evident. Supported by NIH grants NS-08309 and NS-09864.

994 IONIC MECHANISMS OF SLOW MEMBRANE POTENTIAL OSCILLATIONS IN APLYSIA NEURONS. Wolfgang J. Daunicht*$^+$) and Manfred R. Klee*. Dept. Neurobiol., MPI Brain Res., Deutschordenstr. 46, D-6000 Frankfurt/M, FRG. (SPON: R. Eckmiller).

Some identified neurons in the visceral ganglion of Aplysia californica contain a membrane system which gives rise to slow relaxation oscillations of membrane potential. The slow system was investigated by conventional voltage clamp. Steady state and dynamic I-V-characteristics, current relaxation times, and membrane capacity were measured using step and ramp commands in different ionic concentrations of the extracellular solution. The shape of diagrams consisting of the single steady state and a family of dynamic I-V-characteristics was found to give the criterion for the stability of the membrane potential at a constant net current.

After changes of the K^+concentration position and slope of the steady state I-V-characteristics are in accordance with the constant field theory at inward currents, while at zero currents they are not. I-V-diagrams undergo transformations which can be described as contractions in the direction of the voltage axis with increase of K^+concentration and as expansions with decrease of K^+concentration.

In Ca^{++}free solution silent cells tend to start oscillating and oscillating ones tend to become bistable. The transformation of I-V-diagrams caused by reduction of Ca^{++}concentration can be explained by a contraction of dynamic I-V-characteristics in the direction of the voltage axis.

In control solution a reversal potential of the relaxing currents could not be found between -80 mV and -20 mV. Reduction of the Na^+concentration strongly decreases the amplitude of the relaxing currents.

These findings support the hypothesis that in the membrane of the slow system a K^+-Ca^{++} ion exchange controls the diffusion of cations, mainly of Na^+ions. The relaxation could be explained by changes of cation concentrations close to the membrane. Oscillations of membrane potential at constant net current could be due to feed back of K^+concentration changes to the ion exchange process.A numerical model based on this hypothesis predicts the experimental results qualitatively.

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995 SEASONAL OCCURRENCE OF DELAYED RECTIFICATION IN EEL ELECTROPLAQUE. Donald A. Farquharson*.(SPON:F.C.G.Hoskin). Dept. Biol.,Illinois Intitute of Technology,Chicago,IL 60616.

Heretofore,all reports describing eel electroplaques electrophysiologically have characterizied the electrically excitable K^+ channels as being open at the cell's resting potential and as being closed(inactivated)by either a depolarizing voltage step(depolarizing K^+-inactivation)or by a hyperpolarizing voltage step (hyperpolarizing K^+-inactivation),e.g.see Nakamura et al,J. Gen. Physiol. 49:321,1965;or Ruiz-Manresa et al,J. Gen. Physiol. 55:33, 1970. I report here an interesting seasonal variation in the K^+ channel characteristics of electroplaques examined under voltage clamp.Certain eels,shipped to our laboratory in Chicago in the winter months of 1976 through 1978 had electroplaques which displayed a delayed outward current,apparently a K^+current,in addition to the well-known K^+-inactivation phenomenon. This delayed K^+ current was activated when the cells were depolarized. That is, a depolarization of sufficent magnitude resulted in the steady state conductance suddenly decreasing and then,after a delay,increasing with a complex time course. This K^+-activation was qualitatively and quantitatively simular to the delayed rectification of K^+ currents described for the squid giant axon. The K^+channels activated in these winter electroplaques were insensitive to Ba^{++},but were partially blocked by TEA. Non-winter electroplaques have K^+ channels(those inactivated by depolarizing or hyperpolarizing pulses) which are blocked by Ba^{++} and which are insensitive to TEA. This K^+-activation phenomenon in winter electroplaques was also sensitive to the rate of stimulation(i.e. the frequency of a train of depolarizing pulses)and was affected by prepulse conditioning such that hyperpolarizing prepulses increased the delayed current magnitude while depolarizing prepulses decreased the delayed current magnitude. Prepulses also affected the kinetics of the delayed K^+ current activation in a manner somewhat analogous to that found for squid giant axons(Cole and Moore,Biophys. J. 1:1,1960). The pharmacological data collected suggest that these winter electroplaques have two kinds of voltage-sensitive K^+ channels. However, some of the conductance data argue for a single K^+ channel,in winter electroplaques,with complex dynamics. (Research supported by ARO grant DAAG29-78-G-0090)

996 ARRANGEMENT OF MULTIPLE MONOAMINE OXIDASE ENZYMES ACROSS THE OUTER MEMBRANE OF RAT BRAIN MITOCHONDRIA. Robert Faulkner* and Rosa Huang* (SPON: J-Y Wu). University of South Alabama, Mobile, Alabama 36688.

Phospholipases C and D (LC, LD) were used to remove the surface head groups of membrane phospholipids of an intact, purified rat brain mitochondrial preparation. Both LC and LD treatment under conditions of varying lipase concentration and incubation time attacked only phosphatidylcholine (PC) and phosphatidylethanolamine (PE). PC and PE constituted about 80% of the brain mitochondrial phospholipids. After lipase treatment, the bilayer structure of the outer mitochondrial membrane was retained. In the case of LD treatment, MAO-B sites were inactivated to a much greater extent than MAO-A sites. MAO-B sites were more peripheral. MAO-A sites were located below the alcohol moiety of the membrane phospholipids. LC treatment eliminated the phosphoryl alcohols and thus exposed a few Å deeper into the bilayer midline in contrast to the action of LD. The data indicated that MAO-A sites then became accessible. Further, an additional class of MAO-B sites was exposed by the action of LC. (Supported by NIH Grant #NS-144434)

997 NEW NEURITE MEMBRANE IS ADDED AT THE GROWING TIP. E.L. Feldman*, D. Axelrod*, M. Schwartz* and B.W. Agranoff. Neuroscience Laboratory and Biophysics Research Division, University of Michigan, Ann Arbor, MI 48109.

Explant culture of the adult goldfish retina results in vigorous neuritic outgrowth, provided that the optic nerve has been crushed in vivo 10–14 d prior to explantation. We have used this preparation, coupled with a direct membrane marker, Concanavalin A (Con A) to determine the site of new membrane addition in growing neurites.

We had previously determined that Con A labels neurite cell surfaces. In order for Con A to be a useful marker for studies of membrane addition during neurite outgrowth, it was necessary to determine conditions under which the lectin would not be toxic to the culture (i.e., prevent neurite outgrowth) and also would not diffuse into the new areas of growth. Initial studies indicated that high concentrations of Con A were toxic to the culture while lower doses of Con A, which were non-toxic, diffused rapidly into new areas of growth. This low dose of Con A (10 µg/ml) could be immobilized by the subsequent addition of 100 µg/ml of lectin antibody (anti-Con A). This treatment did not prevent growth. Furthermore, the receptor/Con A/anti-Con A complex was not appreciably internalized, making it an ideal membrane marker for the following experiments.

A 3 d culture was labeled with 10 µg/ml Con A. The neurites were then tagged with 100 µg/ml rabbit antibodies to Con A, washed, and left to grow in medium. After 24 h, the neurites were labeled with fluorescent goat anti-rabbit antibodies. Thus, only old membrane that had been tagged 24 h previously with Con A/anti-Con A would now bind the fluorescent antibodies. On the other hand, any membrane that had been added during the 24 h interval would not have the Con A/anti-Con A complex on its surface and, consequently, would not bind the fluorescent antibodies.

Using this paradigm, we observe bright uninterrupted fluorescence of old membrane, while the entire extent of new membrane, including the growth cone, is unlabeled. These results are in agreement with previous studies of Bray (PNAS 65:905-910, 1970), in which inert particles served as stationary markers during growth of cultured rat sympathetic neurons. In the present studies we have used a more direct membrane marker to indicate that the locus of new membrane addition is at the growing end of the neurite. We believe that, during growth, membrane components are transported via axonal flow and fuse with preexisting membrane in the region of the growth cone.

998 BINDING OF QUINACRINE, A FLUORESCENT LOCAL ANESTHETIC PROBE, TO MAMMALIAN AXOLEMMA: EQUILIBRIUM AND KINETIC STUDIES. Marty Greenberg*, Jonathan Freedman*, and Tian Yow Tsong* (SPON: S.E. Poduslo). Dept. Phys. Chemistry, Johns Hopkins Medical School, Baltimore, MD 21205.

Quinacrine was found to bind specifically to the axolemma (axonal membrane) of bovine corpus callosum. We have studied this binding reaction by monitoring fluorescence changes of quinacrine. Local anesthetics compete for binding at equilibrium and in stopped-flow kinetics, and their efficacy of competition correlates with clinical potency. Binding was abolished by trypsin digestion, had an activation energy of approximately 7kcal/mole above 20 degrees celsius and had an apparent K dissoc. of 6 to 7 X 10^{-7} M.

Barbiturates reduced equilibrium binding of quinacrine, although kinetic analysis indicated that quinacrine binding occurred but was subsequently displaced in the presence of barbiturates. Potassium-channel blockers, Tetraethylammonium and 3, 4-Diaminopyridine, also decreased equilibrium fluorescence. Low concentrations of Tetraethylammonium increased the rate of quin-binding by close to two orders of magnitude.

999 K+ EFFECT ON GLIAL AND PERIKARIAL (Na+K+)-ATPase FROM NORMAL AND PATHOLOGICAL HUMAN BRAIN Thierry Grisar*, George Franck*, and A.V.Delgado-Escueta.Dept. of Neurology, Reed Neurological Research Center, Los Angeles, California 90024 and Dept. of Neurology,, University of Liege, Belgium.

The role of glial cells in the active control of extracellular potassium (K_o) has been documented in animals. This is particularly true when considering the sensitivity of the glial (Na+K+) ATPase to K_o (Grisar et al. Brain Res. 1979)

A first attempt in this field is now available in human brains. The effect of K+ on Na+K+ ATPase activity was determined in cell fractions obtained by ultracentrifugation on discontinuous Sucrose-Ficoll gradient from 3 "normal" patients. As in rabbit, the glial fractions exhibit a higher level of enzyme activity than the neuronal fraction. This may be explained by a higher content of membraneous material in glial fractions. However, like in the animals, glial enzyme is markedly activated by K+ ions between 5 to 20 mM while this phenomena is not observed in perikarial fractions. These findings in human brains confirm the observations in animals on the role of glial cells. These "control" brains were compared with those above from one case of psychosis, one case of Alzheimer Disease and one case of Creutzfeld Jacobs Disease (CJD). In the two first cases, enzyme activities were decreased in both fractions while the K+ ion sensitivity of glial enzyme was not observed. These results will be due to the delay between death and autopsy as well as the technical procedures used. However, the loss of sensitivity to K_o in these glial cells remain unexplained. More studies are needed. In CJD no significant changes were observed. These latter findings do not confirm Bignami and Palladini who hypothesized a defect in membrane enzymes in CJD spongeous state.

Reference: Bignami, A and Palladini , G.: Nature (London) Vol.209:413-9.

1000 RECONSTITUTION OF THE GLUTAMATE RECEPTOR-LIKE PROTEIN IN LIPOSOMES. Robert D. Grubbs and Elias K. Michaelis. Dept. HDFL, Univ. Kansas, Lawerence, KS 66045.

Reconstitution of a putative neurotransmitter receptor protein into a liposome with recovery of ionophore function is crucial to the characterization of the protein as the physiologic receptor. The present study represents the first attempt to reconstitute the presumed glutamate receptor protein purified from rat brain synaptic membranes into liposomes of various phospholipid composition by means of equilibrium dialysis or simple room temperature incubation (Racker, 1978).

The glutamate (Glu) binding protein (GBP) was isolated as previously described (Michaelis, 1975). The concentrated GBP was then dialyzed extensively against 50 mM Tris-HCl buffer, pH 7.4. Following a 30 minute incubation at room temperature, a mixture of GBP and phosphatidyl-serine (total volume 200 1) was loaded onto a Sepharose 6B column and eluted with 50 mM Tris buffer. Preliminary evidence indicates that a high molecular weight aggregate of GBP which eluted in the early fractions exhibited high specific activity of L-(^{3}H)-Glu binding. The aggregate also exhibited high affinity binding for (^{3}H)-kainic acid, which is of interest in light of previous studies from this laboratory indicating that the solubilized, non-aggregated GBP does not bind (^{3}H)-kainic acid (Kuonen and Michaelis, 1978).

Several fractions immediately following the GBP aggregate showed little or no L-(^{3}H)-Glu binding when tested on the same day as the reconstitution. When these fractions were tested five days later, they bound considerably more L-(^{3}H)- Glu than the higher molecular weight GBP aggregate. These fractions contained a sizable amount of the phosphatidyl-serine and appear to represent the reconstituted system. Studies are currently underway to determine whether the time delay between reconstitution and the appearance of L-(^{3}H)-Glu binding in these fractions is due to the co-elution of soluble GBP and liposomes which then form a proteoliposome reconstituted system. This increased binding may also be due to the rearrangement of the GBP in the lipid matrix from an initial low-binding to a higher specific binding conformation.

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1001 FREEZE-FRACTURE OF MUSCLE SPINDLES. William R. Kennedy, Donald C. Quick* and Thomas S. Reese. Departments of Neurology and Anatomy, University of Minnesota Medical School, Minneapolis, MN 55455; and Section on Functional Neuroanatomy, NINCDS, National Institutes of Health, Bethesda, MD 20014.

Isolated muscle spindles from humans, cats, and rabbits were freeze-fractured by conventional methods. Prominent features of the capsule are numerous vesiculations and the presence of occluding junctions. The junctions are extensive, in accordance with the known impermeability of the capsule (Kennedy and Yoon, in press, Muscle & Nerve). Occluding junctions are composed of rows of fused particles associated with the protoplasmic (P) fracture faces. Similar, less extensive junctions are seen in the capsule cells immediately surrounding the intrafusal muscle fibers.

No communicating junctions were observed, indicating that they are rare or absent.

Rectilinear arrays of P-face particles were seen on the spindle muscle fibers; their appearance is identical to those found on extrafusal muscle. The arrays on intrafusal muscle fibers are concentrated in areas tentatively identified as post-synaptic membranes of the 'trail' type motor endings. Rectilinear arrays can be seen occasionally on other parts of the muscle fiber surface, but there are few or none in the sensory zones.

Adhering junctions and invaginations (Kennedy, Webster and Yoon, 1975, J. Neurocytol. 4:675) have been observed in the membranes of sensory endings.

1002 DIFFERENT EFFECTS OF TEA VS. 4-AP AND 3,4-DAP ON THE CALCIUM INWARD CURRENT SYSTEM IN APLYSIA NEURONS. Manfred R. Klee* and Yoshimi Ikemoto* (SPON: John M. Sarvey). MPI Brain Res., Dept. Neurobiol., Deutschordenstr. 46, D-6000 Frankfurt-M., FRG.

4-aminopyridine (4-AP) and 3,4-diaminopyridine (3,4-DAP) have been shown to block potassium channels in the squid axon membrane extracellularly, whereas tetraethylammonium (TEA) acts internally; 3,4-DAP was about 50 times more potent than 4-AP. Two types of neurons can be distinguished within the visceral ganglion of Aplysia with respect to the action extracellular TEA and 4-AP have on their action potentials (APs) and membrane currents (Klee, JP,284:125P,1978). To those data the following results were added: i) F cells possess a slow, non-inactivating, calcium-dependent inward current ($I_{Ca}2+$), which is about 1/10 of that seen in S cells (approx. 20 nA). ii) 4-AP and 3,4-DAP with nearly equal potency delay and reduce the outward current in both F and S cells. At the same time the duration of APs is increased but never to that of plateau spikes, unless TEA is added. iii) The most important difference between TEA vs. 4-AP and 3,4-DAP is the effect the latter have on $I_{Ca}2+$. Even in concentrations which have very little effect on the outward current (100-600 uM), both substances can increase $I_{Ca}2+$ by 100% in contrast to TEA, which produces no increase. The increase of $I_{Ca}2+$ could explain why in those cells having small $I_{Ca}2+$ (i.e. F cells) TEA can induce calcium-dependent plateau spikes only in the presence of 4-AP or 3,4-DAP.

1003 FILAMENTOUS PROTEINS OF SYNAPSES IN RAT BRAIN. L. P. Kleine* and H. R. Mahler. (SPON: R. M. Wightman). Chemistry Dept., Indiana University, Bloomington, IN 47405

The presence of filamentous proteins in close association with synaptic plasma membranes (SPM) now appears well established on the basis of both biochemical and morphological studies. Tubulin, filamin, myosin, actin, tropomyosin, calmodulin and perhaps some of the subunits of troponin all have been claimed to form part of the SPM. Synaptosomes, SPM, synaptosomal junctional complexes, post-synaptic densities and post-synaptic membranes have been isolated and analysed for the presence of the above mentioned proteins both qualitatively and quantitatively. The membrane proteins were identified and interrelationships established by comparison to purified reference proteins using the techniques of one-dimensional fingerprinting and two-dimensional gel electrophoresis (isoelectric focusing and SDS-polyacrylamide electrophoresis). (Supported by Research Grant NS 08309 from the NIH)

1004 DIFFERENT EFFICACIES OF *d*- AND *ℓ*-γ-AMINO-β-HYDROXYBUTYRIC ACID FOR GABA RECEPTOR-RELATED BINDING AND CONDUCTANCE INCREASES. D.N. Krause, E. Roberts, A. Mori*†, E. Wong*, Y.-J. Wang*, and K. Ikeda. Division of Neurosciences, City of Hope National Medical Center, Duarte, CA 91010 and †Institute for Neurobiology, Okayama University Medical School, Okayama, Japan.

The structure of the GABA receptor was investigated by determining the relative effects of two stereoisomers of the GABA agonist, γ-amino-β-hydroxybutyric acid (GABOB), in several quantitative receptor-related assay systems. (3S)-(+)-4-Amino-3-hydroxybutanoic acid (*d*-GABOB) was found to be about twice as potent as (3R)-(-)-4-amino-3-hydroxybutanoic acid (*ℓ*-GABOB) in displacing [^{3}H]muscimol from specific binding sites in mouse brain membrane fractions. [^{3}H]Muscimol is thought to bind to the GABA recognition site of postsynaptic GABA receptor-anionophore complexes. A similar order of potency for the GABOB enantiomers was observed for the cerebrovascular GABA receptor recently characterized in our laboratory with [^{3}H]muscimol binding assays using bovine cerebral blood vessels. In contrast to the binding results, *ℓ*-GABOB was significantly more potent than *d*-GABOB in mimicking the postsynaptic action of GABA, which was measured as increases in membrane input conductance in the isolated crayfish stretch receptor neuron. Both GABOB enantiomers have some affinity for GABA transport processes, and *d*-GABOB was found to be more potent than *ℓ*-GABOB in inhibiting GABA uptake into rat brain synaptosomes and Na^+-dependent GABA binding to mouse brain membranes. For this reason, conductance measurements also were made in the presence of 10^{-3}M nipecotic acid or L-α,β-diaminopropionic acid, two specific GABA transport blockers. However, *ℓ*-GABOB was again more potent than *d*-GABOB. The order of potency in the receptor-related binding assays (*d*-GABOB>*ℓ*-GABOB) could not be altered by detergent treatment, changes in buffer composition, or by preparing the tissue at room temperature. The greater effectiveness of *ℓ*-GABOB in the physiological assay does not appear to be due to species differences. *ℓ*-GABOB, but not *d*-GABOB, is an effective inhibitor of induced seizure activity in cat brain (Y. Katayama and A. Mori (1977) IRCS Med. Sci. 5:437) and rabbit motor cortex (H. Aishita, A. Akimoto, T. Makita (1978) IRCS Med. Sci. 6:115). Thus, *d*-GABOB is more potent than *ℓ*-GABOB in membrane binding and uptake systems, while the reverse is found in physiologically responding systems. While the reasons for this discrepancy are unclear, these findings may suggest that the structural asymmetry of the GABA recognition site in vitro is somewhat different than that related to activation of GABA receptors in vivo. (Supported by USPHS grants NS-05695, 12116 and 01615).

1005 ROLE OF PHOSPHORYLATION ON GABA RECEPTOR ACTIVITY. A. Leon*, A. Guidotti, G. Toffano and E. Costa. Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hosp., Washington, D.C. 20032

"GABA-modulin", a thermostable, acidic (M.W. 15,000 dalton) protein isolated from brain, non-competively inhibits the Na^+-independent high affinity binding of GABA to synaptic junction membranes. Brain preparations of this inhibitor block competively cyclic AMP-independent protein kinase in supernatant of brain homogenates. The inhibitory activity of GABA binding and protein kinase was purified by a number of procedures including SDS polyacrylamide gel electrophoresis. When the extracts were purified to apparent homogeneity it was found that the inhibition of protein kinase and GABA binding reside in the same protein. The content of GABA-modulin is higher in preparations of synaptic plasma membranes (SPM) than in those of synaptic junctions (SJ) which were obtained by repeated freezing, thawing and triton X-100 treatments. The high affinity binding of ^{3}H-GABA and the phosphorylating capacity of the SJ is several fold higher than that of SPM. Endogenous phosphorylation and high affinity GABA binding are reduced after recombination experiments with GABA-modulin. The results suggest that GABA-modulin controls GABA receptor function through the phosphorylation of specific membrane protein(s). The identity of this protein is currently under investigation.

1006 DEMONSTRATION AND PROPERTIES OF AN ENDOGENOUS MACROMOLECULAR INHIBITOR OF NEUROTRANSMITTER RECEPTORS. J. E. Leysen*, P. Van Gompel* and P. M. Laduron* (SPON: Steven E. Kornguth) Janssen Research Laboratories, Janssen Pharmaceutica, B-2340 Beerse, Belgium.

Rat brain extracts contain macromolecular components, apparently proteins, which interfere with the binding of ^{3}H ligands to dopamine, serotonin, opiate and most likely other receptors.

The inhibition obtained with a crude rat brain extract appeared to be competitive in the following receptor models with K_i's, expressed in mg protein of a crude extract per ml incubation mixture: 1 mg/ml for ^{3}H-apomorphine and 10 mg/ml for ^{3}H-haloperidol in rat striatum (dopamine receptors); 16 mg/ml for ^{3}H-spiperone in rat frontal cortex (serotonin receptors); 1 mg/ml for both ^{3}H-naloxone and ^{3}H-fentanyl in rat forebrain (opiate receptors).

These components occur in several brain areas, have a molecular weight of over 200 000 and are positively charged at physiological pH. They can be isolated, purified and further characterized using ion exchange and molecular sieve chromatography, SDS and preparative polyacrylamide electrophoresis and preparative isoelectrofocusing.

We hypothesize that these proteins play a regulatory function in the masking and unmasking of receptors. Because of their positive charge they will easily associate with the negatively charged membranes and hence cover receptor sites. They can be removed from these sites by the competitive action of agonists and antagonists. The possible role of these proteins in the induction of receptor supersensitivity will be discussed.

1007 EFFECT OF CHOLINERGICS, D-TUBOCURARINE AND OUABAIN ON THE MEMBRANE POTENTIAL OF SCHWANN CELLS ASSOCIATED WITH THE CRAYFISH MEDIAL GIANT AXON. E.M. Lieberman and K.A. Smiley*, Dept. Physiol., Sch. Med., East Carolina U., Greenville, NC 27834

In an earlier study (Biophys. J. 25(2):305a, 1979) we demonstrated that Schwann cells surrounding the medial giant axon of the crayfish, Procambarus clarkii, could be impaled with microelectrodes and studied electrophysiologically and pharmacologically. The present investigation extends the work to show that the sensitivity of the Schwann cell membrane potential to cholinergic compounds is due to the presence of acetylcholine receptors on the Schwann cell membrane. The resting membrane potential of control Schwann cells was 42.6±0.6mV (n=63). Immediately following superfusion of the axon-Schwann cell preparation with 10^{-7}M ACh the Schwann cell membrane potential hyperpolarized to -56.6±1.0mV (n=7). Identical concentrations of carbachol and nicotine caused hyperpolarization to -57.7±1.4mV (n=11) and -59.4 ±1.0mV (n=7), respectively. Muscarine had no effect on Schwann cell membrane potential when applied at a concentration 10 times greater than the cholinergics. These agents had no discernible effect on the axon resting or action potential. The action of these drugs was long lasting and readily reversible on washing the preparation with drug-free solutions. Two further series of experiments were performed with the cholinergic drugs. First, the axon-Schwann cell preparation was pretreated with 10^{-9}M d-tubocurarine (d-TC) and then superfused with 10^{-7}M nicotine in the presence of d-TC. In this case nicotine had no effect on the Schwann cell potential. In the second series of experiments the Schwann cells were hyperpolarized by 10^{-7}M nicotine and then exposed to 10^{-8}M d-TC in the presence of nicotine. D-TC caused a rapid reversal of the nicotine effect. On the basis of these experiments we conclude that the Schwann cell membrane has nicotinic cholinergic receptors which are responsive to physiological concentrations of acetylcholine. The effect of ouabain on the membrane potential of Schwann cells was also studied. Ouabain (10^{-3}M) caused a hyperpolarization of Schwann cell membrane potential similar to that caused by the cholinergic drugs. Unlike the cholinergic effect the ouabain hyperpolarization lasted only 15-20 min, whereupon a rapid depolarization to zero occurred. The rapid depolarization was likely due to the loss of internal [K]. The hyperpolarization may be due either to an inhibition of a depolarizing electrogenic transport system or to a ouabain-induced acetylcholine release by the axon-Schwann cell system. Supported, in part, by NSF BNS 77-28510.

1008 K^+-STIMULATED PHOSPHORYLATION OF A 41,000 M.W. POLYPEPTIDE IN L8 MYOTUBE MEMBRANES. Gilbert Magilen*, Adrienne Gordon and Ivan Diamond. Department of Neurology, University of California Medical School, San Francisco, California 94143

The acetylcholine receptor (AChR) is reversibly phosphorylated by endogenous enzymes in AChR-enriched membranes from Torpedo and Electrophorus electric tissues. We are presently examining the L8 myogenic cell line to determine whether the mammalian AChR is phosphorylated and to investigate the role of phosphorylation in the regulation of AChR function. Fused L8 myotubes have greater than 15 times more AChR (measured by the binding of [^{125}I] α-bungarotoxin) than undifferentiated myoblasts. We therefore compared phosphorylation patterns of the L8 myoblast and myotube membrane preparations. When membrane fractions from myotubes and myoblasts were incubated with [γ-^{32}P] ATP in the presence of 20 mM Mg^{+2}, several phosphorylated polypeptides were observed after SDS-PAGE and autoradiography. The pattern of polypeptide phosphorylation was both qualitatively and quantitatively different when myoblasts were compared with myotubes. Of particular interest, myotube membranes showed a polypeptide of 41,000 M.W., whose phosphorylation was stimulated approximately 4-fold by KCl (25-100 mM). This effect was not seen with NaCl. Both cAMP and cGMP at 10^{-5}M had no effect on phosphorylation of the 41,000 M.W. polypeptide. Examination of the phosphorylation pattern of myotube membranes in two-dimensional IEF-PAGE autoradiographs (O'Farrell, JBC 250:4007, 1975) demonstrated an approximate isoelectric point of pH 6.7 for the 41,000 M.W. phosphorylated protein. In contrast to the results with myotubes, membranes prepared from myoblasts did not show potassium-stimulated phosphorylation of a 41,000 M.W. polypeptide.

Several lines of evidence suggest that the 41,000 M.W. phosphorylated polypeptide may be the AChR. The acetylcholine binding subunit of the mammalian AChR from many sources has a M.W. around 40,000. The pI and M.W. for the AChR from fetal calf myotubes in culture is the same as that reported here (Merlie et al, JBC 253:2882, 1978). The appearance of K^+ dependent phosphorylation with differentiation may be related to ionic changes which occur in the vicinity of the AChR during activation. Taken together, these findings suggest that the AChR in L8 myotubes may be phosphorylated.

1009 CONDUCTION BLOCK IN PERIPHERAL A AND C FIBRES FOLLOWING ELECTRICAL STIMULATION. B. Matthews* and S.W. Cadden* (SPON: M. Burrows). Dept. Physiol., Univ. Bristol, England BS8 1TD.

Bishop and Heinbecker (*Am. J. Physiol.* 114: 179, 1935) showed that electrical stimulation of a nerve trunk could leave the fibres in a condition in which conduction through the stimulated region was blocked. More intense stimuli were required to block C than A fibres and the intensity of the stimuli determined the duration of block, which could be irreversible. We wished to know whether it was possible to stimulate all the fibres in a nerve without causing this type of block and have determined the relationship between the thresholds for excitation and the intensities of stimuli that cause block in different groups of nerve fibres.

Three groups of electrodes, about 30mm apart, were applied to the exposed saphenous nerve in anaesthetized cats; one pair to the cut central end for recording, and two pairs for stimulation. Conduction block caused by conditioning stimuli applied to the middle electrodes was assessed by recording centrally while test stimuli were applied distally. The proportion of fibres in a group that were conducting impulses was estimated from the integral of the corresponding component of the averaged compound action potential. A stimulus/response curve relating stimulus strength to this integral was first obtained then a supramaximal stimulus for the group of fibres being studied was used in estimating conduction block. Conditioning stimuli were applied at 1/sec for 10 min, interrupted for 15 sec every 2 min for testing. Test stimuli were applied at 1/sec for 10 sec every 2 min during conditioning and also during the subsequent 10 min to monitor recovery.

With 0.1 msec stimuli, Aβ fibres had thresholds between 0.2 and 0.8V. Block of these fibres was first detected with stimuli of 20V and was complete after 10 min at 40V. Provided more intense stimuli were not applied, conduction had returned in 75% of blocked fibres 10 min after stimulation. The corresponding figures for Aδ fibres were: thresholds, 0.5-5V; block, 25-50V. The corresponding C fibre thresholds were between 5 and 50V and some block occurred with stimuli between 50 and 100V, the maximum available. With 1.0 msec stimuli, the C fibre thresholds ranged from 2.5 to 7.5V and conduction block increased progressively from 7.5V to 100V, at which it was 80% complete.

Thus it was possible to excite all A fibres without causing conduction block but the minimum stimulus required to excite all C fibres caused extensive conduction block in A fibres and was at the lower limit of the range that blocked C fibres.

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1010 LIPID-PROTEIN INTERACTIONS IN MEMBRANES CONTAINING THE ACETYLCHOLINE RECEPTOR. Mark G. McNamee, Jeffrey Ellena* and Terrence J. Andreasen*. Dept. of Biochem. and Biophys., UCD, Davis, CA 95616.

Perturbation of the lipid environment of the nicotinic acetylcholine receptor (AChR) in membrane vesicles prepared from Torpedo californica electroplax has profound effects on AChR function including 1) agonist binding and 2) cation permeability control.

Phospholipase A_2 from Naja naja siamensis venom blocks carbamylcholine-induced increases in sodium ion permeability. The inhibition can be correlated with the hydrolysis of phosphatidylethanolamine, and can be prevented or reversed by treatment of the membranes with bovine serum albumin, a protein that can bind the hydrolysis products of phospholipase A_2. The phospholipase A_2 also induces a shift in carbamylcholine binding from low affinity to high affinity, a shift that has been correlated with receptor desensitization.

Exogenous fatty acids can be incorporated into the membranes and at 0° some unsaturated fatty acids (e.g., arachidonic and linoleic acids) block carbamylcholine-induced increases in sodium ion permeability. However, in contrast to phospholipase A_2 treatment, the fatty acids do not induce the increase in agonist affinity. Thus, the fatty acids appear to uncouple ligand binding from ion permeability control by a mechanism different from receptor desensitization. Saturated fatty acids (e.g., palmitic and stearic acids) are relatively ineffective in bringing about inhibition. Further studies indicate that the effectiveness of fatty acids is temperature dependent and a given fatty acid is an effective inhibitor only above its bulk melting point. Spin-labeled fatty acids are now being used to monitor the effects of phospholipase A_2 and exogenous fatty acids on bulk lipid fluidity and on lipid-protein interactions. The fatty acid spin labels can also inhibit AChR ion permeability control in a temperature dependent manner. Interestingly, the effectiveness of the spin labels as inhibitors depends on the location of the nitroxide group along the acyl chain.

1011 CHARACTERIZATION OF THE NA-CA EXCHANGE SYSTEM IN SYNAPTOSOMES AND SYNAPTIC MEMBRANE GHOSTS. Mary L. Michaelis, Elias K. Michaelis, and Sharie L. Myers*. Dept. of Human Development, Univ. of Kansas, Lawrence, Kansas 66045.

The question of how nerve cells maintain low concentrations of free calcium ions in spite of the strong driving force for Ca entry during depolarization has been the subject of several recent investigations. The work of Baker, Blaustein, and their colleagues has revealed the presence of a Ca-Na exchange system in squid axon membranes, and Blaustein has confirmed its existence in synaptosomes from mammalian brain. This exchange system appears to transport Ca against its concentration gradient by the coupled exchange of Ca for Na, thereby utilizing the electrochemical gradient for Na as its energy source. It has been demonstrated in the squid axon that this Na-Ca "carrier" operates in either direction as long as a Na concentration gradient is present. Lithium is not readily exchanged for Ca.

We have confirmed Blaustein's observations of a Na_o-dependent ^{45}Ca efflux in mammalian synaptosomes and have drawn on the pioneering work of Kaback and Kanner in developing a preparation of sealed synaptic plasma membrane vesicles or "ghosts" in which it is possible to study the characteristics of the Na-Ca exchange in neuronal membranes obtained from rat brain. Synaptosomes from ficoll-sucrose gradients are lysed in 10 mM KPO_4 buffer for 15 min at 4°, the synaptic membranes precipitated, and the pellet resuspended in .32M sucrose-.03M K-succinate to a final protein concentration of 15mg/ml. The membranes can then be rapidly frozen in liquid nitrogen and later thawed at 37° for 10 min and allowed to form vesicles in the presence of 50 μM $MgCl_2$. During this step the membrane sacs can be loaded internally with various ions.

The Na-Ca exchange system is most active in freshly-prepared (i.e., not frozen) vesicles, but its activity can be preserved in frozen membranes if they are thawed in the presence of low levels of Mg. Time kinetics studies indicated that the exchange process is linear for about 90 sec in both the fresh and frozen vesicles. Since the membrane sacs can be loaded internally with either Ca or Na, they have enabled us to obtain preliminary results suggesting that the carrier will transport Ca in either direction as long as there is a Na gradient in the opposite direction. Since this preparation is devoid of intracellular organelles or energy-producing capacity, it appears that the Na-Ca exchange system in synaptic membranes does not depend on the presence of intracellular components and apparently relies on a Na gradient for its driving force. (Supported by Institutional Biomedical Research Support Grants RR-07037 and RR-5070G).

1012 ACETYLCHOLINESTERASE: A NEW MARKER ENZYME IN THE ISOLATION AND RESOLUTION OF THE TRIAD STRUCTURE FROM SKELETAL MUSCLE. Robert Mitchell* and Sidney Fleischer* (SPON: N. Ranish). Dept. Mol. Biol., Vanderbilt Univ., Nashville, TN 37235.

The triad structure from skeletal muscle has been isolated by a modification of a procedure previously described by Caswell et al., (Arch. Bioch. Biophys. 176: 417, 1976). Initial studies indicate the presence of acetylcholinesterase (AChE) activity located specifically in the triad fraction and concomitant with other markers described as being present in triad components (Ca^{++} ATPase and pumping, adenylate cyclase, and ^{3}H-ouabain binding). The acetylcholinesterase specific activity in the triad fraction is approximately 60 nmoles/mg-min. Specificity for acetylcholinesterase can be shown using both butyrylcholine and acetylcholine as substrates and by inhibition studies using the AChE inhibitor, BW 284c51, and the BuChE inhibitor, ISO-OMPA. Separation of the structure into its component organelles (i.e., sarcoplasmic reticulum and transverse tubule) can be achieved by first shearing the triad fraction in a french pressure cell followed by isopycnic sedimentation. There is a 10-20 fold enrichment in acetylcholinesterase activity in a light density fraction (equivalent to 25% sucrose density) and co-migrating with known transverse tubule markers (i.e., adenylate cyclase and ^{3}H-ouabain binding). The AChE of the triad exhibits considerable latent activity which can be demonstrated using sucrose as an osmotic effector or the detergents, Lubrol-PX or Triton X-100. This latency is apparent with regard to either substrate or inhibitor and is most fully expressed in fresh preparations. These results indicate that acetylcholinesterase is localized on the inner face of the transverse tubule within the triad structure. (Supported in part by NIH AM 14632 and by Muscular Dystrophy Assoc. of America, Inc.)

1013 STUDIES OF THE INTERACTION OF EEL ACETYLCHOLINE RECEPTOR ANTIBODY WITH HIGHLY PURIFIED BRAIN ACETYLCHOLINE RECEPTOR. Barbara J. Morley and George E. Kemp*. Neurosciences Program, University of Alabama Medical School, Birmingham, Alabama 35294.

Although α-bungarotoxin (Butx) is a specific ligand for the nicotinic acetylcholine receptor (nAChR) in several tissues, it has been suggested that Butx binds to a different molecule in the CNS. Evidence suggesting that CNS nAChRs and the Butx binding site are not equivalent includes: Butx does not prevent the blocking of receptor activation by eel anti-nAChR in the PC 12 nerve cell line, anti-nAChR fails to precipitate ^{125}I-Butx binding site complexes in the PC 12 line, the Butx binding site complex will not block the immunoprecipitation of muscle cell line binding site complexes by anti-nAChR (Patrick & Stallcup, 1977, PNAS, 71, 4689).

We now report the results of studies of the interaction of eel anti-nAChR with a highly purified brain Butx-binding site as part of an investigation of the equivalence of the Butx binding site with brain nAChR.

Purification was achieved by differential centrifugation, α-cobratoxin biospecific adsorption, ion exchange chromatography, and Sepharose 6B gel filtration. The specific activity of the final product was determined to be in excess of 2 pMoles of ^{125}I-Butx sites/μg protein. For immunoprecipitation studies, aliquots of brain toxin-binding site complexes were incubated with dilutions of anti-nAChR (received from M. Eldefrawi) for 0-8 hrs at 4 or 23°C. An excess of goat anti-rabbit IgG (GAR) was added and the samples incubated for 0-24 hrs and centrifuged at 2,000 rpm for 10-240 min. The maximum precipitation was 10% (we obtain 1-2% with non-purified preparations). Additional aliquots were incubated with an excess of anti-nAChR, as above, passed down Sepharose 4B or 6B gel columns and collected in 3 or 5 ml fractions. Each fraction was counted in a gamma counter and the presence of antibody was confirmed by immunoprecipitation. The results clearly indicate a definable peak of antibody associated sites; the number of sites is identical to that found by the standard immunoprecipitation procedure.

In other studies we have shown that antisera prepared against Butx will precipitate 95-100% of the sites; furthermore, anti-nAChR may not prevent immunoprecipitation by anti-Butx.

The validity of our methods has been shown by carrying out these procedures using purified electric fish and denervated muscle nAChR. With these tissues we obtain 60% and 40% immunoprecipitation, respectively. Although we are investigating several interpretations, we currently favor the hypothesis that nAChRs from different sources have dissimilar antigenic determinants.

This research is supported by grants BNS 78-13724 and BNS 78-23604 to Barbara J. Morley and NS 14262 to George E. Kemp.

1014 EFFECT OF INCREASED MEMBRANE CHOLESTEROL CONTENT ON RAT BRAIN SYNAPTOSOMES. Paula North* and Sidney Fleischer* (SPON: A. Burt). Dept. of Molecular Biology, Vanderbilt Univ., Nashville,Tn.37235.

The cholesterol content of isolated rat brain synaptosomes has been increased in vitro by incubation with sonicated liposomes containing a 2:1 molar ratio of cholesterol to egg yolk phosphatidylcholine. Synaptosomes were isolated from rat forebrain by differential and sucrose gradient centrifugation and resuspended in the cholesterol/phosphatidylcholine co-dispersions. Following the incubation, the synaptosomes were collected by centrifugation and washed. Lipids were extracted for cholesterol and phosphorus determination. A three hour incubation at 32°C resulted, for an average synaptosomal preparation, in increasing the cholesterol content of the synaptosome from .158 to .176 mg cholesterol/mg protein and from .64 to .712 mole cholesterol/mole phospholipid. The lipid phosphorus content did not change by more than 3%. A subsequent incubation of the cholesterol-loaded synaptosomes with pure distearoylphosphatidylcholine vesicles decreased the cholesterol content to .150 mg cholesterol/mg protein. Synaptosomes were also incubated with cholesterol/phosphatidylcholine vesicles labeled with either ^{3}H-cholesterol and ^{14}C-phosphatidylcholine or ^{3}H-cholesterol and internally sequestered ^{14}C-dextran. The ratio of ^{3}H to ^{14}C transfered to the synaptosomes was used as a measure of molecular transfer of cholesterol versus that transfered by aggregation or possibly fusion. These analyses of the cholesterol transfer indicate it to be largely consistent with the molecular exchange process described by Bruckdorfer (Eur.J.Bioc.4:512,1968) between liposomes and erythrocyte ghosts. We have now begun to study the effects of increased cholesterol content on synaptosomal plasma membrane-associated activities. We have measured a potassium concentration-dependent synaptosomal membrane potential using the permeant fluorescent dye, diO-C_5-(3), as described by Blaustein (J.Physiol.247:589,1975). Using the level of fluorescence under depolarizing conditions (70 mM K^+) relative to that in a medium containing 5 mM K^+ as a qualitative indication of resting membrane potential, we find that the cholesterol-loaded synaptosomes have approximately 60-80% the control resting potential. Also, cholesterol-loaded synaptosomes depolarized by preincubation without an energy source are less completely repolarized by subsequent addition of glucose than are control synaptosomes. This drop in ability to generate a membrane potential can be roughly correlated with a measured inhibition in ouabain-sensitive (Na^++K^+)ATPase activity in both synaptosomes and isolated synaptic plasma membranes incubated with the cholesterol-containing liposomes. Membrane leakiness as measured by lactate dehydrogenase occlusion was not increased by the liposome treatment. (Supported in part by NIH AM 14632).

1015 RECONSTITUTION AND PURIFICATION OF A SYNAPTOSOMAL ATP-DEPENDENT CA^{++} TRANSPORT COMPONENT. Diane Papazian*, Hannah Rahamimoff*, and Stanley M. Goldin*. (SPON: G.D. Maxwell). Depts. of Pharmacology and Neurobiology, Harvard Medical School, Boston, Mass. 02115.

Recently several groups have presented evidence for the existence of a non-mitochondrial, ATP-dependent Ca^{++} transport system derived from lysates of synaptosomes. This transport system is presumably involved in regulation of Ca^{++} levels in nerve terminals, thus regulating the release of neurotransmitter. Identification and purification of this Ca^{++} transport system is a prerequisite for its immunocytochemical localization in nerve terminals and for further study of its molecular mechanism; this should increase our understanding of the dynamics of Ca regulation of neurotransmitter release.

A synaptosomal ATP-dependent Ca^{++} uptake system (H. Rahamimoff and E. Abramovitz (1978) FEBS Lett. 89, 223) was reconstituted into artificial vesicles by a cholate dialysis procedure employing an 80-fold excess of exogenous phospholipid. Under these conditions, most of the ∿500Å diameter vesicles that result would be expected to have only one or at most a few membrane proteins. The vesicles containing an ATP-dependent Ca^{++} uptake system were purified from the bulk of the preparation on density gradients by increasing their density due to the ATP-dependent intravesicular precipitation of Ca^{++} oxalate; a 100-fold purification of the transport system resulted. The purified Ca^{++}-transporting vesicles contained two major protein components, of M_r 94,000 and 140,000. These components are believed to be responsible for Ca transport in this synaptosome-derived membrane fraction.

This demonstrates the successful use of a new approach, "transport specificity fractionation" (Goldin and Rhoden (1978) J. Biol. Chem. 253, 2575), for the identification and purification of a class of neuronal ion transport proteins--to our knowledge, no comparable degree of purification of any ion transport protein from the CNS has yet been demonstrated.

1016 EFFECT OF VASOPRESSIN ON THE PENETRATION OF ^{14}C-UREA INTO BRAIN COMPARTMENTS PROTECTED BY BARRIER SYSTEMS. Z. Parandoosh* and C.E. Johanson* (SPON: J.A. Madsen). Dept. of Pharmacology, University of Utah College of Medicine, Salt Lake City, Utah 84132

Vasopressin increases the permeability of some epithelia to urea. Since the choroidal epithelium is relatively impermeable to urea, it is conceivable that vasopressin could alter the permeability of the choroidal membrane (and perhaps the cerebral capillaries) to urea. Using an in vitro as well as an in vivo preparation, we have investigated the effects of various doses of vasopressin on the uptake of radiourea by tissues protected by the blood-CSF barrier (choroid plexus) and the blood-brain barrier (cerebellum and cerebral cortex).

Lateral ventricle choroid plexus (LVCP) from adult rats was incubated in artificial CSF with or without vasopressin (100 mU/ml); plexus tissue from one lateral ventricle served as control for tissue taken from the contralateral ventricle.

^{14}C-urea	^{14}C-urea spaces in LVCP	
Incubation Time	Control	Vasopressin
2 min (Preincubated)*	52.3 ± 2.5	51.6 ± 0.1
3 min (Preincubated)*	60.5 ± 3.1	57.6 ± 0.6
5 min (Preincubated)*	55.1 ± 2.6	54.8 ± 2.9
5 min	64.6 ± 1.0	64.2 ± 3.8
15 min	65.7 ± 1.8	52.3 ± 1.7**

*LVCP preincubated for 15 min with vasopressin before exposure to ^{14}C-urea. Values are means ± S.E. for 3-6 rats. **P<0.05, t test.

There is no evidence from the in vitro studies that vasopressin enhances the uptake of ^{14}C-urea from incubation medium; moreover, under some conditions, the ^{14}C-urea space in LVCP may be decreased by vasopressin.

In in vivo studies in which ^{14}C-urea was injected intraperitoneally into animals receiving an intravenous infusion of vasopressin, there was no increase in the 1-hr ^{14}C-urea space in either the cerebral cortex, cerebellum, LVCP or cisternal CSF at the lower doses of vasopressin (750 to 950 mU during 1-hr infusion); at higher doses of vasopressin (1400 to 1500 mU) a 40 to 80% increase in the 1-hr ^{14}C-urea space was observed in these CNS compartments, possibly due to a vasopressin effect related to an increase in arterial blood pressure (to 150 torr) and cerebral blood flow. (Supported by NIH Grants #NS 13988 and GM 07579)

1017 REDISTRIBUTION OF Na AND K IN RAT CNS DURING RESPIRATORY ACIDOSIS AND ALKALOSIS. Lynn K. Pershing* and C.E. Johanson* (SPON: E.C. Beck). Dept. of Pharmacology, University of Utah College of Medicine, Salt Lake City, Utah 84132

Acute distortions of acid-base metabolism can profoundly alter the distribution of Na and K in some tissues. To evaluate the separate influences of plasma [HCO_3] and [H] upon Na-K distribution in CNS tissues, previous studies involving metabolic acid-base distortions (Fed. Proceed. 38: 375, 1979) were extended to include respiratory acidosis and alkalosis.

Anesthetized (150 mg ketamine/g) Sprague-Dawley rats (400-500 g) were tracheotomized and normoventilated on a rodent respirator with air (control) or 5% CO_2 (acidosis), or hyperventilated with air (alkalosis). Animals were sacrificed by exsanguination at 1 hr, at which time blood from the abdominal aorta was analyzed for pH and pCO_2.

Treatment	pH	pCO_2	HCO_3
Control	7.40 ± .02 (12)	37.4 ± 1.8 (12)	25.6 ± 0.5 (12)
Acidosis	7.20 ± .03 (15)	67.1 ± 4.1 (13)	26.8 ± 0.9 (12)
Alkalosis	7.60 ± .01 (13)	22.6 ± 1.5 (10)	20.9 ± 0.6 (10)

Various CNS tissues were sampled and analyzed for Na and K by flame photometry: lateral and fourth ventricle choroid plexus (LVCP and 4VCP), spinal cord (SC-white matter) and cerebral cortex (CC-gray matter). Data below are means for tissue K/Na .

Treatment	LVCP	4VCP	CC	SC
Control	96.6/64.2	106.1/68.6	109.4/44.3	73.5/31.5
Acidosis	*121.7/55.5*	*121.3/67.4	103.4/41.7	72.7/30.1
Alkalosis	104.8/59.8	111.3/66.9	104.8/43.8	75.2/33.6

(P≤0.05, Student's t test, control vs. respiratory distortion)

Changes in CSF and plasma electrolytes were not significant by Student's t test. LVCP [K] increased significantly by 23% while [Na] decreased by 14% during acute acidosis, yet neither CC nor SC displayed any electrolyte changes with the treatments. Contrasts between the responses of neural and choroidal tissues are probably attributable to differences in the chemical composition of the interstitial fluid in contact with the parenchymal cells associated with the blood-CSF and blood-brain barrier systems. Since in both respiratory and metabolic acidosis the elevated plasma [H^+] produces similar changes in LVCP [Na] and [K], it is possible that extracellular [H^+] influences a K-transferring pump at the basolateral surface of the choroid plexus epithelium. (Supported by #NS 13988 and GM 07579)

1018 INTERMITTENT RESPONSE OF SCIATIC AXONS TO REPETITIVE STIMULATION IS GOVERNED BY THRESHOLD SHIFTS. Stephen A. Raymond and Kenneth McLeod*. Research Laboratory of Electronics, MIT, Cambridge, MA 02139

At regions of low conduction safety in dendrites and axons, subthreshold influences (e.g. extrinsic currents, slow potentials, variations in firing threshold) can modulate conduction of impulses. In excised sciatic nerves, continuous stimulation of the whole trunk with brief current pulses leads to a periodic responsiveness of single fibers. Long trains of one-for-one responses alternate with long periods of failure for pulses having a constant amplitude and duration. The phenomenon is called intermittent responsiveness (intermittent conduction is a corresponding phenomenon occurring at regions of marginal conduction safety such as bifurcations rather than at stimulating electrodes). It occurs over a broad range of frequencies (500 Hz to less than 0.3 Hz) and stimulus magnitudes (2% below the charge delivered per threshold stimulus at rest ($\Delta Q/Stim_R$) to 150% or more above the $\Delta Q/Stim_R$).

Conduction latency, which parallels threshold in a variety of experimental conditions, was measured during intermittent responsiveness. The results indicated that threshold rises during the "response periods", and that it recovers during the "off periods". Latency measurements also showed that the initial threshold levels at the beginning of a response period are well below the level of stimulus pulses. However, as the response period becomes prolonged, the latency becomes progressively longer. At the end of the response period, the average value and variance of latency reached those associated with threshold level stimulation at rest, indicating that the threshold had risen to the point where each stimulus was marginal.

Latency shifts and direct measurement of threshold during the off period show that threshold is initially well above the level of stimulation. The threshold recovers rapidly at the beginning of an off period, but descends less steeply as it nears the resting level. As the threshold approaches the level of the maintained stimulation, it again becomes possible for one of the stimuli to be successful, initiating an on period.

Measurements were made of "periodicity" as defined by the cycle times between response periods and off periods. Periodicity was a function of both the frequency and magnitude (pulse current X pulse duration) of stimulation pulses. The results match with periodicity-frequency and periodicity-magnitude relations obtained from a numerical model of the threshold curves subjected to similar conditions of stimulation.

The activity-dependence of threshold appears to be sufficient to account for intermittent responsiveness.

1019 IDENTIFICATION OF EXTERNAL SURFACE PROTEINS OF SYNAPTIC PLASMA MEMBRANES BY PHOTOLABELING. James C. Schaeffer and Daniel J. Bergmann*, Dept. of Chemistry, University of Missouri, Kansas City, Mo. 64110.

In order to investigate the molecular basis of the numerous functions associated with synapses, it would be useful to establish the orientation of proteins found in synaptic plasma membranes. Previously, Wang and Mahler (J.Cell Biol.,1976) have employed lacto-peroxidase iodination while Smith and Loh (J.Neurochem.,1977) have used proteolytic digestion to study this problem. Since both of these methods have drawbacks, we have investigated the topography of synaptic plasma membranes by the photolabeling technique. In our studies, we used a tritiated, photoactivated, membrane impenetrable label: 3-(4-azido-2-nitroanilino)propyl trimethylammonium iodide.

Crude synaptosome preparations from rat brain were labeled at 4°C to insure that the impenetrable label was not internalized by a non-specific cationic transport process and to minimize synaptosomal membrane alterations. After the labeled synaptosomes were thoroughly washed, they were lyzed with 5mM Tris, pH 8.1 and the resulting synaptic plasma membranes were isolated by the procedure of Jones and Matus(Biochim.Biophys.Acta, 1974). The labeled membranes were solubilized and extensively dialyzed to remove lipids. Membrane bound proteins were resolved into eighteen bands ranging in molecular weight from 15kD to 243kD by SDS-gel electrophoresis (5.6% gels). The gels were sliced into 1 mm thick sections and the tritium content of each section determined.

Proteins with apparent molecular weights of 135kD 108kD, 65kD, 54kD and 35kD were heavily labeled while several other gel regions were less prominently labeled. These data suggest that proteins with a wide range of molecular weights are exposed on the surface of synaptosomes. Parallel dark controls showed identical Coomassie Blue staining patterns and contained only 3% of the radioactivity of the irradiated samples. Thus irradiation was necessary for covalent bond formation and labeling does not alter apparent molecular weights.

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1020 HISTOFLUORESCENT IDENTIFICATION OF α-BUNGAROTOXIN BINDING SITES IN THE GOLDFISH VISUAL SYSTEM, M. Schwartz*, D. Axelrod*, E.L. Feldman* and B.W. Agranoff. Neuroscience Laboratory and Biophysics Research Division, University of Michigan, Ann Arbor, MI 48109.

Recent studies suggested the existence of bungarotoxin (α-Butox) binding sites in the goldfish tectum. However, it is not yet clear whether these sites are presynaptic or post-synaptic. The present fluorescent microscopic studies support evidence for the presence of presynaptic α-Butox binding. Binding of rhodamine-conjugated α-Butox (R-α-Butox) was observed on both optic nerve sections and on neurites of cultured retina explanted from adult goldfish whose optic nerve had been crushed 10-14 d previously. Optic nerve sections were obtained from adult goldfish and were fixed in a mixture of ethanol, formaldehyde and acetic acid. R-α-Butox binding to cultures fixed under mild conditions was high, while the binding to unfixed tissue was very low. In both optic nerve sections and neurites in culture, preincubation with 10^{-7}M unlabeled α-Butox blocked the binding of 10^{-7}M R-α-Butox. The pharmacological profile of R-α-Butox binding to the neurites in culture was investigated in the presence of neuroactive drugs. D-α-Tubocurarine and carbamylcholine were effective in inhibiting the binding while atropine was ineffective. These experiments have shown that α-Butox binding is present in optic nerve axons and the in vitro results indicate that re-establishment of synaptic connections is not necessary for its presence. Both in vivo and in vitro, these axonal sites are not accessible from the exterior of the cell. These axonal α-Butox binding sites may play a role other than in events related to neurotransmission.

1021 MEMBRANE POTENTIAL AND TAURINE TRANSPORT IN A CULTURED GLIOMA CELL LINE. B. Seligmann*, W. Shain, and D. L. Martin*. Neurobiol. Dept., Armed Forces Rad. Res. Inst., Bethesda, MD 20014 and Univ. Md., Dept. Chem. 20742.

High-affinity sodium-dependent transport of taurine by a glioma cell line (LRM 55) has recently been described (Martin & Shain, J. Biol. Chem., in press). Since sodium-dependent transport mechanisms for neutral amino acids may involve the cotransport of sodium with the substrate, they are potentially electrogenic. Therefore we have investigated the relationship between taurine transport and membrane potential in LRM 55 cells.

Membrane potential was measured by intracellular recording and also calculated from the distribution of the tritiated molecular probe triphenylmethylphosphonium ion (TPMP+). The average recorded membrane potential was -43 ± 3 mV. The membrane potential decreased in a non-Nernstian manner as the potassium concentration was increased. Thus the membrane of these cells did not act as a pure potassium electrode. Valinomycin (5×10^{-7} M) hyperpolarized the membrane potential to -63 mV in normal potassium solutions and made the dependence of membrane potential on external potassium more nearly Nernstian. Ouabain (10^{-3} M) or taurine (10^{-3} M) had no significant effects. However, if cells were treated for 30 min with ouabain and then exposed to taurine, a depolarization was observed.

TPMP+ required 160 min to equilibrate across the membrane. The dependence of TPMP+ distribution on potassium was similar to the recorded membrane potential, but the absolute calculated values were substantially greater. The calculated membrane potential at normal potassium was -78 mV. If calculated values were corrected for the amount of TPMP+ remaining in fully depolarized cells, the corrected values agreed more closely with those recorded directly. Valinomycin increased the amount of TPMP+ accumulated by cells in agreement with its effect on the intracellularly recorded membrane potential. However, ouabain caused a consistent small decrease in the amount of TPMP+ accumulated by cells, which was not consistent with intracellular recordings. Because of the kinetics of the TPMP+ distribution and the large amount of membrane potential insensitive TPMP+, we conclude that this probe is of limited value in this system.

The effect of membrane potential on taurine transport was measured in normal- and low-sodium solutions. Depolarization with potassium in normal-sodium solutions had no effect on the initial rate of taurine uptake, nor did hyperpolarization with valinomycin. In low-sodium solutions, increasing potassium inhibited the initial rate of uptake but valinomycin still had no effect. Ouabain did not affect the initial rate of uptake unless the cells were first incubated for greater than 30 min with ouabain, even though ouabain inhibited sodium and potassium fluxes (Na^+-K^+ATPase activity) within 15 min. We conclude that alteration of membrane potential of LRM 55 cells at physiological sodium concentrations has no effect on transport.

1022 STIMULATION BY CALMODULIN OF [Ca^{2+}-Mg^{2+}]ATPase AND Ca^{2+}-DEPENDENT CALMODULIN-REQUIRING PROTEIN KINASE IN RAT BRAIN SYNAPTIC PLASMA MEMBRANES. R. Sorensen* and H. R. Mahler Chemistry Dept., Indiana University, Bloomington, IN 47405

The calcium binding protein, calmodulin, initially identified and described as a cytosolic component of brain capable of modulating the activity of cyclic nucleotide phosphodiesterase has been shown to be capable also of modulating the activity of several other enzymes from the same source, including the [Ca^{2+}-Mg^{2+}]ATPase [Sobue *et al.*, FEBS Lett. 99 (1979) 199] and a Ca^{2+}-dependent protein kinase [Schulman & Greengard, Proc. Natl. Acad. Sci. 75 (1978) 5432]. We now describe the conditions under which calmodulin and calcium can modulate these activities in a purified synaptic membrane fraction from rat brain cortex.

Maximal stimulation of the [Ca^{2+}-Mg^{2+}]ATPase occurred at 3-8 μM Ca^{2+} in the presence of 1 mM Mg^{2+} to a level of 76 nmol P_i hydrolized x min^{-1} x mg^{-1} protein above the basal, Mg^{2+}-requiring activity (380 nmol P_i x min^{-1} x mg^{-1}). Addition of calmodulin at 20 μg x ml^{-1} resulted in maximal additional stimulation over the whole range of [Ca^{2+}], with no further increase at higher concentrations. This enhancement by calmodulin was dose dependent with a threshold at 6 μg x ml^{-1} and half-maximal stimulation at 8.5 μg x ml^{-1} (membrane protein concentration 150-360 μg x ml^{-1}).

In the same buffer (50 mM PIPES, pH 7.0, 100 mM NaCl, 20 mM KCl, 1 mM $MgCl_2$, 1 mM DTT, 0.5 mM $EGTA_2$, 0.2 mM ouabain), maximal stimulation (three-fold) of the Ca^{2+}-activated, calmodulin-dependent, protein kinase required 32 μM Ca^{2+} in the presence of 200 μg x ml^{-1} calmodulin (protein concentration ~1.5 mg x ml^{-1}; reaction terminated after 10 s; specific activity 0.32 μmol phosphate incorporated x min^{-1} x mg^{-1}). As with the ATPase, stimulation of kinase activity by calmodulin was dose-dependent but with half-maximum stimulation at 50 μg x ml^{-1}. The membrane-bound, endogenous substrates for this protein kinase, as identified by SDS-PAGE and autoradiography, included at least 12 major components with apparent molecular weights (x 10^{-3}) of 220, 205, 200, 195, 190, 155, 115, 100, 64, 59, 50 and 48. Among these, different subsets appeared to become phosphorylated at low vs high levels of both Ca^{2+} and calmodulin. In particular, p48, p115, p190, p195, p200 and p205 were the principal components phosphorylated at low (≤1.5 μM) Ca^{2+}; p50 and p100 required high Ca^{2+} (≥8.0 μM) and low (≤2 μg/ml) calmodulin, while p59 and p64 made their appearance only at high levels of both Ca^{2+} and calmodulin (>10 μg/ml). Finally, p115 was present only at low, but not at high levels of Ca^{2+}. We are currently exploring the possibility that these differences are due, at least in part, to localization of the proteins on pre- vs post-synaptic membranes. (Supported by Research Grant NS 08309 from the NIH)

1023 ELECTRON MICROSCOPIC STUDIES OF ACETYLCHOLINE RECEPTOR (AcChR) TOPOLOGY IN POST-SYNAPTIC MEMBRANES FROM TORPEDO. Paul A. St. John, Jonathan B. Cohen, Daniel A. Goodenough*. Depts. of Anat. & Pharmacol., Harvard Med. Sch., Boston, MA 02115

The polypeptide compositions of post-synaptic membranes isolated from Torpedo electric tissue and of the nicotinic cholinergic receptor isolated in detergent from that tissue differ mainly in a 43,000 dalton peptide which is present in the membranes, but absent from the isolated AcChR. In order to understand the topographic arrangement of the polypeptides contained in the membranes, and to be able to interpret experiments with membrane labeling, methods have been developed for studying the sealing and the inside-out/outside-out orientation of isolated membrane vesicles.

Sealing of membranes was studied with protein tracer molecules and electron microscopy. When membranes are mixed with ferritin (450,000 daltons) or horseradish peroxidase (HRP,40,000 daltons) and subsequently fixed for EM, over 90% of membranes are found to exclude the tracer protein from their interiors. When membranes are fixed briefly, and then exposed to 0.1% saponin, focal holes are formed and over 95% of the vesicles are found to contain ferritin or HRP in their interiors. It appears that during the isolation procedure the membranes are formed into vesicles impermeable to large proteins and that they can be made permeable without being completely dissolved.

Orientation of vesicles was examined with two different techniques. First, freeze-etching showed that the rosette structure peculiar to the true outer surface of the post-synaptic membrane in situ was found on the outer surfaces of isolated membrane in over 85% of the cases. Second, when non-detergent treated membranes were incubated with α-bungarotoxin and toxin was localized indirectly with ferritin-conjugated antibody, label was found on the outer surfaces of over 95% of the membranes greater than 0.1 μm diameter. Taken together, these results indicate that most of the isolated membranes are oriented extracellular side-out.

In order to determine whether the AcChR is exposed on both sides of the membrane, membranes which had been made permeable with saponin were labeled with antiserum raised against AcChR purified from detergent extracts of Torpedo electric tissue. Under these conditions, label was found on both the inside and outside of those membranes which were labeled. We conclude that the acetylcholine receptor is exposed on both sides of the membrane.

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1024 ROWS OF DIMERIC-PARTICLES WITHIN THE AXOLEMMA AND JUXTAPOSED PARTICLES WITHIN GLIA, INCORPORATED INTO A NEW MODEL FOR THE PARANODAL GLIAL-AXONAL JUNCTION AT THE NODE OF RANVIER. Clayton A. Wiley* and Mark H. Ellisman (SPON: C. Spooner). Dept. Neurosci., Sch. Med., UCSD, La Jolla, CA 92093.

Using freeze-fracture techniques, we have analyzed the glial-axonal junction (G-A-J) between Schwann cells and axons in the peripheral nervous system, and oligodendrocytes and axons in the central nervous system. We have identified a new set of dimeric-particles arranged in circumferential rows within protoplasmic fracture faces (P-faces) of the paranodal axolemma in the region of glial-axonal juxtaposition. These particles were observed in both aldehyde-fixed and non-fixed preparations. The dimeric-particles are 260 Å in length, composed of two 115 Å subunits. The rows of dimeric-particles within the axonal P-face leave complementary rows of pits within the external fracture face (E-face) of the paranodal axolemma. These axonal particles are positioned between rows of 160 Å particles occurring in both fracture faces of the glial loops in the region of the G-A-J. In addition to these previously described 160 Å particles, we observed a new set of 75 Å particles within the glial P-faces of the G-A-J. These 75 Å glial particles form rows that are centered between the rows of 160 Å particles and therefore are superimposed over the rows of dimeric-particles within the paranodal axolemma. Our new findings are interpreted with respect to methods of specimen preparation as well as a potential role for the paranodal organ in saltatory conduction. We conclude that this particle rich junction between axon and glia could potentially provide an intricate system of ion exchange between the two cells.

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1025 STUDIES OF THE LOCALIZATION OF CALMODULIN AND ITS BINDING PROTEIN IN BRAIN. John G. Wood, Robert Wallace*, John Whitaker and W.Y. Cheung*. Dept. Anat., Univ. Tenn. Center Health Sci., Dept. Biochem., St. Jude Children's Research Hospital and V.A. Hosp., Memphis, TN

Antibodies to a calcium dependent activator protein (calmodulin) and a protein (CaM-BP_{80}) which binds to calmodulin and blocks its activator effect on several enzyme systems have been used to localize these proteins in mouse brain. A dinitrophenyl derivative of purified calmodulin and native purified CaM-BP_{80} were used to immunize rabbits and the antisera were extensively characterized. In order to perform immunocytochemical experiments, C57 Bl/6J mice were perfused through the heart with a fixative containing 4.0% paraformaldehyde and 0.1% glutaraldehyde in 0.12M phosphate buffer, pH 7.2. The brain was stored overnight in paraformaldehyde only. Forty micron slices were obtained with a vibratome apparatus and incubated in rabbit anti-calmodulin (1:200) or rabbit anti-CaM-BP_{80} (1:400) for 30 min in phosphate buffered saline (PBS). After washing 3 min in PBS the slices were incubated in peroxidase conjugated Fab of goat anti-rabbit IgG followed by a 3 hr wash and development of the peroxidase. At the light microscopic level the slices revealed specific staining within the cell somata and processes of large cells within the basal ganglia, cerebellum and cerebral cortex. In the cerebellum these labeled cells were the characteristic neuronal cells of this structure; no glial elements reacted with antibody. At the electron microscopic level the label was restricted to neurons and was concentrated at the postsynaptic density (PSD) and along microtubular profiles of the postsynaptic dendrite. The pattern of localization with both antisera appeared identical. Biochemical studies have also shown calmodulin is associated with the PSD (J. Cell Biol. 79:96a). The localization of calmodulin and CaM-BP_{80} at the PSD and along postsynaptic microtubules implies these proteins play a role in regulating enzymes involved in neurotransmission and in microtubular function. USPHS NS-12590, NS-08059, AM-05689; Sloan Foundation.

MEMORY AND LEARNING

1026 BRAIN MODEL: A BRIDGE FROM PSYCHOLOGY TO PHYSIOLOGY. J.H.Andreae* (SPON:J.D. Loeser).Dept.Elec.Eng.,Univ.Canterbury,New Zealand.

To connect psychological behaviour with physiological structure, a dual model of the brain is proposed which comprises a well-tested artificial intelligence system, called PURR-PUSS(Andreae: "Thinking with the Teachable Machine",Academic Press.1977), and related neurochemical circuits. Starting with a first assumption that intelligence is the main aspect of human behaviour to be modelled, 3 necessary conditions for intelligence follow, namely, an "open" system, learning from experience, and the self-setting of goals. These 3 conditions imply (i) a "body" to connect the "brain" to the real world so as to avoid the limitations of a formal system, (ii) a "multiple context", comprising short most-recent sequences of events from speech, vision, proprioception, &c., to represent the "here and now", and (iii) "novelty goals", which are events in context that have occurred only once.

The second assumption is that the model must operate at a speed comparable with that of human behaviour so that psychologists can experiment with it. Speed is achievable by using the computer science technique of "hashing". Each new event is stored in LTM (long term memory) at a place determined by hashing its associated context, or event-sequence, in the multiple context. If the associated context occurs again, the context-hashing retrieves the same event together with any other events which had been stored with the same context as "predictions" to be used by the "brain's" decision processes. Context-hashing gives a speed of operation independent of the size of LTM.

Conventional neuron "circuits" allow the multiple context to be translated into biological hardware, but the seeking of novelty goals requires a more subtle mechanism involving the leak-back of chemical code molecules from neurons representing novel events through the neural network to neurons representing current events. This leak-back of "novelty" biases the majority-evidence decisions towards actions which lead the system to novelty goals.

On the organization side, context-hashing corresponds to genetically-controlled neuron growth and the adaptive formation of synapses. The multi-channel interconnection of "brain" and "body" via the multiple context results in a variety of functional and modal subdivisions of the neural network. In this way, the intelligence and speed demanded of the psychological model are related to the neurochemical circuitry and organization of the physiological part of the dual model.

The psychological model has been subject to extensive interaction and teaching, while the operation of the neurochemical circuits has been tested by computer simulation. It remains for foundations to be established at each end of this "bridge" from psychology to physiology.

1027 PUROMYCIN DOES NOT OBLITERATE ALL THE DIFFERENT MEMORIES WHICH OCCUR IN A GIVEN TRAINING SITUATION. D.A. Barraco, E.M. Eisenstein, and K.L. Lovell. Departments of Physiology and Biophysics and the Neuroscience Program, Michigan State University, E. Lansing, MI 48824.

A new one-session training procedure for cockroaches, in which animals are trained to turn right or left to avoid a shock has been developed. This paradigm was utilized to investigate the effects of puromycin, a protein synthesis inhibitor, on learning and memory. The drug was injected before training in does causing approximately 70% protein synthesis inhibition. The number of correct turns and the time taken for the animal to proceed down the runway were the two behavioral parameters measured in the paradigm. In control animals the number of correct choices and the runway time both increased with succeeding trails during training. In addition, control animals showed excellent retention of these responses 5 hours later during testing (n=23). Puromycin also had no effect on aquisition of the two responses. However, upon testing, experimental animals showed a significant retention deficit of the correct turn response but not of the behavioral modification evidenced by increased runway time (n=31). Thus, puromycin may show specificity for the different types of behavioral plasticity that occur in any training situation and that may be mediated at different levels of the central nervous system. (Support in part by the Biomedical Research Support Grant to the College of Natural Science).

1028 VERY LONG SHORT TERM MEMORY IN RATS: EFFECTS OF HIPPOCAMPAL LESIONS. William W. Beatty. Dept. Psychol., NDSU, Fargo, ND 58105.

Rats were trained on a win-shift food searching strategy in an 8-arm radial maze to nearly errorless performance at 0 delay and with a delay of 1 min imposed between the 4th and 5th choices. Then they received dorsal, ventral or combined dorsal and ventral hippocampal lesions or neocortical lesions or control operations. Neurologically intact controls displayed virtually perfect spatial memory (i.e., better than 90% correct on the first 4 choices after the delay) for delays ranging from 1 min to 4 hr; some rats showed comparably excellent memory for 8 hr. Memory deteriorated at 12 hr delays and essentially disappeared by 24 hr. Preliminary results indicate that ventral hippocampal lesions have little effect on spatial memory while dorsal hippocampal lesions cause serious deficits in spatial behavior at the 0 delay condition and deficits in spatial memory in animals that successfully reacquire criterion performance at 0 delay.

1029 ENGRAM LATERALIZATION IN THE INTACT AND SPLIT-BRAIN CHICK. Graham A Bell* and David Ehrlich* (SPON: David G Satchell) Dept.Behav.Biol., Res.Sch.Biol.Sci., Australian National Univ., Canberra, Australia 2601.

Young chicks were trained to suppress their tendency to peck a small bead after it had been dipped in a noxious substance, methyl anthranilate (MeA). Retention was measured by the proportion of a group of chicks pecking an identical clean target. Chicks were able to discriminate between the previously tainted bead and a novel one of different colour, for up to 24 hr after the learning trial. There was good interocular transfer for the task when chicks were trained and tested monocularly.

Recent work with this task using intracranial injection of ouabain or cycloheximide (Bell and Gibbs, Neurosci.Lett., 1979, in press) suggests that monocular learning results in a localized engram in the forebrain hemisphere contralateral to the trained eye. Interocular transfer of the MeA task in the intact chick therefore requires interhemispheric readout of the unilateral engram by the trained hemisphere. A bilateral engram can become established as a consequence of interocular transfer testing; the readiness of engram duplication depends on the recency of formation of the original trace.

We lesioned the dorsal supra-optic commissure (DSO) in three day old chicks 4 hr after monocular learning of MeA-avoidance, and following 48 hr of monocular exposure to an imprinting object. A sham operated control group was taken from the same hatch, similarly housed and trained.

At 24 hr after operation the sham group (n=15) showed good retention of the MeA task with the trained and untrained eyes, and discriminated between the previously tainted target and a novel one. The split-brain group (n=25) showed good retention and discrimination with the trained eye. When tested with the untrained eye, chicks failed to show retention and pecked the learning and novel targets with the same probability. This suggested that monocular learning of MeA-aversion established a lateralized engram and that access to it depends on an intact DSO.

Interocular transfer of imprinting failed in the split-brain and sham groups 24 hr after operation, but succeeded in some individual chicks from both groups, on three subsequent testing days. It appears that imprinting is more sensitive to suture reversal than the MeA task. Further work is required to resolve whether monocular imprinting establishes a bilateral engram or whether retrieval of a unilateral engram is achieved independently of the DSO.

1030 CHOLINERGIC BLOCKADE OF THE CAUDATE NUCLEUS AND AUTOSHAPING IN RATS. Federico Bermúdez-Rattoni* and Roberto A. Prado-Alcalá. (SPON: J.A. Roig). Dept. Physiol., Sch. Med., Natnl. Univ. of México, P.O. Box 70250, México 20, D.F., México.

Commonly, studies on the acquisition of instrumental behaviors involve "shaping" of the animal's responses by the experimenter. Thus, acquisition becomes the result of an interaction between the animal's learning capabilities and the experimenter's ability to shape. Autoshaping procedures, on the other hand, represent an important technical advancement with respect to the method of successive approximations, since the behavior of the animals may change within a standarized experimental environment, without a direct intervention of the experimenter. We studied the effects of cholinergic blockade of the caudate nucleus on the acquisition of lever pressing behavior. Animals were trained in a Skinner box; on day 1, a light-bulb situated above a centrally located liquid dispenser was turned on and then 0.3 ml of water were delivered. This sequence was repeated 50 times with an intertrial interval (ITI) of 30 sec. Twenty-four hr later a light-bulb placed above a lever was turned on 50 times (ITI 30 sec). If during these periods a rat pressed the lever the liquid dispenser was lighted and water was delivered. If the animal pressed while the lever was not lighted, the response was not reinforced. Bilateral microinjections of scopolamine (30 µg into each cannula) or saline solution (NaCl) were performed in independent groups 6 min before the second session, into the anterior caudate nucleus (ACN), the posterior caudate (PCN) or into the parietal cortex (Ctx); there was also an unimplanted (UI) control group. Those groups injected with scopolamine in ACN and PCN showed an impairment in the acquisition of lever pressing, as compared with the Ctx, NaCl and unimplanted groups. These results suggest that cholinergic activity of the CN is involved in the acquisition of instrumental behaviors.

1031 DISTRIBUTION AND RESPONSE CHARACTERISTICS OF RAT MEDIAL GENICULATE NEURONS WHICH SHOW ASSOCIATIVE CHANGE DURING DIFFERENTIAL CONDITIONING AND REVERSAL. D. Birt and M. E. Olds*. Div.of Biol., California Institute of Technology, Pasadena, CA 91125.

We recently reported that in a conditioning paradigm which eliminated a number of previously demonstrated non-associative factors, a proportion of neurons in medial geniculate of rat showed short latency associative change. The present experiment was designed to determine the distribution of such units throughout the subdivisions of the medial geniculate and to identify response characteristics which might differentiate these units from units not showing associative change.

The multiple unit responses on 117 probes distributed throughout MG were recorded during differential appetitive conditioning, extinction, and successive reversals. Seventy-three of these met criteria for responsiveness and stability of recording and were further analyzed. 11 units which showed enhanced response to the positive stimulus relative to the response to the negative stimulus throughout 4 or more consecutive reversals were considered to be associative. These units were largely localized in the far posterior portion of MG where 25% (9 of 36) units were associative. Only 5% (2 of 37) units in the remainder of MG were associative. Associative units were further differentiated from non-associative units by differences in the temporal pattern of their initial response prior to conditioning. Units with a more sustained response were more likely to show associative change than those with a very transient response and the magnitude of change was positively related to the initial duration of response. 71% (5 of 7) of units which initially were responsive for more than 80 ms showed large magnitude associative change during conditioning. Only 9% (6 of 66) of units which were initially responsive for 80 ms or less showed associative changes and these were of small magnitude.

These associative neural changes were shown not to result from behavioral feedback since their latency (as early as 16 ms after stimulus onset) preceded behavioral response and since they were not directly correlated with behavioral change. They appear more directly related to processing of the significance of stimulus information.

The fact that these changes are localized to a discrete portion of MG with known differential afferents and efferents and that associative units have different temporal response patterns which are easily identified leads to the potential for identifying the neural circuitry involved using single unit analysis techniques. (Supported by NSF BNS 77-22289)

1032 CHANGES IN RESPONSIVENESS TO GLABELLA TAP AMONG NEURONS IN THE SENSORIMOTOR CORTEX OF AWAKE CATS. J. Brons*and C.D. Woody. Depts. Anatomy & Psychiatry, Brain Research Institute, Mental Retardation Research Center, UCLA Medical Center, Los Angeles, CA 90024.

Unit responses to glabella tap stimulation were studied in 128 neurons of the sensorimotor cortex in 4 awake cats. The glabella tap, used as an unconditioned stimulus (US) during classical conditioning, elicits an unconditioned eyeblink response of 7-9 msec latency by activation of a brainstem reflex arc (Woody & Brozek, J. Neurophysiol. 32: 704-716, 1969). The effects of the tap US on cortical responsiveness were examined over a two day period (up to 400 tap trials per day, 5 sec ITI). Overt habituation of the eyeblink response occurs at ITI's of 1-2 sec or less.

Unit activity was recorded intracellularly or extracellularly with 15-50 MΩ, 1.4M K+- citrate filled glass microelectrodes. On the first day of recording, out of 55 neurons, 29% showed increased activity to tap; 36% showed decreased activity; and 35% were unresponsive. On the second day, out of 73 units, 55% showed increased activity, 27% decreased activity, and 18% were unresponsive. Analysis indicated that the proportion of cells responding with increased activity had increased significantly from the first day to the second ($P<.01$, X^2 test). PST histograms of the responses to the tap revealed that the mean onset latencies (9 msec for increased activity, 13 msec for decreased activity) and peak magnitudes of the averaged unit responses remained unaltered over the two days. Intracellular recordings indicated that the increases and decreases in evoked activity were supported by EPSP's and IPSP's, respectively.

These data provide evidence that repeated presentations of a glabella tap over a period of two days increases the proportion of cells within the motor cortex responding to the stimulus with increased activity. Furthermore, this US which reinforces eyeblink conditioning produces both EPSP's and IPSP's in the motor cortex. (Supp. by AFOSR 76-3074 and BNS 78-24146.)

1033 THE ROLE OF EXTRACELLULAR CALCIUM IN HABITUATION AND TRANSDUCTION OF ELECTRICAL AND MECHANICAL STIMULI IN THE CILIATED PROTOZOAN, SPIROSTOMUM AMBIGUUM. Donald G. Brunder* and E. M. Eisenstein. Biophysics Dept., Michigan State Univ., East Lansing, MI 48824.

Previous work shows that Spirostomum ambiguum responds to mechanical (vibratory) and electrical stimulation by rapidly contracting. Repeated mechanical but not electrical stimulation (rate 0.1 Hz) leads to a decrement in the probability (habituation) of the contractile response. The independence of the two stimulation modes with respect to habituation suggests separate sites for mechano- and electro-transduction (Osborn et al., Behav. Biol. 8:655,1973). Earlier studies have also indicated that extracellular calcium is important in mechanotransduction (Osborn et al., Behav. Biol. 8:665,1973).

The present study is concerned with the effects of extracellular calcium on the transduction and habituation processes in Spirostomum. Calcium was manipulated by incubating the protozoans in media containing different calcium concentrations and by the addition of the calcium chelator ethyleneglycol-bis (ß-amino-ethylether)N,N'-tetraacetic acid (EGTA). The results indicate that increasing the calcium concentration increases responsiveness to mechanical stimulation over the concentration range studied (0-0.5mM). Initial responsiveness (minute 1 of the stimulation period) to electrical stimulation was depressed in media to which no calcium was added. Such media also resulted in a response decrement to electrical stimulation not seen when free calcium was present. The data suggest that free calcium is the main factor in responsiveness as determined by manipulation of calcium and EGTA levels. EGTA-containing media in which no free calcium was present generally decreased responsiveness to mechanical and electrical stimulation.

The results are consistent with previous studies in supporting the role of extracellular calcium in mechano- and electrotransduction and possibly habituation. In addition it was shown that the lack of extracellular calcium causes a decrement of the contractile response to electrical stimuli; the unavailability of extracellular calcium may be an important feature in the mechanism of habituation to mechanical stimulation as well. (Supported in part by the Biomedical Research Support Grant to the College of Natural Science; D. G. Brunder is supported by funds provided by Osteopathic Medicine).

1034 EFFECTS OF L-PROLINE, PROLINE ANALOGS AND OTHER AMINO ACIDS ON MEMORY IN THE CHICK.

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The amnestic effect of L-proline (L-PRO) (Van Harreveld and Fifkova, Brain Res. 81, 455, 1974) has been confirmed by two laboratories (Science 193, 242, 1976; Neurosci. Lett. 6, 355, 1977). The latter concludes that the amnestic effect results from interference with brain uptake of amino acids, rather than with glutamate release as postulated by Van Harreveld and Fifkova. Structure-function relationships may clarify the issue. The L-PRO effect is stereospecific and dependent upon molecular size (Brain Res. 156, 256, 1978). We have reported the amnestic effect of DL-3,4 dehydroproline and L-baikiain. We now present data on additional amino acids. We injected chicks intracerebrally with 10 µl/hemisphere of 300 mM amino acid, 1 min after one-trial training to suppress the peck response to a bead. Suppression was conditioned by coating the bead with an aversive liquid (methyl anthranilate). Retention was tested 24 hr later using the uncoated bead; increased peck scores (mean sq. rt. of pecks in 10 sec) indicate impaired memory. The experiment was in block design. The results with D-proline and cycloleucine are similar to those with no injection, eliminating trivial factors, such as injection itself, as causative of memory impairment. The results with L-isoleucine, and with L-serine and L-threonine (albeit borderline), indicate that imbalance of free amino acids in the ventricular fluid does not necessarily impair memory. Of interest is that the amino acids found by Van Harreveld (Neuroscience Abst., this volume) to resemble L-PRO in their dose-response effect upon spreading depression in the chick retina (DL-3,4 dehydroproline, L-baikiain, 4-hydroxy-L-proline) also have an amnestic effect. The amnestic effect of amino acids which do not resemble L-proline in the retina experiment (glycine, L-homoserine, L-glutamine) may result from different mechanisms.

Amino Acid	Peck Score[a]	Amino Acid	Peck Score[a]
4-Hydroxy-L-PRO	1.20*[c]	L-Serine	1.05
L-PRO	1.17*[c]	L-Threonine	0.97
Glycine	1.12*	L-Pyroglutamic Acid	0.92[c]
L-Homoserine	1.09*	Cycloleucine	0.57
D-PRO	0.59[c]	L-Isoleucine	0.71[bc]
		No injection	0.72[b]

* Differ significantly from D-PRO ($p<0.05$; t-test).

[a] N=41-50, except L-isoleucine (N=134). The range of SEM's was 0.08-0.17 in present experiment.

[b] Data from previous experiments. SEM's=0.06-0.10.

[c] EEG records show no seizure or isoelectric activity in these amino acids or in L-baikiain or DL-3,4 dehydroproline; remaining amino acids being studied.

1035 THE NATURE OF REMOTE MEMORY IMPAIRMENT IN THE AMNESIC SYNDROME.

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Performance of amnesic patients on tests about public events or public figures has previously been evaluated, usually in a single population of patients. The present study assessed memory for photographs of famous individuals who came into prominence at various times from the 1930s through the 1970s. We studied the amnesic patient N.A., a group of alcoholic Korsakoff patients, and three groups of control Ss.

Three major findings emerged: 1) the pattern of impairment demonstrated by N.A. differed markedly from that demonstrated by the Korsakoff patients. N.A. scored at least as well as 16 matched controls on photographs of individuals who were known prior to the onset of his amnesia in 1960. N.A. was severely impaired with photographs of individuals who were known only in the 1960s or 1970s. In contrast, Korsakoff patients were impaired over the entire range of the test, and this deficit was graded such that their performance was poorer for the recent time periods than for remote periods. 2) Yes/No and multiple-choice cuing improved both amnesic performance and control performance, but did not eliminate the amnesic defect. 3) A group of 16 alcoholic inpatients performed at a level intermediate between Korsakoff patients and 16 matched non-alcoholic inpatients. This finding provided support for the notion that the marked temporal gradient of impairment demonstrated by the Korsakoffs might in part reflect the gradual onset of anterograde amnesia over many years. Taken together, these findings emphasize the difference between etiologically distinct amnesias and provide additional evidence for continuity between Korsakoff patients and chronic alcoholics.

1036 AMYGDALA INDUCED AMNESIA GRADIENTS OR ALTERATION OF PERCEIVED AVERSIVENESS? Jeffrey D. Cross and Irving J. Goodman. Dept. of Psychology, West Virginia U., Morgantown, W. Va. 26506

There have been numerous reports of the disruptive effects of electrical brain stimulation (ESB) on memory. The stimulus parameters for ESB in these studies varied widely with regard to both stimulus intensity and duration. The vast majority of these investigations employed some form of avoidance performance as the lone dependent variable. Impaired performance of the avoidance task after some post-treatment delay indicates retrograde amnesia caused by the ESB. Several authors have reported a positive rela-tionship between the intensity of the ESB and the amount of retrograde amnesia observed. The results of these studies are typically referred to as "amnesia gradients". Theoretical explanations of how a memory trace can be more-or-less impaired by varying intensities of ESB are based on the concept of partial destruction of the short-term memory consolidation process. An alternative interpretation of these results is suggested by the present study which proposes alteration of affective quality of memory as the process involved in so-called amnesia gradients.

Thirty adult male hooded rats were anesthetized and implanted unilaterally with a bipolar nichrome electrode in the amygdala. Following a short recovery period, the animals were reduced to 80% of their ad lib. body weight and trained in a step-down apparatus. Training consisted of placing the animal in an enclosed platform chamber and opening a guillotine door. Opening the door permitted the animal to descend from the platform and cross a grid floor to a food cup mounted on the wall. Upon reaching a performance criterion, a 2 mA footshock followed immediately by ESB was administered on the next trial to all animals. The intensity of the ESB was at one of four levels: 0uA; 25 uA; 50 uA; 100 uA. The duration of the single pulse was 0.5 msec. Following the treatment trial, all animals were tested for recall deficits 24 hours later by the administration of a single avoidance trial. During training, treatment, and test trials, the heart rate of each animal was recorded. Heart rates (bpm) were calculated for the five seconds immediately prior to the opening of the door (PRE) and the first five seconds after the opening of the door (POST) for pretreatment and recall test trials. Graded step-down latencies were observed across groups; ESB intensity and step-down latency were inversely related. Heart rate change scores (post H.R. - Pre H.R.) were positively correlated with avoidance step-down latencies. These findings are better explained by alteration of affective quality of the aversive ("to be stored") event rather than the complete or partial destruction of the memory trace.

1037 OPIATES LEAD TO PERSISTENCE OF SPATIAL HABITS WITH SOCIAL REWARDS. Fatma G. DeEskinazi and Jaak Panksepp. Dept. Psychol., Bowling Green State Univ., OH 43403

To evaluate the effects of brain opiate systems in habit-formation, 15 day old rats injected with saline, morphine (0.5-1.0 mg/kg) or naloxone (1 mg/kg) were tested for acquisition and extinction of spatial discrimination in a T-maze using either access to home cage or food as rewards. Although morphine animals ran significantly slower than controls during the first two days of acquisition, they did not differ in terms of errors or trials to acquire the habit. Naloxone animals took reliably fewer trials to reach criterion and made fewer acquisition errors than controls. During 12 days of extinction, morphine treated animals sustained their behavior practically undiminished, running as fast and as correctly, on the last day of extinction as on the first. Saline animals showed a gradual rise in running times till day 5, whereupon latencies became stable. Naloxone animals received only 7 days of extinction, because by the end of 70 trials performance was completely extinguished. The maintenance of the spatial habit by morphine was not state-dependent in that morphine was as effective given only during extinction as during both acquisition and extinction. To determine whether these results are specific for social rewards, animals were tested in the same task using food reward. There was almost no difference in number of trials to criterion by saline and morphine animals, and morphine did not slow the initial acquisition running time. Although morphine did sustain fast running times during extinction, correct-choice behavior was not sustained, suggesting that opiates lead to persistence of social habit more than food-rewarded habits. The capacity of morphine to sustain social-habits is probably mediated by opiate receptors, since the effect was eliminated by pretreatment with naloxone. To further evaluate the perseverative behavior produced by morphine, rats were tested in a position reversal task using social rewards. Whereas controls reversed side preferences within 2 days, morphine animals required 5 days to acquire the reversal. These results indicate that morphine has a strong capacity to modify the expression of learned behaviors, and suggests that brain opiate systems may be important in formation and maintenance of habits, expecially social-habits.

1038 A MODEL FOR PERCEPTUAL CODING BASED ON EEG ANALYSIS. Walter J. Freeman, Dept. Physiology-Anatomy, University of California, Berkeley CA 94720.

Rabbits were implanted with 8x8 electrode arrays spaced .5 mm epidurally on the olfactory bulb. Maps of the EEG rms burst amplitudes showed that (a) amplitudes in waking, motivated rabbits were non-uniform; (b) contour plots showed foci resembling islands with patterns unique to each animal; (c) patterns remained stable for months if no training to olfactory stimuli was undertaken; (d) the patterns changed with discriminant training to give a CER or CR to a warning odor paired with an aversive stimulus; (e) the new pattern restabilized in 1 or 2 further sessions but changed again on training to a new warning odor; and (f) after each new discrimination was established, the new EEG pattern was present whether or not the odor was present.

The results were taken to mean that the EEG patterns were mainly determined by expectation rather than by stimulation. During training a template of synaptic connections is laid down for the warning odor. Thereafter, when the animal searches for that odor, the template is activated. This gives rise to the EEG burst pattern, which conforms to a percept rather than a recept.

A set of nonlinear differential equations, based on behavioral studies of evoked potentials, EEG and unit activity (Freeman, W.J. Mass Action in the Nervous System, Academic Press, New York, 1975), was solved to simulate bulbar space-time activity patterns. It is inferred that (a) during training the template forms by 2-4 fold strengthening of excitatory synapses between co-activated mitral cells, and constitutes a probability map of the variety of spatial forms a given odor can take; (b) when the animal becomes selectively attentive to the stimulus, the template is sensitized by background input from olfactory and other senses, under centrifugal control; and (c) when any portion of the template is stimulated, the entire set of neurons comprising it is activated through the mutually excitatory connections. Thus the template is a generalization over all input for an odor during training, is sensitive to any subset whether or not it occurred during training, and serves to fill in missing detail for small or large input subsets. When a subsidiary mechanism for habituation is introduced, the output for adventitious odors is attenuated, and the pattern of output thereafter reflects the form of the template rather than the pattern of the input, as observed in the EEG.

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1039 β-ADRENERGIC MANIPULATION WITHIN THE AMYGDALA ALTERS HEART RATE CONDITIONING IN THE RABBIT. Michela Gallagher, Bruce S. Kapp, Robert C. Frysinger,* and Peter R. Rapp.* Dept. of Psychology, University of Vermont, Burlington, VT 05401.

Previous research conducted in this laboratory to assess the involvement of specific amygdala systems in the development of heart rate conditioning demonstrated that lesions confined to the central nucleus of the amygdala produce impairment of conditioned bradycardia in rabbits (Kapp et al., Neurosci. Abs., 1978). The present investigation was aimed at determining whether catecholamine systems which provide a particularly rich innervation of the central nucleus contribute to heart rate conditioning. To this end the effects of injections of the β-adrenergic antagonist dl-propranolol into the region of the central nucleus on heart rate conditioning were assessed. New Zealand rabbits were surgically prepared with cannulae positioned at the dorsal surface of the central nucleus. Two weeks following surgery all subjects were trained using a standard Pavlovian conditioning procedure. All drug injections were delivered bilaterally in a 1.0 µl volume immediately prior to the conditioning session.

Compared to a vehicle injected control group, a group receiving injections of the β-adrenergic antagonist, dl-propranolol, into the central nucleus exhibited significant attenuation of the conditioned bradycardia response. This effect of dl-propranolol appears to be due to the specific β-adrenergic blocking activity of the drug because a group receiving the same dose of d-propranolol did not exhibit a comparable conditioning deficit. In addition, combined administration of dl-propranolol and l-isoproterenol, a β-adrenergic agonist, reduced the conditioning impairment observed following administration of dl-propranolol. Finally, data obtained from subjects receiving injections of dl-propranolol into other sites within the amygdala complex or into sites dorsal to the central nucleus of the amygdala support the interpretation that the effects of dl-propranolol on heart rate conditioning in this research are due to interference with β-adrenergic activity within the region of the central nucleus.

Recent neurochemical investigations have revealed that the central nucleus receives adrenergic projections from the catecholamine containing cells in the medulla as well as from the locus coeruleus (Fallon et al., J. Comp. Neurol., 1978, 180, 509-532). The innervation from the medulla may in part derive from cells in the A2 region of the nucleus tractus solitarius, a region involved in cardiovascular regulation. Our results, therefore, may reflect the effects of interference with an A2-amygdala system.

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1040 DIFFERENTIAL FOOD AVERSION LEARNING BY THE TWO HALF-BRAINS OF MONOCULARLY TRAINED CHICKS. Karen E. Gaston* (SPON: R. W. Sperry). Division of Biology, California Institute of Technology, Pasadena, CA 91125.

It has been shown that young chicks avoid drinking a novel green sucrose solution which has been paired once with delayed LiCl-induced illness, and that this conditioned aversion is mediated by the visual (color) cue and not by taste (Gaston: Behav. Biol. 20: 441, 1977). Tests with monocularly trained chicks demonstrated good interocular transfer of the aversion learning (Gaston: Behav. Biol. 24: 272, 1978). To examine the possibility that novel taste might play some role in acquisition and/or interocular transfer of the visual aversion, the present experiment evaluated the effects of monocular training with novel color in combination with either novel or familiar sucrose taste.

Ten-day-old chicks with one eye occluded were made sick with LiCl after drinking green sucrose solution. Half the animals had been pre-adapted to the sucrose taste. The chicks were tested the following day, with the trained or the untrained eye open, for learned aversions to green or uncolored sucrose. Chicks tested with the trained eye displayed a marked aversion to the colored sucrose, but not to uncolored sucrose, regardless of the taste variable. These results demonstrate that taste adaptation did not interfere with monocular learning and that novel taste was not required for acquisition of the visual aversion. In contrast, animals tested with the untrained eye showed a significant aversion to either colored or uncolored sucrose, but in each case only if the taste was novel during training. The avoidance of uncolored sucrose clearly represents a taste aversion which was acquired only by the hemisphere deprived of direct visual information during training.

The present results, in combination with those of the previous related studies, suggest that the seeing whole or half-brain could utilize only the novel visual information (color) for acquisition and expression of an illness-induced aversion. However, when one eye was occluded and vision through that eye thus eliminated, then the non-seeing half-brain was able to use the novel taste information in forming an aversion. These findings indicate that, in monocularly trained chicks, each half-brain can independently acquire an illness-induced food aversion, with learning in the seeing and non-seeing hemispheres mediated by novel color and taste cues, respectively.

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1041 BRAIN NOREPINEPHRINE AND RETROGRADE AMNESIA. Paul E. Gold and James M. Murphy. Dept. Psychology, University of Virginia, Charlottesville, VA 22901.

Most candidates for neurobiological correlates of retrograde amnesia (e.g., seizures, or transmitter or protein synthesis inhibition) have only limited generality across amnestic agents. Recently we found that brain norepinephrine (NE) concentrations are sensitive to several training-treatment combinations. The extent of a transient decrease (maximal 10 min after training) in NE content appears to be a predictor of later retention performance in a variety of situations. Brain NE concentrations decrease by 20% after a training footshock adequate for good retention. Under conditions in which a larger (30-40%) or smaller (0-10%) decrease occurs, later retention performance is poor.

The present experiment examined the relationship between the effects of supraseizure electrical stimulation of frontal cortex on retention and on brain NE content. Rats received footshock (3 ma, 2 sec) in a one-trial inhibitory (passive) avoidance task. Five sec after training, the animals received frontal cortex stimulation (2.5 or 5.0 ma, 1 sec) through chronically implanted screw electrodes. Other animals received no training, training, or stimulation alone. A set of animals in each group was tested for retention performance 24 hr later; both cortical stimulation intensities resulted in brain seizures and in poor retention performance (retrograde amnesia).

Ten minutes after training and/or treatment, the remaining animals in each group were decapitated and the brains were removed and dissected into forebrain and brainstem samples. NE concentrations were measured with a PNMT-linked radiometric assay.

Control NE values were 515±30 and 550±40 ng/gm for forebrain and brainstem samples respectively. Consistent with our previous findings, footshock reduced forebrain NE content by 17% and brainstem NE by 22% as measured 10 min after training. Frontal cortex stimulation administered after avoidance training significantly altered this normal NE response to footshock. In the forebrain, the NE response after footshock was potentiated (32% decrease) while in the brainstem the response was attenuated (2% decrease). Although it is difficult to explain the opposite direction of the effects of stimulation on the two brain regions, the findings are consistent with the general view that a transient posttraining 20% decrease in brain NE content, presumably reflecting NE release, is optimal for memory storage processing. Treatments that result in significant deviations from this pattern appear to produce retrograde amnesia.

Supported by research grants BNS 76-80007 (NSF) and MH 31141 (NIMH).

1042 HOW DOES A BRAIN BUILD A COGNITIVE CODE? Stephen Grossberg. Dept. Math., Boston Univ., Boston, MA 02215.

This talk addresses the question: where codes of sensory or cognitive events can develop through experience, how are globally self-consistent hierarchies of such codes established and maintained? How is the local ignorance of each feature detector reconciled to yield global consensus? How is a coding error corrected when no individual cell knows that one has occurred? Previous work has shown that feedforward coding mechanisms are either unstable in a complex input environment, or must be shut off by a chemical switch that is insensitive to the behavioral meaning of the code. This paper indicates how competition between afferent data and learned feedback expectancies, or templates, can stabilize the code by buffering committed populations against continual erosion by new environmental demands. The gating phenomena that result lead to dynamically maintained critical periods, whether or not a chemical switch also exists, and to attentional phenomena such as overshadowing in the adult. The functional unit of cognitive coding is suggested to be an adaptive resonance, or amplification and prolongation of neural activity, that occurs when afferent data and efferent expectancies reach consensus through a matching process. The resonant state is a global, context-dependent representation of the data in STM. It embodies the perceptual event, or attentional focus, and its amplified and sustained activities are capable of driving slow LTM changes in the codes and expectancies that define the network. Mismatch between afferent data and efferent expectancies yields a global suppression of activity, and triggers a reset of codes in STM, as well as a rapid parallel search for uncommitted cells. This is accomplished by a nonspecific arousal mechanism that is gated by a chemical transmitter system, probably catecholaminergic, whose relative states of accumulation at antagonistic pairs, or dipoles, of on-cells and off-cells can shift the pattern of STM activity across a field of feature detectors. In particular, a sudden arousal increment in response to an unexpected input, or mismatch, can selectively reverse, or rebound, these relative activities in a graded fashion across all cells, thereby suppressing incorrectly classified populations. The cellular field is hereby conditionalized, and a rapid parallel scheme of hypothesis testing is elicited. A source paper is in Progress in Theoret. Biol., Vol. 5. R. Rosen and F. Snell, Eds. New York: Academic Press 1978.

1043 ENRICHED AND IMPOVERISHED ENVIRONMENTAL REARING: SELECTIVE ALTERATIONS OF PARADOXICAL SLEEP AND MEMORY. Baruch M. Gutwein* and William Fishbein. Psychobiology Lab., Dept. Psych., The City College of New York, New York, N.Y. 10031.

Mice were reared in either enriched, social control or isolate environments for 30 days. Environmental enrichment results in a significant and selective increase of paradoxical sleep (PS, or REM sleep) and also enhances recall of a multiple-trial, brightness discrimination task 28 days after training. Conversely, isolate reared mice exhibit a decrease in PS and impaired task performance relative to controls. These results support the hypothesis that PS plays an integral role in the maintenance and stability of long-term memory.

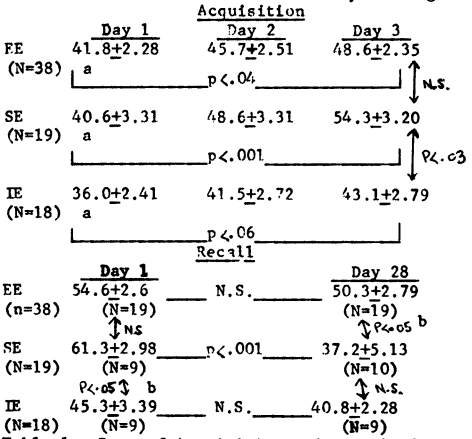

Table 1. Rate of Acquisition and Recall of Y-maze training (mean percent discriminations± SEM) in Enriched, Social Control, and Impoverished reared mice.

a. ANOVA, linear trend component for acquisition.
b. Duncan Range Test.

1044 POSTNATAL MALNUTRITION AND ITS EFFECTS ON FOOD MOTIVATION OF ADULT RATS: IMPLICATIONS FOR LEARNING STUDIES. Edward S. Halas, Patricia A. Burger*, and Harold H. Sandstead*. Dept. Psychology, University of North Dakota and Human Nutrition Lab., USDA, Grand Forks, N. D. 58202

Several studies have shown that prenatal and postnatal malnutrition impaired subsequent performance of animals on learning tasks that involved food motivation. These experiments are difficult to interpret because the food motivation of animals which have been exposed to early malnutrition may not be the same as normal animals. Two different types of postnatal malnutrition were studied. Ten pregnant Long-Evans rats were given a zinc deficient diet (ZD) and deionized water on the day of delivery. The dams were kept on the diet until the pups were 22 days old. Since zinc deficiency causes anorexia, a second group of 10 dams (PF) were fed the same quantity of the diet as was consumed by their pair mates (ZD). The PF dams were given 25 ppm/Zinc in their water. A third group of 10 dams (AL) were fed the diet ad libitum plus the zinc supplemented water. After weaning, all pups were fed Chow ad libitum plus tap water. At 90 days of age, the offspring began their food motivation test. Six males and 6 females were selected from each of the 3 dietary groups for a total of 36 rats. Once a week for the next 8 weeks, each pup was deprived of food for 24 hours and tested for food motivation in an operant box using a progressive ratio schedule. The ratio was increased by one after each reward. The "breaking point" was defined as failure to bar press for 15 min. The PF rats, both males and females, had significantly higher breaking points than the AL males and females. The higher food motivation of the postnatally malnourished rats (ZD and PF) has definite implications for learning studies. Performance on a learning task is affected by two critical variables: learning ability and motivation. When learning is studied, the motivation of the animals must be constant, otherwise a difference in performance between groups cannot be attributed solely to a difference in learning ability. These difficulties of interpretation are further complicated when the Yerkes-Dobson law is taken into consideration.

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1045 THE ROLE OF THE HIPPOCAMPAL FORMATION IN HUMAN MEMORY: A MODEL. Eric Halgren. Brain Research Institute,UCLA,Los Angeles,CA 90024.

The preservation of general intellectual processes with the narrow exception of Recent Memory (RecM) after bilateral Hippocampal Formation (HCF) damage implies that those processes are located elsewhere: the model assumes that they are performed by the neocortex (NC) and that the brain processes correlated directly with the specific content of a conscious experience lies in a cell-assembly(CA) distributed throughout widespread NC areas. Residual excitation intrinsic to just-activated NCCAs underlies Short Term Memory. Remote Memory (RemM), also intact after HCF lesions, procedes using cognitive processes such as logical deduction, acting upon general knowledge of the world and specific knowledge of the item to be recalled. When a NCCA is activated, a portion of its efferents converge on a group of Hippocampal Gyrus (HCG) neurons. Very powerful and long-lasting post-tetanic-potentiation is known to occur within the HCF. As a result of this potentiation, a HCGCA activated by a certain set of afferents will fire when a subset of these afferents are later active, i.e., when a semantic cue is re-experienced. Persistent or repeated activation of an HCGCA will evoke progressively stronger excitation of the associated HCF neurons in the same lamella. There are several ways whereby the activation of an HCFCA by a cue could promote the activation of the NCCA associated with its other afferents: (1) direct projections of HCF neurons to NC, several of which have described, although they have not yet been demonstrated to be topographically reciprocal; (2) direct antidromic activation of NCCA synapses upon the HCGCA, perhaps only when the HCGCA is sufficiently excited by positive feedback via the HCF: (3) direct and indirect subicular cortex (HCF) efferents to the nonspecific thalamic nuclei may help focus attention on the relevant NCCA; and (4) Brainstem, hypothalamic, and septal projections of CA3 (HCF) neurons may help release interference from prepotent RemMs. These mechanisms, even if weak and nonspecific, would, in concert with the aforementioned cognitive processes involved in both RecM and RemM, lead to the activation of further cues. A cyclical process would thus be initiated that, if successful, would culminate in an avalanche of cues resulting in the reactivation of the sought-for experience as well as in a feeling of recognition.

Our model is consistent with existing data, integrates with neural and cognitive models elaborated at different levels of analysis, suggests experiments, and makes testable predictions. (Supported by grants NSF-BNS77-17070 and NIH-NS02808. My thanks to Thomas Babb and Paul Crandall)

1046 THE EFFECT OF CAROTID LIGATION ON STRIATAL CATECHOLAMINES AND BEHAVIOR IN THE GERBIL. J.D. Irvin, W.H. Vogel, B.R. D'Amore*, R.K. Ladman* and J.L. Alderman. Depts. of Neurosurgery and Pharmacology, Jefferson Medical College of Thomas Jefferson University., Philadelphia, Pa. 19107.

The effect of unilateral carotid ligation on striatal catecholamine fluorescence (CAF), learning and memory was studied in the gerbil. Animals were anesthetized with ether, the left carotid artery exposed and ligated, and sacrificed at 5 and 15 minutes, 24 and 48 hours and up to 3 weeks post-ligation. The left and right caudate (LC, RC) were examined for CAF by the Falck-Hillarp condensation technique. Learning and memory were studied with a shuttle box by conditioned avoidance response (CAR) acquisition and retention. Animals were observed from 24 hours to 17 days post-ligation for behavioral changes. Although no alterations in parenchymal CAF were observed 5 or 15 minutes post-ligation, there was a marked decrease, more so in the LC than RC after 24 hours, and a further drop by 48 hours. By 3 weeks, there was a return of CAF in the LC and RC. Acquisition of the CAR was delayed by 20% (from 97 CAR/animal to 77 CAR/animal) in animals tested 48 hours post-ligation, and by 28% (from 71 CAR/animal to 51 CAR/animal) when tested 15 days after ligation. Memory seemed little affected by ligation; animals tested at different times between 2 hours and 10 days after surgery showed no decrease in CAR's. The data indicate that biphasic changes occur in caudate CAF of both hemispheres after unilateral carotid ligation which are apparently not correlated with either learning nor memory behavior.

1047 CHOLINERGIC AND ADRENERGIC MODULATION OF CYCLOHEXIMIDE-INDUCED MEMORY IMPAIRMENT. Stanley J. Jackson and Herbert P. Alpern, Dept. Psych., Univ. Colo., Boulder, CO 80309.

A number of physiological mechanisms, such as protein synthesis, and the activity of several neurotransmitters, have been implicated in the formation and maintenance of memory. The primary evidence linking protein synthesis to memory comes from experiments utilizing drugs such as anisomycin, cycloheximide, and pyromycin which inhibit protein synthesis and impair memory. Recent evidence suggests that these agents could also be disrupting memory by several secondary actions that result in altered synaptic efficiency. This experiment was designed to investigate how cycloheximide-induced disruption of memory and learning might be modulated in ways that would support an interpretation based on the secondary actions of protein synthesis inhibitors.

Male and female HS/Ibg mice were trained in a T-maze, with footshock motivation, on a position task. Training continued until the subjects attained a criterion of 5/6 correct responses, or a maximum of 15 trials. One hr after initial training subjects were given 10 trials to the opposite maze arm, and 22 hrs later were retention tested over a block of 10 trials. A 5-min inter-trial interval was used throughout. With respect to drug manipulation individual groups were administered cycloheximide, anisomycin, or the vehicle 30 min prior to initial training, reversal training, or retention testing. All other agents (oxotremorine, clonidine, isoproterenol, and scopolamine) were administered 15 min prior to reversal training.

Neither anisomycin nor cycloheximide had any effect on acquisition of the initial problem. Both agents produced a deficit in acquisition of the reversal problem, which in general was also reflected during retention testing. A sex-dependent protection of the disrupting effects of cycloheximide was observed when noradrenergic agonists were given in conjunction with cycloheximine. Oxotremorine disrupted performance when given with cycloheximide. Scopolamine in conjunction with cycloheximide tended to attenuate the disruption of behavior.

These findings demonstrate that cholinergic and noradrenergic agents can modulate the disrupting effect that cycloheximide has on learning. With respect to the aspect of learning and memory that is impaired, it is clear that cycloheximide did not affect acquisition of the initial problem but did affect the acquisition and retention of the reversal problem. These results suggest that cycloheximide either induced perseveration of the initial behavior or enhanced proactive interference. Therefore, cycloheximide may influence both learning and memory. [Supported by NIMH Grant MH 11167 and NIGMS Grant GM 07305.]

1048 LEFT VISUAL FIELD IDENTIFICATION AND BILATERAL CROSS-INTEGRATION IN SPLIT-BRAIN HUMANS. Larry E. Johnson* (SPON: James Bonner). Division of Biology, California Institute of Technology, Pasadena, CA 91125.

Split-brain humans can verbally categorize left visual field (LVF) stimuli and cross-compare visual stimuli presented simultaneously in the LVF and RVF. This contrasts with the theory of 'complete disconnection' in which communication between the two cerebral hemispheres following commissurotomy is severely limited.

All the split-brain subjects tested (4), when required to distinguish tachistoscopically presented letters and numbers from patterns, or identify single digit numbers as greater or less than '5', or odd-even (choice discriminations), can do so very accurately in both visual fields using either manual or verbal responses. And, like normal or partially split controls, they also respond as fast to stimuli in one field as the other. When asked to name stimuli, however, LVF accuracy varies between subjects and differs for various stimuli and sample sizes. Yet, in spite of these individual differences and in contrast with normals and a partial split, all of the split-brain subjects are alike in that they require significantly longer to name, whether correctly or incorrectly, LVF as compared with RVF stimuli.

When stimuli (colors, patterns, numbers, or letters) are presented simultaneously to the two visual fields, the ability to manually or verbally categorize two stimuli as same or different seems to be dissociated from the ability to name the stimuli.

There thus appears to be several mechanisms or pathways by which visual information can pass between the hemispheres of a split-brain subject: one which handles choice discrimination tasks very efficiently, one which operates when stimuli are to be named (but usually permits accurate LVF responses only in the youngest subjects), and one which allows bilateral stimuli to be compared as same or different. There is also evidence that some of these mechanisms may interfere with each other when tasks are combined.

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1049 A QUANTITATIVE REGIONAL ANALYSIS OF PROTEIN SYNTHESIS FOLLOWING CYCLOHEXIMIDE INJECTIONS INTO THE RAT BRAIN: POSSIBLE UTILITY FOR THE STUDY OF MEMORY. Raymond P. Kesner and Lester M. Partlow. Depts. Psychol. and Pharm., Univ. of Utah, Salt Lake City, UT 84112.

Previous work has shown that bilateral injection of 10 or 20 µg of cycloheximide (CHX) into the amygdala, but not into the internal capsule, resulted in a time-dependent disruption of long-term retention of passive avoidance training, even though total brain protein synthesis was inhibited by less than 6%. In the present autoradiographic study, rats were subcutaneously injected with L-[methyl-^{14}C]-methionine following intracranial administration of CHX via implanted cannulas in either the amygdala or internal capsule. Autoradiograms from different brain levels were analyzed by use of an image-analyzing computer to quantitate the regional inhibition of protein synthesis. Control experiments showed that the image analyzer produced accurate optical density measurements and that these values were linearly related to the amount of radioactivity. Data indicate that the results obtained with this new histoanalytic technique do not differ from traditional biochemical techniques and the degree of inhibition of protein synthesis within the amygdala, internal capsule, caudate, cortex, hippocampus, thalamus, hypothalamus, and half brain following 20 µg CHX injection into the amygdala is both replicable and internally consistent. Furthermore, a dose-dependent relationship exists between degree of inhibition and amount of CHX injected. In addition, following amygdaloid injections of 20 µg CHX, a profound inhibition of protein synthesis (~60%) is found within the amygdala and internal capsule, while similar injections into the internal capsule result in marked inhibition in the internal capsule, but very little, if any, inhibition in the subjacent amygdala. In general, this new histoanalytic technique provides for a new quantitative approach for analyzing autoradiograms. The technique appears to be accurate, reliable and can be applied to the study of behavioral functions. More specifically, we will suggest that based on autoradiographic and behavioral data amygdaloid injection of CHX impairs memory by virtue of its action upon amygdala function.

1050 FACILITATION OF EYE-BLINK CONDITIONING BY HYPOTHALAMIC STIMULATION. H-J. Kim and C.D. Woody. UCLA Medical Center, Los Angeles CA. 90024.

More than 1000 pairings of click (CS) and glabella tap (US) may be needed to produce stable, short-latency conditioned eye blink responses in the cat (Woody et al. J. Neurophysiol. 1974). Some time ago Murphy and Gellhorn (J. Neurophysiol. 1945) found that hypothalamic stimulation (HS) could facilitate the production of electrocortically-elicited movements in an "enduring" manner. Voronin (Proc. Intl. Union Physiol. Sci. 1974) reported that adding HS to an auditory CS and US consisting of electrical stimulation of the motor cortex resulted in rapid acquisition of a short-latency conditioned startle response. He has suggested that the effect is associative.

We report the following preliminary results of pairing click CS with glabella tap (ISI, 340 ms) and HS (240 ms after tap). A hiss of intensity comparable to click CS was also presented 4 sec after HS as a discriminative stimulus (DS).

1. CRs emerged within 30-50 pairings or less.
2. The onset latencies of the major blink responses to CS, measured electromyographically, ranged between 80 and 320 msec.
3. CRs were extinguished when click and hiss were presented alone.
4. Responses elicited by the DS were smaller and less frequently observed than those elicited by the CS. Initially, before any associative pairing, subliminal myographic activity was greater to DS than to CS.
5. CRs were also smaller and less frequently observed when delivery of HS was moved to before click CS, tap, and hiss DS.
6. The effect of HS was location-dependent within the hypothalamus and was not seen with stimulation of the adjacent optic chiasm, which produced pronounced blinking.
7. Other effects were also produced including some sensitization, a facilitation of the response to glabella tap, and small, short-latency (onset: 15-50 msec) responses to click and frequently to hiss.

Intracellular recordings obtained from awake cats during these procedures indicate that the major effects of HS on the activity of cells in the motor cortex are: a) early excitation, b) late inhibition, and c) later excitation. (Supp. by AFOSR 76-3074 and NSF BNS 78-24146.)

1051 TIME DEPENDENT EFFECT OF POSTTRIAL AMYGDALOID LESIONS ON RETENTION OF AN INHIBITORY AVOIDANCE RESPONSE. K.C. Liang*, James L. McGaugh, Joe L. Martinez, Jr., Robert A. Jensen, and Beatriz J. Vasquez. Department of Psychobiology, School of Biological Sciences, University of California, Irvine, CA 92717, U.S.A.

There is considerable evidence that retention of learned responses is impaired by posttrial electrical stimulation of the amygdala. These findings suggest that the amygdala may be involved in modulating memory storage processes. An alternative possibility is that the amygdala is in some way involved in the permanent storage of memory. If the amygdala is involved primarily in modulating storage, then the impairing effect of a lesion given after training should decrease as the time between training and the lesion is increased. To investigate this issue, bipolar electrodes were implanted bilaterally into the amygdalae of male ARS Sprague-Dawley rats (60 days old). At least 8 days following surgery, the animals were trained on an inhibitory avoidance task using a 2 mA, 2 sec footshock, and tested after one of three retention intervals: 4 days, 7 days, 12 days. Five groups tested at each retention interval: unimplanted control (UC), implanted control (IC), pretraining lesion (IL), and delayed posttraining lesion (DL) (2 days before testing). The time between the training experience and delayed posttraining lesion was 2 days, 5 days, or 10 days for the various training retention intervals used. Control animals from these 5 groups were trained without footshock and tested at the 12 day retention interval. Bilateral amygdaloid lesions were made with radio frequency current (1.5-2.0 mA, 30 sec). Histological results showed the lesion area included primarily the lateral, basolateral, and basomedial nuclei as well as part of the pyriform cortex. As is shown in the table, a retention deficit occurred only when the amygdala was lesioned prior to, or within 2 days, following training. This time-dependency supports the notion that the amygdala is involved in memory modulation rather than as a site of memory storage.

Median Retention Latency (sec)

Retention Interval	UC	IC	PL	IL	DL
4 days	600[a]	417.4	56.9[a]	17.6[a]	42.5[b]
7 days	600[b]	185.9	8.9[a,c]	6.7[a,c]	59.6
12 days	600[a]	100.5	11.7[a,c]	9.8[b,c]	80.6
12 days no shock	10.5	2.3	2.3	2.7	2.4

a: $P < .02$ different from IC; b: $P < .05$ different from IC; c: $P < .02$ different from DL.

Supported by UPHS grants MH 12526, AG 00538; BNS 76-17370 and a grant from the McKnight Foundation (all to JLMcG).

1052 DISRUPTION OF RABBIT HIPPOCAMPAL ACTIVITY AND CONDITIONED BEHAVIOR FOLLOWING ADMINISTRATION OF DELTA-9-THC. Laura A. Mamounas*, Donald J. Weisz*, Stephen D. Berry and Richard F. Thompson (Dept. Psychobio., UCI, Irvine, CA 92717).

Previous studies have shown strong correlations between hippocampal activity and classical conditioning of the nictitating membrane (NM) response in rabbit. Hippocampal physiology is known to be altered following administration of delta-9-tetrahydrocannabinol (THC), the major psychoactive constituent of marihuana. To examine further the relationship between hippocampal activity and NM conditioning, acquisition and performance of the NM response were correlated with hippocampal EEG and multiple unit activity (MUA) following i.v. administration of THC.

Microelectrodes were implanted bilaterally in the dorsal CA1 region in rabbit hippocampus. MUA and EEG were recorded prior to, during, and following the conditioning paradigm in which a tone CS was paired with a corneal air puff UCS to evoke NM extension. Daily, 117 trials with a 60 sec average ITI and 250 msec ISI were presented. Samples of hippocampal activity were recorded prior to and 10, 60 and 130 min following injection of drug vehicle, 0.5 mg/kg THC or 1.0 mg/kg THC. Frequency and amplitude of EEG waves were analyzed using a zero crossing program. The MUA from hippocampus will also be analyzed and will be discussed in relation to EEG and behavior.

Behavioral analyses indicated that there was a significant increase ($p < .05$) in trials to criterion (8 conditioned responses (CR) out of 9 consecutive trials) for animals in the 1 mg/kg THC group. In addition to the behavioral effects there was a significant shift ($p < .01$) in EEG frequencies. For the 10 min and 60 min post-drug samples there were shifts from pre-drug values of 4-8 Hz waves to both lower (0-4) and higher (12-22) frequencies. By 130 min post-drug, the EEG frequencies were returning to pre-drug levels. In a second experiment, administration of 1 mg/kg THC abolished CRs in animals conditioned in a non-drug state. The drug effect on CRs persisted for almost the entire conditioning session the first day of THC administration and for approximately one-half the session on the second day. By the third day only a slight effect on the presence of CRs was observed.

The concomitant impairment of hippocampal activity and NM conditioning following THC administration is consistent with findings of hippocampal involvement in learning.

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1053 DIAZEPAM IMPAIRMENT OF DELAYED-RESPONSE PERFORMANCE IN YOUNG AND OLD RHESUS MONKEYS. John G. Marriott, Joanne S. Abelson* and Raymond T. Bartus. Pharmacology Department, Warner-Lambert/Parke-Davis Research Laboratories, Ann Arbor, MI 48105.

Benzodiazepines have been found to impair short-term memory (STM) in humans. The effects of diazepam on a non-human primate model of STM were examined to explore further the amnestic effects reported in humans. Both young and aged subjects were studied to test for possible age differences in response to diazepam treatment.

In Experiment I, five young rhesus monkeys (5-8 years, 4.5-5.1 kg) were tested on an indirect delayed-response task. The Automated General Experimental Device (AGED) was used to measure the animals' ability to recall the spatial position of a stimulus presented prior to retention intervals of various durations. Two retention conditions were used: a 0-sec, non-memory control condition; and a 60 sec, STM-dependent condition. Diazepam, at doses of 2.5, 5.0, and 10.0 mg/kg administered orally 30 min prior to behavioral testing, produced a dose-related decrease in delayed response accuracy only at the 60 sec, memory-dependent retention interval (drug X condition interaction, $p<.01$). However, no impairment was found on the 0-sec control condition, indicating that memory per se was affected by the drug and not non-specific, performance aspects of the task.

In Experiment II, six aged rhesus monkeys (18+ years, 4.2-5.6 kg) were tested on the same task using the same apparatus as in Experiment I. However, three retention conditions were used: a 0-sec, control condition; a 'short' delay, adjusted for each individual animal to yield a control performance level of 60-80% correct (range = 10-30 sec); and a 'long' delay, yielding a 40-60% correct performance level (range = 20-60 sec). Diazepam (1.25 and 2.5 mg/kg) administered 30 min prior to testing produced declines in performance only on the two memory conditions ($p<.01$). These effects are similar to those found in young animals, although more severe. Again, the amount of memory impairment was dose-dependent with higher doses of diazepam producing greater disruption of delayed-response performance.

Thus, the short-term memory impairments produced by diazepam in these studies on young and old monkeys appeared similar to those reported in humans. They also correspond to the well-known impairments of STM produced by the administration of anticholinergic drugs to animals and humans. Since diazepam is reported to block the release of acetylcholine in the brain, the amnestic effects of diazepam may be due to its effects on the cholinergic system. Benzodiazepine-induced impairments of STM may provide a useful model of memory impairments commonly associated with human brain disorders.

1054 ADRENAL MEDULLARY CATECHOLAMINES ARE NECESSARY FOR AMPHETAMINE-INDUCED ENHANCEMENT OF LEARNING IN RATS. Joe L. Martinez, Jr., Beatriz J. Vasquez, Robert A. Jensen, Rita B. Messing, Henk Rigter*, K.C. Liang*, and James L. McGaugh. Department of Psychobiology, School of Biological Sciences, University of California, Irvine, CA 92717, U.S.A.

We investigated, (1) the effects of 4-OH amphetamine (4-OH AMPH) a drug with limited ability to cross the blood brain barrier, on retention of an inhibitory avoidance task, (2) the effects of 4-OH AMPH on retention in rats sympathectomized with 6-hydroxydopamine (6-OHDA), (3) the learning ability of sham operated and adrenal demedullated (ADXM) rats, and (4) the effects of 4-OH AMPH and d-amphetamine (AMPH) on retention in ADXM rats.

In all experiments male ARS Sprague-Dawley rats (90 days old) were trained in a one trial inhibitory avoidance task with a 72 hr training-testing interval. In the first experiment rats received either SAL, .05, .21, .82, 3.3, 6.5, or 13.1 mg/kg (i.p.) 4-OH AMPH immediately following training, and were trained using a 500 μA, 1 sec footshock. A .82 mg/kg dose significantly facilitated retention performance ($p < .01$). In the second experiment rats were pretreated with 100 mg/kg 6-OHDA (i.v.) 24 hrs prior to training. Immediately following training they received either SAL, .05, .21, .82, or 3.3 mg/kg 4-OH AMPH. Two footshock levels, 500 μA or 750 μA were used. Pretreatment with 6-OHDA made the rats more sensitive to the effects of 4-OH AMPH. At the 750 μA footshock, a dose of .21 mg/kg 4-OH AMPH enhances retention ($p < .05$). Thus, 4-OH AMPH enhances retention performance and this effect is not dependent upon the integrity of the sympathetic nervous system.

Other experiments addressed the question of whether adrenal catecholamines are necessary for the action of 4-OH AMPH on memory. In one study, learning characteristics of SHAM operated and ADXM rats was studied. The operations were performed 7 days before training. A 500 μA and 750 μA footshock was used. In these conditions, retention performance of SHAM and ADXM rats was not different. In the final experiment ADXM rats were given either 4-OH AMPH (SAL, .21, .82, or 3.3 mg/kg) or AMPH (SAL, .25, 1, or 4 mg/kg) immediately following training. ADXM abolished the facilitatory effects of both 4-OH AMPH and AMPH, suggesting that adrenal catecholamines are necessary for the enhancement of retention. Further, 4-OH AMPH and AMPH enhance memory processes, in this particular task, at equimolar doses (Neurosci. Abs. 1978, 4, 261), even though only AMPH readily crosses the blood brain barrier.

[Suported by UPHS grants MH 12526, AG 00538; BNS 76-17370 and a grant from the McKnight Foundation (all to JLMcG). We thank Tracy Hannan for her technical assistance].

1055 PLASMA CATECHOLAMINE RESPONSES TO TRAINING AND POSTTRIAL MEMORY MODULATORS. Richard McCarty and Paul E. Gold. Dept. Psych., Univ. Virginia, Charlottesville, VA 22901.

Several recent reports suggest that the sympathoadrenal medullary system may play an important role in modulating memory processing and in mediating the effects of various treatments on memory. These findings led us to examine plasma catecholamine responses to several treatments known to modulate memory processes.

In the first experiment, male Sprague-Dawley rats were trained in a one-trial inhibitory avoidance task (3 ma, 2 sec FS). Five sec after training, rats received 5 ma, 1 sec electrical stimulation of frontal cortex (FCX) through chronically implanted cortical screw electrodes. This stimulation is sufficient to produce widespread brain seizures and retrograde amensia. Other groups included home cage controls, rats exposed to the training apparatus without footshock (FS) or FCX, and rats that received FCX only. Plasma samples (0.5 ml) were obtained from previously implanted tail artery catheters immediately and 5, 10, 20, and 40 min after the various treatments. Plasma levels of EPI and NE were determined using a COMT radiometric-thin layer chromatographic procedure.

The results indicated that FS or FS + FCX resulted in an approximately 10-fold increase in plasma EPI immediately after the treatment. FCX alone did not result in an increase in plasma EPI concentration above that of non-footshocked or non-stimulated animals. The concentrations of EPI for all groups returned to basal values within 40 minutes. A similar pattern, but of lesser magnitude, was observed for plasma levels of NE.

In a second experiment (without training), posttraining injections of ACTH (0.3 or 3.0 I.U./rat, s.c.) or an ACTH analog (Organon 2766: 125 or 250 mg/kg) failed to alter either plasma EPI or NE concentrations. An EPI injection (s.c.) at a dose (0.1 mg/kg) that enhances later retention performance resulted in an increase within 5 min in plasma EPI concentrations from 230 pg/ml to approximately 1000 pg/ml that was maintained for 40 min. A higher dose (0.5 mg/kg) that can produce retrograde amnesia resulted in plasma EPI concentrations of approximately 4000 pg/mg during the 40 min after injection. Plasma NE concentrations did not change following the injection.

These results indicate that peripheral adrenergic responses do occur with training. Furthermore, an injection of EPI at a dose that enhances retention performance mimics the extent of the endogenous responses to a training FS. However, other treatments that modulate retention (i.e., FCX or ACTH) may not involve sympathoadrenal medullary activity.

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1056 MEMORY, TESTABILITY & AFFECT IN LATE-ONSET DEMENTIA. Miller, N.E. Ctr. for Studies of the MH of the Aging, NIMH, Rockville, MD.

Behavioral measures of memory are important tools in the assessment of treatment efficacy in senile dementia. Yet clinical researchers often report that aged patients with altered brain function are untestable on both standard and newer experimental tests of memory. As part of a continuing investigation of memory impairment in late life psychiatric disorders, a series of information processing tests were administered to subjects positive for diffuse, chronic, organic brain disease. 11 Ss with altered brain function and 17 controls aged 50 and up were tested on a choice reaction time memory scanning task derived from Sternberg. Digit strings ranging in length from 1 to 6 digits were displayed in a sequential format for 3 seconds, followed by a 2 second delay. A single digit was then presented and the S was to decide whether the digit had appeared in the preceding sequence. Despite the presence of a 2 second delay and a warning buzzer, demented Ss appeared to have difficulty distinguishing where the stimulus set ended and the test probe began. They manifested a random level of performance, coupled with signs of fluctuating arousal, labile attention, psychomotor restlessness, impaired object constancy (& catastrophic anxiety in a few cases). It was not possible to determine whether the Ss had failed to understand the nature of the task or whether their primary memory abilities were impaired to such an extent that they could not maintain the stimuli in mind even briefly. Accordingly, systematic modifications were introduced to simplify the nature of the experimental task & to provide for the emotional security of respondents. 35 dements & 33 age-matched controls were tested on a modified scanning task in which the memory set was presented in a simultaneous horizontal array so that distinctions between stimuli & probe were highlighted. Anxiety & restlessness were specifically reduced by having the examiner remain in close physical & visual proximity to the S & by providing immediate verbal feedback regarding response accuracy following the completion of each trial. Performance of Ss with altered brain function was found to be significantly better than chance when both the affective parameters & simplification of experimental task were presented concomitantly. Neither modification alone resulted in improvement. These findings suggest that by lowering anxiety & restlessness, providing for object constancy & the reward aspects of reinforcement & ensuring that level of task difficulty is appropriate, aspects of the test environment that have a deleterious effect on performance are reduced. Once testability was possible, the results revealed serious deterioration in primary memory with senile dementia.

1057 DEGREE OF MEMORY IMPAIRMENT IN MONKEYS RELATED TO AMOUNT OF CONJOINT DAMAGE TO AMYGDALOID AND HIPPOCAMPAL SYSTEMS. Mortimer Mishkin and Richard C. Saunders*. Lab. Neuropsychology, NIMH, Bethesda, MD 20205.

Complete destruction of both the amygdala and hippocampus in monkeys yields a memory impairment resembling the clinical syndrome of global anterograde amnesia (Mishkin, Nature 273: 297, 1978; Spiegler & Mishkin, Neurosci. Abstr. 5: 1979). Two new experiments show that the effect of the combined removal is not all-or-none, but is graded in relation to the amount of conjoint damage to the two systems.

In both experiments, rhesus monkeys were trained preoperatively on a one-trial visual recognition task requiring memory of single objects for 10 seconds each, retrained on this task postoperatively, and then given a performance test in which their one-trial recognition ability was taxed with longer delays (up to 2 minutes) and longer list lengths (up to 10 objects). Their average scores on this performance test were compared with those of previously studied groups that had been given either separate amygdaloid and hippocampal removals (average score, 90%) or combined removals (average score, 60%).

In the first experiment, bilateral amygdalectomy was combined with unilateral hippocampectomy, and vice versa. Both groups obtained average scores near 75%, midway between those of the comparison groups. The results support the view that the amygdala and hippocampus are equally important for recognition memory and that there is a quantitative relationship between the amount of damage to the two structures and impairment of this function. The same conclusion could apply to man (cf. Penfield & Mathieson, Arch. Neurol. 31: 145, 1974).

In the second experiment, bilateral amygdalectomy was combined with bilateral transection of the fornix, a major hippocampal-diencephalic pathway; and bilateral hippocampectomy was combined with bilateral transection of the stria terminalis, a major amygdaloid-diencephalic pathway. Again both groups obtained average scores near 75%. These results point not only to joint participation by the amygdala and hippocampus in short-term memory, but to a wider, limbic-diencephalic circuit underlying this process (cf. Gaffan, JCPP 86: 1100, 1974).

1058 SCOPOLAMINE EFFECTS ON LEARNING AND RETENTION PERFORMANCE IN RATS. D. E. Moss, J. B. Rogers, P. L. Peck*, R. R. Salome*, and E. Hall*. Department of Psychology, University of Texas at El Paso, El Paso, Texas 79968.

Rats injected with 2 mg/kg scopolamine HBr i.p. 20 min before training on a Y-maze brightness discrimination task learned the task to a criterion of 10/10 in an average of 54 trials while placebo injected controls showed nearly identical learning with an average of 49 trials. Rats injected with scopolamine HBr before training, however, showed amnesia when retention tested without any drug or 20 min after an i.p. injection of 0.5 mg/kg physostigmine salicylate at either 1 day ($p<.05$) or 2 weeks ($p<.01$) after training. In contrast to these results, however, animals trained after 2 mg/kg scopolamine HBr and retention tested at either 4 or 6 weeks after training showed good retention when tested either without any drug or after 0.5 mg/kg physostigmine salicylate. A factorial analysis of variance for the 3 drug conditions (control, scopolamine before training only, and scopolamine before training and physostigmine before the retention test) and the 5 retention test intervals (1 day, 2, 4, 6, or 9 weeks) showed the interaction between drug condition and time was highly significant ($p<.001$). The occurrence of amnesia after training under one drug condition (scopolamine) and retention testing under another drug condition (either no drug or an antagonistic drug, physostigmine) is consistent with a "state dependent" explanation of amnesia. The reappearance of memory at the longer time intervals (4 to 6 weeks after training) with retention testing conducted under the same altered drug states, however, is not consistent with a "state dependence" explanation of amnesia. Insofar as amnesia was not produced by pretraining injections of methscopolamine nitrate or injections of scopolamine HBr immediately after training, the observed temporary scopolamine-induced anterograde amnesia was attributed to central nervous system effects of scopolamine that occurred during the learning process itself. These results support the idea that the learning process initiates a time-dependent change in a brain neurotransmitter system, probably the cholinergic system, that is necessary for memory recall. In addition, these results suggest that the function of the neurotransmitter system must be within some optimum limits for normal memory function and the presence of scopolamine in the brain during training seems to change the formation of the memory trace so that it is temporarily outside of the optimum limits and, therefore, memory cannot be recalled for a period of time after training.

1059 ENHANCEMENT OF 24-HOUR RETENTION IN PREWEANLING RATS BY POST-TRAINING HORMONE ADMINISTRATION. James M. Murphy, Susan G. Cooley*, and Paul E. Gold. Dept. of Psychology, Gilmer Hall, Univ. of Virginia, Charlottesville, VA 22901

Immature animals have poorer long-term memory than adults, and the rate of forgetting consistently decreases during early development. Recent evidence indicates that adult memory can be modulated (i.e., enhanced or impaired) by a number of posttraining treatments, including peripheral injections of some hormones such as epinephrine (E), which may mimic the endogenous release of E that occurs with more intense training. Because immature animals may not respond to stressful stimuli with an adult-like release of various hormones, the present study examined the possibility that the poor memory of young rats could be enhanced by post-training injections of agents previously demonstrated to facilitate memory in adults.

Rats 20 days of age and older demonstrated reliable 1- and 24-hr retention after training on a one-trial, shock-motivated (.6 mA/.8 sec) inhibitory-avoidance task, whereas 16 day olds retained the task for 1 but not 24 hr, and 14-day-old rats failed to show reliable retention at either test interval. We next examined the effects of several posttrial treatments on 24-hr retention performance of 16 day olds. Separate groups received subcutaneous injections immediately after training. Saline controls did not evidence 24-hr retention and were comparable to untreated 16 day olds. Posttrial E enhanced retention at .1 and 1 mg/kg, whereas groups receiving lower and higher doses (.01, .05, and 2 mg/kg) did not differ from the saline group and E no-shock controls. Norepinephrine (NE) also enhanced retention at 1 mg/kg but not at lower doses. These results concur with previous findings in adult rats that posttrial E and NE can facilitate memory of an inhibitory-avoidance task. However, ACTH and amphetamine also enhance retention in adults but failed to alleviate the poor memory of 16 day olds. This suggests that some memory modulatory systems may mature at different rates.

We had previously observed that transient posttraining decreases in forebrain NE concentrations in adults proved a reliable index of training-related stress and an accurate predictor of retention performance. In the present study, assay for post-training NE in 16 day olds indicated an adult-like response to the stress of footshock. In contrast to the adult findings, NE levels failed to differentiate between training conditions that predicted poor (footshock alone) and good (footshock + E) memory. These results suggest that this brain NE response occurs independent of 24-hr retention capacities in immature rats.

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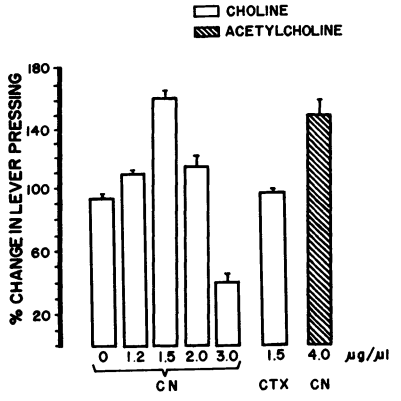
1060 EVIDENCE THAT HIRANO BODIES IN HUMAN BRAIN ARE PARACRYSTALLINE RIBOSOMES. L.V. O'Brien*, K. Shelley*, J. Towfighi* and A. McPherson* (SPON: S.H. Miller). Departments of Biological Chemistry and Pathology, The Milton S. Hershey Medical Center, Pennsylvania State University, Hershey, PA 17033.

Hirano bodies appear in certain forms of presenile and senile dementia such as Pick's and Alzheimer's diseases. They are spindle-shaped highly ordered inclusion bodies, 4 μm to 30 μm, that occur predominantly in the pyramidal layer of the hippocampus, particularly Sommer's sector area, and are associated with both neurons and glial cells. Ultrastructurally they appear as paracrystalline arrays of alternating filaments and electron dense particles occurring in the perikaryon adjacent to the nucleus. These particles have a highly polar distribution within the bodies with respect to the filaments. Orientations have been obtained that show the bodies to be composed of sheets of electron dense particles disposed in a two dimensional rhombic lattice with translations of about 130 Å. The size, distribution, staining characteristics and other morphological features suggest a striking similarity to membrane-bound ribosomes and we believe the Hirano bodies represent a crystalline form of rough endoplasmic reticulum. An Optronics high speed microdensitometer interfaced to a PDP11/40 computer was used to convert periodic images in electron micrographs to digitized data arrays which were subsequently analyzed by sequential Fourier transform methods. A digitally filtered and averaged image of the electron dense particle was produced. This image is virtually identical to that obtained for the 60s subunit of ribosomal particle from rat liver and shows its distinctive asymmetric "skiff" shape. Histochemical analysis of formalin fixed, paraffin embedded autopsy samples included specific staining for nucleic acids. Positive results were obtained with toluidine blue, ethidium bromide, and acridine orange. Concomitant light and fluorescence microscopy shows that the Hirano body binds acridine orange and fluoresces with a red component as expected for RNA; nuclei fluoresce yellow-green which is characteristic of DNA. Nissl substance in adjacent cells also fluoresces red. Negative results were obtained when the bodies were stained for acid mucopolysaccharides with colloidal iron and alcian blue. In conclusion, evidence is presented that Hirano bodies are ordered, crystalline arrays of membrane-bound ribosomes. The hippocampal region is associated with the consolidation of short term to long term memory and the predominant symptom of senile dementia is the impaired capacity to consolidate new memory. Thus the possibility arises that these bodies represent a form of ribosomal storage and the ribosomes are not available for the synthesis of memory associated proteins.

1061 CHOLINERGIC STIMULATION OF THE CAUDATE NUCLEUS AND OPERANT BEHAVIOR, IN CATS. Roberto A. Prado-Alcalá and Guillermo G. Cobos-Zapiaín*. Dept. Physiol., Sch. Med., Natnl. Univ. of México, P.O. Box 70250, México 20, D.F., México.

To test the hypothesis that cholinergic activity of the head of the caudate nucleus (CN) is involved in the processes underlying instrumental performance, the effects of microinjections of several doses of choline into this structure on lever pressing behavior (CRF schedule) were assessed. A dose dependent modification of performance was found: small doses improve while large doses impair lever pressing; choline applications into the parietal cortex were without effect. The facilitatory effects were reproduced by microinjections of acetylcholine into the CN. These data further support our working hypothesis.

Figure 1.- Bilateral microinjections (5 μl) were performed 10 min before experimental sessions. Results are expressed as % change relative to control sessions. CN, caudate nucleus, CTX, parietal cortex.



1062 FACILITATION OR DISRUPTION OF MEMORY BY ELECTROCONVULSIVE SHOCK (ECS): THE ROLE OF THE LOCUS COERULEUS (LC). Lia Prado de Carvalho*, Patricia J. Kubanis*, Steven F. Zornetzer. Dept. Neuroscience, Coll. of Med., University of Fla., Gainesville, FL 32610.

The role of the LC in complex behavior remains elusive. Recent studies from our laboratory reported that unilateral, but not bilateral, post-training LC lesions resulted in a dramatic (7 days) extention of the susceptibility period of recent memory to ECS-produced retrograde (RA) amnesia. The present study extends the analyses of the role of the LC in memory by investigating the effect of pre-training LC lesions on the consequences of ECS upon memory.

Male swiss mice (n=291) received small electrolytic lesions (500 uA anodal current for 10 sec.) delivered through twisted nichrome wire electrodes (125 um). Either 2 or 14 days after lesions, mice were trained in a single trial inhibitory avoidance task (FS 300 uA) and immediately returned to their home cages. After either a 24 hr or 14 day post-training period, transcorneal ECS or sham ECS (15.0 mA for 200 ms) was administered. All mice were tested for retention of the inhibitory avoidance response 24 hrs after ECS.

The results indicate that in all cases unilateral LC damage results in an extended susceptibility to ECS-produced RA. These results are compatible with, and extend, previous data from our laboratory using post-training lesions.

The most interesting result from this experiment is that ECS resulted, paradoxically, in facilitation of performance ($p = .04$) in mice receiving bilateral, but not unilateral, LC damage 28 days prior to ECS. Bilaterally, but not unilaterally lesioned animals not receiving ECS, had accelerated forgetting when compared to control lesioned mice ($p = .01$). This facilitated performance in bilaterally lesioned mice receiving ECS appears to result from the stimulation of the residual memory remaining after 14 days of accelerated forgetting in similarly-lesioned mice not given ECS. Control lesioned mice, either with or without delayed ECS, showed good retention. These data will be discussed in terms of the possible dynamic changes occuring in neurotransmitter systems mediating memory storage processes, and the role of both the LC and of ECS in altering these dynamic changes.

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1063 AMYGDALOID MULTIPLE UNIT ACTIVITY DURING CLASSICAL CONDITIONING IN RABBITS. Russell T. Richardson* and Richard F. Thompson. (SPON: Pauline I. Yahr) Dept. of Psychobio., Univ. of Calif., Irvine, CA 92717.

Experimental manipulations of the amygdala can alter learning processes. For example, Kapp et al. (Neurosci. Abst. 3:236, 1977) have shown that lesions of the amygdala can retard conditioning of the nictitating membrane reflex in rabbits. To further investigate the role of the amygdala in learning, we recorded multiple unit activity bilaterally from the amygdalae of New Zealand white rabbits during classical conditioning of the nictitating membrane reflex. The training consisted of a 1 KHz tone (CS) presented 250 msec prior to a 100 msec puff of air to the cornea (US). One group of animals received two days of random presentations of the CS and US prior to two days of paired presentations. The other group received only two days of paired training. Multiple unit counts were made for 250 msec prior to CS onset (PreCS period), 250 msec after CS onset (CS period), and 250 msec after US onset (US period). All CS and US period counts were expressed as percent increases over the preceding PreCS period count.

The multiple unit activity during paired stimulus presentations showed a strong increase (121%) in the US period. However, this increase was not significantly different from that seen during unpaired stimulus presentations, and it did not show consistent changes as animals acquired the conditioned response. There was an average increase of 34% in activity during the CS period on paired training days, but, again, this response was not significantly different from the response on unpaired training days. Several cell populations showed a significant increase in firing in the CS period on both paired training days, and most of these populations were located in or adjacent to the cortical nucleus of the amygdala. Activity during the PreCS period showed a highly significant ($p<.001$) increase in firing over the training session on both paired and unpaired days. The CS and US period counts also increased over the training days, but because they were assessed relative to the PreCS counts, their activity showed no change over the day.

These findings indicate that, in contrast to the hippocampus, (Berger & Thompson, Brain Res. 145: 323, 1978), multiple unit activity in the amygdala does not display changes which can be strongly correlated with acquisition of the conditioned response.

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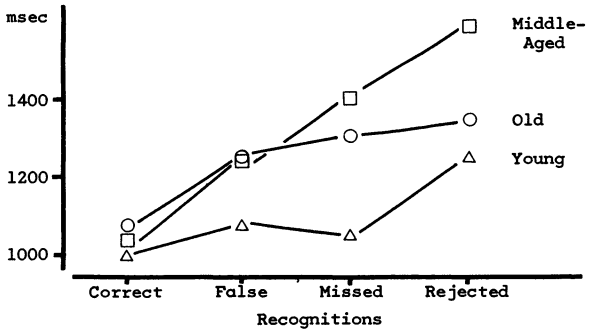
1064 RESPONSE DELAY WITH INCORRECT DECISIONS IN NONVERBAL RECOGNITION MEMORY. Walter H. Riege and Howard F. Wallach.* VA Med. Center Sepulveda & Dept. Psychiatry, Sch. Med., UCLA, CA 91343

It is commonly observed that it takes longer to recall verbal information as a person grows older. There is a reduction in processing effort which may be related either to the rate of scanning of items in memory, to the verbal memory load, or to the decision making (Fozard & Poon, 1978). We hypothesized that the perceived correctness or incorrectness of a recognition response influence decision times and that these would be age dependent.

Young, Middle-Aged, and Old persons (N = 57) were asked to answer as quickly as possible during recognition of 10 visual nonverbal designs recurring randomly in a series of 40. From unweighted means and signal detection analyses we observed that age groups differed in the decision criteria adopted for recurrent recognition ($F = 3.89$, df 2, 54; $p < 0.05$) but not in response times. Regardless of age, however, the response times differed significantly among correct, false, missed and rejected recognitions ($F = 14.00$; df 3, 54; $p < 0.01$). More specifically, the average time for correct recognitions was smaller ($p < 0.01$) than that for false or for missed recognitions. The results argue for a response delay of approximately 100 msec independent of age when nonverbal items fail to meet the parity check for recognition.



AVERAGE RESPONSE TIMES FOR RECURRENT RECOGNITION

1065 POST-STIMULATION ADMINISTRATION OF EITHER ACTH OR CORTISONE HAS INFLUENCES ON TIME-DEPENDENT PROCESSES UNDERLYING KINDLING. Robert P. Rose* and Wagner H. Bridger. Depts. of Neurosciences and Psychiatry, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

"Kindling" is a progressively augmenting convulsive response to intermittent focal electrical stimulation of brain. As a form of neuronal plasticity, kindling may depend on similar underlying mechanisms as other plastic processes, including memory consolidation. Much recent work has pointed to an important modulatory role for hormones upon synaptic plasticity. We have reported that hypophysectomy leads to alterations in kindling rate. The present study examines the effects of ACTH and cortisone on kindling of intact animals.

Male albino rats bearing implanted depth electrodes in basolateral amygdala of one side were subjected to daily electrical stimulation (150 uA, 60 Hz., 1 msec. pulses, 2 sec. train duration). Polygraph recordings of cortical (via calvarium screws) and amygdala activity were made before and after stimulation. After-discharge durations and all behavioral manifestations of seizure activity were noted. Daily stimulation was continued until animals manifested tonic-clonic convulsions on three successive days. Animals were given IP injections of ACTH (.03, .3, 1.0, or 3.0 IU/ animal), cortisone (2.5, 10 or 25 mg./ animal), or saline- either immediately or 4 hours after the seizure evoked by each daily stimulation had subsided. Animals were sacrificed after kindling was completed, and histology was performed (cresyl violet) to confirm electrode localization.

Administration of both ACTH and cortisone had significant dose-dependent influences on the rate of kindling in these animals- if given immediately after each stimulation. If the administration was delayed 4 hours, it no longer altered kindling rate. Therefore, it appears that these hormones can act in a retroactive manner upon processes initiated by the preceding stimulation in a way analogous to their action upon memory consolidation processes following training. These findings provide another example of hormonal modulatory influences upon neuronal plasticity, as well as supporting evidence for the strength of kindling as a model of processes underlying memory consolidation.

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1066 LONG TERM RETENTION DEFICITS IN RATS WITH LESIONS OF THE DORSAL HIPPOCAMPUS. Susan J. Sara and Michele David-Remacle. Michotte Ctr. Behav. Biol., U. Louvain, Pellenberg,Belgium.

Facilitation of a visually discriminated avoidance learning was observed in rats one week after they were lesioned in the dorsal hippocampus. While perseveration of a spatial strategy acquired during a pre-training session occurs in sham-operated and cortically lesioned controls, no such perseveration was observed in hippocampals. Elimination of the pre-surgical shaping session reduced the number of spatial strategies in all groups, but differences in trials to criterion and percentage of place strategies remained. Retesting animals 3 weeks after training showed that hippocampals and corticals had poorer retention than shams, while there were no differences in retention groups tested 24 h after training.

If the lesion is made four weeks prior to training, the same effect is seen on rate of acquisition and on perseveration of spatial strategies as when the lesion precedes the training by one week. Thus the behavioral differences seen after the 3 week training to test interval cannot be due to a recovery of hippocampal function.

A further study examined the effect of the lesion on long term retention of the discrimination acquired before surgery. It was found that there was no retention deficit under these conditions. The similarity between these results and the clinical hippocampal syndrome suggests that this preparation might be useful as an animal model for pharmacological studies aimed at alleviation of amnesia.

The nature of the memory deficit (e. g. increased rate of decay or retrieval dysfunction) remains to be investigated. Studies are presently under way to determine under what conditions this deficit can be reversed by increasing retrieval cues at retention testing or by using a reactivation procedure during the training to test interval.

1067 PARADOXICAL SLEEP AND MEMORY: LONG-TERM DISRUPTIVE EFFECTS OF ANISOMYCIN. Priyattam J. Shiromani, Baruch M. Gutwein* and William Fishbein. Psychobiology Lab., Dept. Psych., The City College of New York, New York, N.Y. 10031.

The effects of the protein synthesis inhibitor Anisomycin (ANI) on Paradoxical Sleep (PS or REM sleep), slow wave sleep (SWS), and retention of one-trial inhibitory avoidance training was examined in mice in three separate experiments. In experiment 1, mice injected with ANI 120 mg/kg and 210 mg/kg exhibited reduction of PS for 9 consecutive hours and ANI 40 mg/kg treated mice for 6 consecutive hours with no PS rebound in all three groups. ANI increased SWS commencing 3 hr. post-injection,continuing for 9 consecutive hr. and then returning to saline control levels. This effect was not dose-dependent. In experiment 2, part a, ANI 120 mg/kg and ANI 210 mg/kg but not ANI 40 mg/kg impaired retention measured 72 hr. after training. In experiment 2, part b, ANI 120 mg/kg and ANI 210 mg/kg induced amnesia from 3 to 9 hr. post-training but ANI 40 mg/kg was effective only from 3 to 6 hr. In experiment 3, the gradient of memory trace susceptibility to disruption by ECS was extended to 3 hr. post-training in mice given immediate post-training injections of ANI 40 mg/kg. ANI 20 mg/kg or ANI 10mg/kg alone or in combination with ECS was ineffective in extending lability of the memory trace. The results of this study indicate that PS in the 3 hr. period after aversively motivated training is not essential for memory processing. We suggest that memory stability and maintenance is dependent on PS occurring over a protracted time period.

1068 NEUROCHEMICAL EFFECTS OF AVERSIVE LEARNING IN RATS. D.O.Souza*, E.Elisabetsky*, R.D.Dias*, I.Izquierdo. Dept. Bioquímica, Inst. Biociências, UFRGS (centro), 90000-Porto Alegre, RS, Brasil.

Adult female Wistar rats were submitted to the following behavioral treatments in a shuttle-box: 1. pseudoconditioning (5-sec tones and 1 mA footshocks at random); 2. Pavlovian conditioning (tone-shock pairings on every trial regardless of responses made to the former); 3. avoidance without CS-US pairing (each tone followed at a randomly variable interval of 5 to 35 sec by a shock unless there was a shuttle response to the tone); 4. tones alone; 5. shocks alone. Inter-trial interval was 10-40 sec in all cases. Sessions were 5 or 25 min long. Two biochemical parameters were measured in hippocampus, caudate nucleus, rest-of-the-brain, and liver of these animals: a) in vivo incorporation of 32-P to non-histone acid-extractable chromosomal proteins (NAEP); b) in vitro incorporation of 14-C-leucine to total protein. Data were expressed as dpm/mg NAEP / dpm/mg total protein in the 32-P experiments, and as dpm/mg protein / dpm in total homogenate in the 14-C experiments. Statistics were by multiple-range analysis (Null hypothesis rejected at 1 or 5% level). There was an increased phosphorylation of hippocampal and caudate NAEPs after 5 min of Pavlovian or avoidance learning, and of NAEPS from the rest-of-brain fraction after 25 min of shocks alone. No change relative to intact controls was detected in the other groups. An increased incorporation of 14-C-leucine into hippocampal and caudate total proteins was observed after 25 min of avoidance training, 1 hr later in animals submitted to either tones or shocks alone, and 2 hr later in rats submitted to the Pavlovian procedure. These results argue against a specificity of the biochemical parameters examined in relation to learning or memory processes; but point out, instead, to a selective effect of the learning factors studied on their timing, and to a possible inhibitory influence of pseudoconditioning on their occurrence. The latter is of importance since pseudoconditioning has recently been shown to be an inseparable component of aversive learning, classical or instrumental. (Supported by CNPq, FAPERGS, and FAPESP, Brasil).

1069 ASSOCIATIVE MEMORY SEVERELY IMPAIRED BY COMBINED AMYGDALO-HIPPOCAMPAL REMOVALS. Brenda J. Spiegler* and Mortimer Mishkin (SPON: B. J. Richmond). Lab. Neuropsychology, NIMH, Bethesda, MD 20205.

Damage limited to the hippocampus of monkeys has failed to reproduce the profound anterograde amnesia that has been attributed to such damage in man. By contrast, combined damage to the monkey's hippocampus and amygdala does appear to reproduce the syndrome. A severe memory loss following combined amygdalo-hippocampal ablation was found recently on a visual recognition task in which the animal had to decide on the basis of a single previous experience whether a test object was familiar or novel (i.e., had previously been presented or not) (Mishkin, Nature 273: 297, 1978). The present study demonstrates that the impairment following the combined limbic lesion is not limited to such stimulus recognition memory, but extends to stimulus-reward associative memory as well.

The rhesus monkeys that served as subjects were part of an earlier study of associative memory (Spiegler & Mishkin, Neurosci. Abstr. 4: 263, 1978) in which the animals had to decide on the basis of a single previous experience whether a test object was positive or negative (i.e., had previously been baited or not). It was found in that study that bilateral lesions of either area TE or the amygdaloid complex produced a marked but recoverable deficit, whereas bilateral lesions of either area TEO or the hippocampal complex had only a mild effect. In the present experiment each animal received an additional bilateral lesion, the combined damage being confined either to the temporal cortical area or to the temporal limbic area. Thus, animals originally given TE lesions were now given adjacent TEO lesions, and vice versa; similarly, animals originally given amygdala lesions now received adjacent hippocampal lesions, and vice versa.

The effect of an additional cortical lesion was indistinguishable from the effect of that particular lesion given by itself. That is, the addition of a TE to a TEO removal produced the same marked-but-recoverable deficit as a TE removal alone, while the addition of a TEO to a TE removal produced the same negligible effect as a TEO removal alone. (Only when a bilateral prestriate ablation was added in a third stage did a severe and lasting deficit ensue.) By contrast, the serial limbic lesions produced a seemingly permanent loss irrespective of the sequence of lesions. None of these animals reestablished one-trial learning of object-reward associations within the 2000-trial limit of postoperative testing. Apparently, the amygdala and hippocampus can serve as partial substitutes for each other in a limbic memory mechanism, such that only their combined removal will yield an irrecoverable memory loss.

1070 ANTEROGRADE AMNESIA AND MEMORY FOR CONTEXTUAL INFORMATION: Larry R. Squire, Lynn Nadel* and Pamela C. Slater*. Dept. Psychiat., Univ. Calif. San Diego, La Jolla, CA 92093.

Confusion about the temporal order of events has sometimes been regarded as the fundamental defect in amnesia. Accordingly, it has been suggested that amnesia might reflect a selective defect in assigning context to items that are to be remembered. An alternative hypothesis is that in normal memory as well as in amnesia contextual information is simply more fragile than other kinds of information.

We tested the patient N.A., who has chronic amnesia for verbal material, a group of psychiatric patients receiving bilateral electroconvulsive therapy, and matched control patients. Subjects read two lists of sentences 3 min apart, and then at varying times afterwards were given a yes/no recognition test and were also asked to judge which of the two lists the sentences had appeared on. For all cases, contextual information was deficient in amnesic patients. However, when the recognition memory of control Ss had declined as a result of forgetting to a point where it was equivalent to the recognition performance of amnesic Ss, contextual judgments of control Ss declined such that they matched the levels observed in amnesia. Thus, contextual information is fragile in amnesic patients, but it is fragile as well in normal Ss during the course of forgetting. The evidence suggests that the difference between normal and amnesic Ss may be understood in a quantitative way, and may resemble the difference between normal Ss tested shortly after learning and normal Ss tested long after learning.

1071 ADRENERGIC ANTAGONISTS: ATTENUATION OF RETROGRADE AMNESIA. Debra B. Sternberg and Paul E. Gold. Dept. Psych., Gilmer Hall, Univ. of Virginia, Charlottesville, VA 22901.

Previous results (Gold and Sternberg, Science, 201, 367-9, 1978) indicate that when injected 30 min before training, the α-adrenergic antagonist, phenoxybenzamine (2 mg/kg), attenuates retrograde amnesia produced by most classes of treatments (i.e., supra-seizure frontal cortex stimulation, sub-seizure amygdala stimulation, cycloheximide, pentylenetetrazol, and diethyldithiocarbamate). The present study employed several adrenergic antagonists in order to assess the generality of these findings.

Animals were trained on either a one-trial inhibitory (passive) avoidance task or a visual discriminated avoidance Y-maze. Thirty min prior to training animals received an injection of the β-adrenergic antagonist propranolol (0.5 mg/kg), saline, or one of the following α-adrenergic antagonists: phentolamine (10 mg/kg), piperoxane (5 mg/kg), or phenoxybenzamine (2 mg/kg). Shortly after training, the animals received frontal cortex stimulation (5 ma/1 sec) through implanted cortical screw electrodes. The results indicate that pretreatment of rats with any of these α- and β-adrenergic antagonists attenuates the production of retrograde amensia.

In addition, we examined the effects of α- and β-adrenergic antagonists on amnesia produced by sub-seizure amygdala stimulation. Thirty min before training, animals received injections of either phenoxybenzamine (2 mg/kg), propranolol (0.5 mg/kg), or saline. Animals were trained on either of the tasks described above. Shortly after training, the animals received electrical shock of the amygdala (60 μa, 100 Hz, 0.1 msec monophasic pulses, 10 sec train duration) through bilaterally implanted electrodes. Twenty-four hours later, the animals were tested for retention. Consistent with the findings described above, pretreatment with the α- or β-adrenergic antagonists resulted in attenuation of amnesia produced by amygdala stimulation in both learning situations.

Thus it appears that several adrenergic antagonists can attenuate the amnesias produced by different treatments. These findings add further support to the view that there may be a common adrenergic mechanism underlying retrograde amnesia produced by many amnestic treatments.

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1072 THE EFFECTS OF CHRONIC ADMINISTRATION OF AN ANTICHOLINERGIC ON VERBAL MEMORY IN PARKINSON'S DISEASE PATIENTS. Karl Syndulko, Eugene R. Gilden*, Edward C. Hansch*, Janet A. Lemmon*. Alfred R. Potvin, and Wallace W. Tourtellotte*. Neurology Svc., VA Wadsworth Med. Ctr., Los Angeles, CA 90073.

Anticholinergics have a long history of use in the treatment of symptoms of Parkinson's disease and are currently in common use either alone, or more often in combination with other antiparkinsonism medications. Recent studies have suggested that the putative neurotransmitter acetylcholine (Ach) may also play an important role in certain types of memory function. Acute injections of central acting Ach agonists have been shown to facilitate performance on memory tasks, while acute administration of Ach antagonists appears to disrupt memory functions transiently, particularly those involved in acquisition of new information. In a double-blind, cross-over study the therapeutic efficacy of the anticholinergic, benztropine mesylate (Cogentin [R] was compared with placebo in Parkinson's disease patients on their best dosage of levodopa-carbidopa combination (Sinemet [R]. Each of 29 male patients (age 45-80 yrs) with idiopathic Parkinson's disease was given incremented dosages of the anticholinergic (max. 2 mg) or placebo over a ten week period, washed out for 5 weeks then crossed-over to either placebo or the anticholinergic for another ten week trial. The patients were tested for acquisition of new verbal information before, during, between and after the two ten week trial periods. On each occasion, they were given four trials to learn a list of twenty words. Patients showed small, but signficant impairment of acquisition and recall while on the anticholinergic as compared to their performance while on placebo. These results with chronic administration of an anticholinergic extend previous findings that implicate central Ach in memory mechanisms, and also suggest that other chronically administered anticholinergics used in medical treatment should be carefully evaluated for their effects on memory function.

1073 PHASESHIFT INDUCED AMNESIA: EFFECTS OF SHOCK INTENSITY AND ACTH 4-10. Walter N. Tapp* and Frank A. Holloway. Dept. Neurosci., N.J. Med. School, E.Orange, NJ 07018 and Dept.Psychiatry & Behav.Sci.,Univ. Okla. Health Sci.Ctr.,Oklahoma City, OK 73190.

Recent studies (Neurosci. Abstr. 3: 241, 1977) showed that phase-shifting circadian rhythms shortly after passive avoidance training can produce retrograde amnesia in rats. In order to further analyze this phenomenon, the effects of shock intensity and ACTH 4-10 (Org OI 63) were studied.

Male, albino rats were entrained to LD 12:12 prior to passive avoidance training. Rats were trained in a one-trial passive avoidance task with one of two levels of footshock (1.4 mA or 0.7 mA). Shortly after training, the LD cycle of phase-shifted groups was shifted by 12 h. Phase-shifted rats were housed in cages equipped for photocell activity recording. Activity records provided a measure of the progress of the phase-shift. Rats were tested 7 days after training, after the phase-shift was complete. One hour prior to testing, rats were injected with ACTH 4-10 (Org OI 63) (100 μg/rat, s.c.) or with an equivalent volume of vehicle.

As in earlier experiments, phase-shifting circadian rhythms after training with 1.4 mA impaired retention performance ($p < 0.01$). In contrast, phase-shifting circadian rhythms after training with 0.7 mA facilitated retention performance ($p < 0.01$). These differences in the effects of phase-shifting after different shock intensities suggest that phase-shifting alters memory by acting as a non-specific stressor.

ACTH 4-10 given 1 h prior to testing facilitated retention performance of a 1.4 mA, phase-shifted group ($p < 0.01$), suggesting that the disruptive effects of phase-shifting are due to retrieval effects. ACTH 4-10 further improved the performance of a 0.7 mA, phase-shifted group ($p < 0.05$). However, ACTH 4-10 did not facilitate the performance of 0.7 mA, unshifted controls, suggesting that phase-shifting facilitates retention by enhancing storage.

Phase-shifting appears to facilitate or disrupt memory as a result of its stressful consequences. However, phase-shifting appears to alter different memory processes depending upon whether it facilitates (storage) or disrupts (retrieval) retention.

1074 RESPONSES OF SINGLE HIPPOCAMPAL NEURONS DURING CLASSICAL CONDITIONING. Richard F. Thompson and Theodore W. Berger. Dept. of Psychobio., Univ. of Calif., Irvine, CA 92717 and Dept. of Psych., Univ. of Pittsburgh, Pittsburgh, PA 15260.

Responses of over 130 single hippocampal neurons recorded during classical conditioning of the nictitating membrane (NM) response in the rabbit were examined. All neurons were first classified as showing antidromic (and thus identified as pyramidal cells) or orthodromic activation following single shock stimulation of the fimbria-fornix. Antidromic identification required short (<3msec) latency activation, low variability in latency and ability of such units to follow high frequency stimulation. Orthodromic classification was made on the basis of longer (>3msec) latency activation, greater variability in latency and an ability to follow only low frequencies of stimulation. All cells were analyzed with respect to several pretraining characteristics of spontaneous activity (i.e., complex vs. simple spike patterns, spontaneous rate, etc.), as well as their subsequent firing patterns recorded during conditioning trials.

Clear correlations were found to exist between cell type as defined by fornix stimulation and their classifications according to the above criteria. The majority of cells identified as pyramidal neurons (i.e., antidromically activated) had lower (<10/sec) spontaneous rates and showed complex modes of unit firing during spontaneous periods. Orthodromically activated cells tended to have high (>10/sec) spontaneous rates and did not show complex modes of discharge. Within conditioning trials, the majority of pyramidal cells increased rate of discharge and showed patterns of unit firing that corresponded closely to the topography of conditioned NM movement. The majority of cells showing orthodromic activation inhibited during conditioning trials. Of cells that could not be activated in any manner after fornix stimulation, a subset having very low (<1/sec) spontaneous rates and showing complex modes of unit firing maintained or decreased firing rate during training trials. The specific pattern of increased unit activity seen for pyramidal neurons was shown to be learning-dependent. That is, pyramidal cells in animals given unpaired control training showed little or no increase in firing rates during CS-alone or UCS-alone trials. In addition, the majority of pyramidal cells recorded from conditioning animals on the first day of training showed growth in the amount of unit increase across initial paired trials.

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1075 SINGLE DIGIT FLEXION-EXTENSION IN THE MONKEY, CALLITHRIX JACCHUS. Thomas J. Tobias and Dao Tien Duong.* C. and O. Vogt Institute for Brain Research, and IV Department of Anatomy, University of Duesseldorf Moorenstrasse 5, 4000 Duesseldorf, West Germany.

The recent application of intracellular recording techniques in operantly reinforced animals engaged in forelimb movement has led to a search for a paradigm which might produce a more tranquil behavioural response than the commonly employed lever press, thereby permitting neuronal penetrations of longer duration (Tobias, 1975,1977; Matsumura,1977). While some improvement might be expected from employing floating electrodes (Woodbury,Baker and York, Voronin, King and Skinner), it would seem reasonable to assume that a narrowly delimited movement of the forelimb might reduce vibrations affecting recording stability.

In the present experiment we report the feasibility of training marmosets to flex and extend a single forelimb digit without employing a manipulandum. These small (300 g) South American monkeys have been selected for several reasons: completely lissencephalic dorsal aspect of the cerebral cortex (35mm A-P), small heart with concomitant reduction in cerebral pulsation, and high fecundity. Of six animals, two, serving as controls, were trained to press a lever using only one finger. The remaining monkeys were reinforced with water on a variable ratio schedule, for alternating their finger in a slot (10mm x 3mm) past either the 4th and 2nd, or the 4th and 6th of eight infrared detecting pin photodiodes, positioned parallel to and 3 mm below the slot in an array 2.5 mm apart. Use of more than one digit was discouraged by occurence of a tone instead of water upon interruption of more than three of the photodiodes. Total responses were counted and stored in RAM for subsequent display on an oscilloscope following each 2 hour session. After 1-3 months of daily training it was possible to obtain from all four animals an uninterrupted pattern of movement throughout the session (2000responses). Photographs of the hand taken at 300 msec intervals reveal the distal and middle phalanges of a single finger serving as a radius moving circumferentially in a 120 degree arc extending from a horizontal position at the distal tip of the slot to a vertical position at the proximal edge. The next step will compare intracellular recording stability in both groups, an experiment which is about to commence.

1076 DELAYED RESPONSE LEARNING AND BRAIN BIOGENIC AMINES IN CATS. Luc Vachon*, Andrée G. Roberge and James Everett*. Dépt. Bioch., Fac. Méd., U. Laval, Québec, CANADA, G1K 7P4.

In an attempt to determine the involvement of CNS biogenic amines in short term memory and learning processes, the effects of a delayed response task (DR) on norepinephrine (NE), dopamine (DA), serotonin (5-HT) and 5-hydroxy-indolacetic acid (5-HIAA) concentrations were investigated in normal cats. Moreover, two groups of cats have received Metergoline (Liserdol, Farmatalia) (14 µg/kg/day) or L-DOPA (30 mg/kg/day) daily, beginning four days before the training period. Cats were given 48-trials a day in a modified WGTA apparatus with two food wells, 24 of which were at 0 sec. delay, and 24 trials with delays varying according to individual performance (titration technic). Treated cats were trained 3 hours after drug administration.

At 0 sec. delay, Metergoline has not produced any change in performance (number of good responses) neither in response latencies. Three of the eight L-DOPA treated cats have not responded at all, whereas cats who have responded showed no difference in performance, but a significant increase in response latencies, suggesting a non mnesic effet of L-DOPA. With delays longer than 0 sec., normal, Metergoline and L-DOPA treated cats have showed a progressive improvement of their performance during the first six days. A plateau is then reached for the controls and L-DOPA treated cats whereas Metergoline treated cats have shown an unexplained decrease in performance. L-DOPA treated cats showed longer response latencies whereas Metergoline treated cats have not shown any significant difference from controls.

Biochemical assays, done on different structures in the brain, show that training on DR in normal cats produces a significant increase of 5-HT and 5-HIAA content in the piriform lobe and mesencephalon (without raphe nuclei), a decrease of NE in the piriform lobe and frontal cortex and of DA content in the piriform lobe. The interaction of training on treatments was also biochemically investigated. The results show that DR training interacted with Metergoline effects on 5-HT and 5-HIAA concentrations in the mesencephalon and on NE and DA content in the frontal cortex and piriform lobe, respectively. On the other hand, such an interaction was also found in L-DOPA treated cats, for NE and DA concentrations in the frontal cortex and piriform lobe, respectively.

These results suggest a selective involvement of the cerebral 5-HT, NE and DA in DR training. A biochemical equilibrium between these neurotransmitters based on different neuroanatomical pathways is suspected: the mesolimbic system and the mesencephalic reticular formation deprived of its raphe nuclei in term of inputs and outputs.

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1077 EFFECTS OF L-PROLINE, SOME PROLINE ANALOGS AND SOME OTHER AMINO ACIDS ON SPREADING DEPRESSION IN THE CHICKEN RETINA. A. Van Harreveld. Division of Biology, California Institute of Technology, Pasadena, CA 91125.

It has been suggested (Van Harreveld, 1978) that spreading depression (SD) in the chicken retina can be based on two mechanisms. In one a release of glutamate from the cellular element is primarily involved, in the other a release of K^+. The concept of two mechanisms of SD in the retina can explain the effect of L-proline on this phenomenon. L-proline has been found to suppress SD in the chicken retina at low concentrations (1.5-2.5 mM), at higher concentrations (5-7 mM) it again allows SD in a larger percentage of the experiments. At still higher concentrations (10 mM) it again suppresses SD. The low concentration effect has been postulated to be due to a competition of L-glutamate and L-proline for glutamate receptors. The L-proline attached to these receptors would tend to release intracellular K^+ by an increase in the neuronal membrane permeability. This would facilitate the K^+-based SD observed at L-proline concentrations of 5-7 mM. A similar effect, inhibition at low concentration, and SD at moderate concentration was observed in experiments with a number of proline analogs, such as 3,4 DL-dehydroproline, 4 L-hydroxyproline (both with a 5 member ring), and L-baikiain (which has a 6 member ring). D-proline was without effect as was DL-pipecolic acid (which differs from L-baikiain in the absence of a double bond in the ring). L-azetidine-2-carboxylic acid with a 4 member ring inhibits SD in rather high concentrations. It is of interest that L-proline and those of its analogs which have a biphasic dose effect on SD, exhibit an amnestic effect on one-trial avoidance learning in the chick. A number of other compounds have been tested such as L-glutamine, L-isoleucine, L-norleucine and L-pyroglutamate which did not affect SD in the chicken retina.

1078 PAPAVERINE FACILITATES PASSIVE AVOIDANCE IN MICE.
John W. Villiger,* and Douglas L. Chute, Dept. Psych. Univ. of Otago, Dunedin, New Zealand.

Because of our interest in cAMP as a second messenger potentially mediating memory we decided to test the behavioural effects of the phosphodiesterase inhibitor, papaverine HCl. A preliminary experiment with 8 female NHI/a mice indicated a significant ($p<.025$) performance facilitation in passive avoidance. In a more complete study, 63 male mice of the same strain received a 1 mA 1.5 sec duration footshock after stepping through a guillotine doorway. This shock level was chosen so that undrugged animals showed an intermediate performance level in the 24-hr retention (latencies approx. 300 sec). Maximum latency was 600 sec. Depending upon group designation animals received a 50 mg/kg I.P. injection of papaverine HCl or an equivalent volume of the NaCl vehicle. Group P-20 was injected with drug 20 min prior to training. This time allowed animals to recover from the overtly observable sedative effects of papaverine. Group P+O received the drug immediately *after* acquisition. Group S+O received saline. Group P+60 was given papaverine 60 min after acquisition. A drug side effect control group received the drug but no footshock (PNFS+O).

All groups except PNFS+O showed significant learning ($p<.001$) as measured by increased retention test latencies. However groups differed in retention performance where a Kruskal-Wallis ANOVA showed a significant ($H=22.69$, df 4, $p<.001$) effect across groups. Group P-20, S+O and P+60 showed no significant differences between their respective median latencies (208 sec, 242 sec, 187 sec). Group P+O showed significant performance facilitation (at least $p<.05$) compared to all other groups. (md. Lat 355 sec). Because papaverine was administered after acquisition, and because of the apparent time dependent effect with no observable drug side effects at retention testing we speculate that papaverine may facilitate memory consolidation and/or retrieval. Whether this occurs as a result of changes in blood flow, cAMP or some other effect is as yet undetermined.

1079 Pathophysiologic Correlates of Cognitive Function
Bruce T. Volpe* William Hirst* Michael S. Gazzaniga (SPON:F.Plum). Division of Cognitive Neuroscience,Cornell University Medical School and Rockefeller University, New York,N.Y. 10021.

Two unusually impaired amnesic patients have been extensively evaluated using current methods of cognitve psychology, and their disorders have been subsequently correlated with computerized tomography (CT). Results based on a variety of paradigms revealed that the memory disorder rested in the processes underlying memory function. The first patient,a fifty two year old woman,recovered from a dense retrograde and anterograde amnesia to be left with a devastating inability to remember the temporal order of events. Although she performed substantially below a control group when free recalling a list of unrelated words,her recognition for items in the list was unimpaired. However her recognition for the temporal relation between these items was severely impaired,as evinced by her chance performance on the part of a task in which random queries probed the relative recency of previously presented items. Her CT scan revealed the clips from a successful repair of a ruptured peri- callosal artery aneurysm. Reminiscent of the transient Korsakoff-like syndrome described in the post cingulectomey state,she suffered a qualitatively similar yet permanent injury. The second patient, a fifty six year old man,had severe anterograde amnesia and performed like case H.M. on traditional tests. Analysis on another level however,suggested that impaired transfer from short term to long term memory,commonly called consolidation block could not completely explain this disorder. Techniques,such as semantic ordering tasks and cueing,successfully and predictably facilitated remembering,so that the situation may be viewed more productively as a failure of information processing,either involving encoding or retrieval modes. In spite of this profound cognitive impairment there was no evidence for generalized dementia or destruction of the hippocampus. In sum our evidence is consistent with the hypothesis that a variety of central brain structures and not necessarily the hippocampus are involved in the memory process. (Aided by USPHS Grant No.25643,and the McKnight Foundation).

1080 RECALL IN AMNESIC PATIENTS AS CONTROLLED BY ACQUISITION STRENGTH.
C. Douglas Wetzel & Larry R. Squire. Psychiatry Dept., Univ. of California & Veterans Hospital, La Jolla, CA, 92161.

Amnesic patients benifit from partial information cues to the extent that they initially acquire information. After a single presentation of a word list, control subjects showed better recall with meaningful semantic retrieval cues, compared to recall with either a rhyme cue or a cue consisting of the initial two letters of the word. By contrast, amnesic patients performed poorly and showed equivalent retrieval under all cue conditions. This amnesic pattern of performance could be mimicked in the recall of control subjects who were tested one day after learning instead of 1 minute after learning. In addition, amnesic patients given repetitions of the learning list could mimic the pattern of control subjects tested 1 minute after a single list presentation. Thus, with greater acquisition strength, the amnesic patients showed the normal pattern of performance: superior retention of semantically cued words.

The results suggest that the pattern of performance in amnesic patients can be largely determined by the strength of initial acquisition. As in other studies of anterograde amnesia, apparently qualitative differences between amnesic patients and control subjects can often be understood as quantitative differences in memory strength.

1081 IMPROVEMENT OF MEMORY IN FOUR DIFFERENT SITUATIONS BY SELF-STIMULATION.
Norman White and DanielCoulombe*, Dept. of Psychol., McGill Univ., Montreal, Canada.

Four experiments demonstrating the improvement of memory by contiguous, but non-contingent electrical self-stimulation of the brain (ESSB) are described. The fact that four learned behaviors of such diversity are affected in similar ways by post-training ESSB shows the generality of the phenomenon. The subjects in all experiments were rats with chronic electrodes implanted in the dorsolateral quadrant of the lateral hypothalamus; all bar pressed for ESSB at a rate of at least 180 responses in 5 min. In all experiments the rats were trained on a task in a test apparatus and were then allowed to respond 1000 times for ESSB in a different apparatus. Testing on the learning task was done 24 hrs. later. 1) Secondary Reinforcement. 48-hour deprived rats were allowed to lick a drinking tube 100 times; each lick produced a 0.1 sec tone (paired condition). A control group heard tones randomly throughout the session and licked 100 times (unpaired condition). 24 hrs. later all rats were tested with a dry drinking tube; a tone accompanied each lick. The rats trained in the paired condition with immediate ESSB made significantly more licks to extinction than a group of rats trained in the unpaired condition with immediate ESSB or than a group trained in the paired condition that received ESSB 2 hrs. after training. 2) Conditioned Emotional Response. On day 1 rats were water deprived and placed into a black cage where they heard ten 10 sec tones. On day 2 they were placed into a white cage and given 2 tones with foot shock during the last 0.5 sec of each tone (paired). The control group was given 2 tones and two shocks randomly (unpaired). On day 3 each rat was placed into the black cage with a water spout. When 10 sec of continuous drinking was completed the tone came on for 10 sec. In 10 such trials drinking was supressed by the tone in the paired/immediate-ESSB group, but was not suppressed in the unpaired/immediate-ESSB group or in a paired/delayed-ESSB group. 3) Acceleration of Extinction. Food deprived rats were trained to bar press for food. They were given one session of extinction training, followed by ESSB which they obtained by running in a square-shaped runway. On a second extinction session 24 hrs later the rats failed to show spontaneous recovery as compared to rats that ran for ESSB in the pre-optic area, and rats that received no ESSB. 4) Conditional Discrimination. Food deprived rats were given 12 massed trials in a T-maze. With a white card at the choice point, food was always on the right; with a black card food was on the left. 24 hrs later rats which bar pressed for ESSB after the 12 training trials made significantly more correct choices in the T-maze than rats which did not receive ESSB.

MONOAMINERGIC SYSTEMS

1082 DOPAMINE AND CYCLIC NUCLEOTIDE HISTOFLUORESCENCE IN RAT CAUDATE-PUTAMEN COMPLEX. Marjorie A. Ariano[1] and Larry L. Butcher[2]. Molecular Biology, University of Southern California (1), Los Angeles, CA 90007; Department of Psychology and Brain Research Institute, UCLA (2), Los Angeles, CA 90024.

Using a glyoxylic acid procedure for catecholamines (de la Torre and Surgeon, Histochemistry 49: 81-93, 1976) and a fluorescence immunohistochemical technique for cyclic nucleotides (Steiner, et. al., Adv. Cycl. Nuc. Res. 7: 115-155, 1976), we have determined at the light microscopic level, possible morphologic relationships among striatal tissue elements containing dopamine; cyclic 3',5'-guanosine (cyclic GMP); and cyclic 3',5'-adenosine monophosphate (cyclic AMP). In all experiments, the intensity of fluorescence was greater for cyclic GMP than for cyclic AMP. Furthermore, in material stained for both cyclic nucleotides and for Nissl substance, cyclic AMP appeared primarily associated with neuronal somata whereas cyclic GMP was predominantly associated with processes. Large lesions of the medial forebrain bundle, field H_2 of Forel, and adjacent regions at the level of the subthalamic nucleus resulted in complete loss of dopamine terminals from the caudate-putamen complex, but cyclic GMP and cyclic AMP staining was essentially unaffected as assessed histochemically. We conclude from these data that striatal cyclic nucleotides are associated with caudate-putamen cell bodies and processes originating in that extrapyramidal structure and not with afferent dopamine fibers deriving from the mesencephalon.

This research was supported by grants USPHS AM 05925 (MAA) and USPHS NS 10928 (LLB).

1083 LOCUS COERULEUS LESIONS AND CHRONIC RESERPINE TREATMENT: EFFECT ON ADRENERGIC AND MUSCARINIC RECEPTORS IN RAT BRAIN. S.P. Banerjee, V.K. Sharma*, M. Ganapathi*, R. Busto* and S.I. Harik. Dept. Pharmacology, Univ. of Rochester Medical Center, Rochester, New York 14642, and the Dept. of Neurology, Univ. of Miami School of Medicine, Miami, Florida 33101.

Bilateral, unilateral or sham locus coeruleus lesions were made stereotoxically in Wistar rats by local microinjection of 6-hydroxydopamine (or saline for sham operations). Two weeks after the lesion, the animals were killed and the cerebral cortex and hippocampus were obtained bilaterally. The cerebellum was divided at the midline. For the chronic reserpine treatment experiment, rats were injected intraperitoneally with the drug once daily for 10 days (4 mg/kg was administered on the first day and 1 mg/kg on subsequent days). α- and β-adrenergic (α- and β-AR) and muscarinic cholinergic receptors (MCR) respectively were studied by measuring the specific binding of radiolabeled dihydroergocryptine, dihydroalprenolol and quinuclidinyl benzylate in the particulate fractions of different areas of rat brain. Unilateral LC lesions increased binding to α-AR (15%), β-AR (45%) and MCR (10%) in cerebral cortex. In the hippocampus, unilateral LC lesions decreased binding to α-AR (23%) and MCR (10%) and increased binding to β-AR (61%). Chronic reserpine treatment increased binding to β-AR by 70% in cerebral cortex and 26% in hippocampus and decreased binding to MCR in these two areas of brain by about 10%. Bilateral LC lesions increased binding to β-AR by 32% in cerebral cortex and 53% in hippocampus. Only the changes in the levels of β-AR were statistically significantly different following chronic reserpine treatment and unilateral or bilateral LC lesions. Scatchard analysis revealed that alterations in the levels of β-AR in cerebral cortex and hippocampus were due to changes in the number of binding sites so that there was a 50% increase in B_{max} after bilateral LC lesions as compared to 166% increase seen after chronic reserpine treatment in the cerebral cortex. Neither functional denervation nor structural denervation affected binding to α-AR, β-AR and MCR in the cerebellum. It may be concluded that dysfunction of central noradrenergic nerves mainly leads to augmentation of the levels of β-AR in the cerebral cortex and hippocampus. There are quantitative differences in the magnitude of changes in the levels of β-AR following functional denervation as opposed to structural denervation. This may suggest the presence of presynaptic receptors. In the cerebellum, denervation does not produce an increase in the level of β-AR. (Supported by the Jack Wechter Memorial Fund and HL-18185).

1084 NON-COMPETITIVE INTERACTION OF ERGOT ALKALOIDS WITH DOPAMINERGIC RECEPTORS. M. J. Bannon, K. L. Marek* and R. H. Roth. Depts. Pharmacology and Psychiatry, Yale Univ. Sch. Med., New Haven, CT 06510

Some of the ergot alkaloids seem to possess dopamine (DA) agonist activity. Yet their onset is slow and duration of action prolonged compared to other DA agonists such as apomorphine. The activity of various ergot alkaloids at the presynaptic DA receptors was assessed by measuring dopa accumulation in selected regions of rat brain following inhibition of dopa decarboxylase with Ro4-4602, and the cessation of impulse flow in dopamine neurons induced by γ-butyrolactone (GBL)(Walters and Roth, Naunyn-Schmiedeberg's Arch. Pharmacol. 296: 5, 1976). Ergocornine (2.5 mg/kg, 40 or 95 minutes), lergotrile (5 mg/kg, 40 or 95 minutes), bromocryptine (10 mg/kg, 95 minutes only) and apomorphine (2 mg/kg, 95 and 65 minutes) were all found to possess DA agonist activity in this model, i.e., to reverse the GBL-induced increase in dopa levels in the caudate nucleus. Haloperidol (1 mg/kg) administered 5 minutes prior to ergot administration effectively blocked the agonist effects of the ergots and apomorphine at the DA receptor. However, when given 55 minutes after the agonists, haloperidol did not reverse the action of bromocryptine or ergocornine, although lergotrile's effect was partially reversed, and the effect of apomorphine was completely antagonized. Likewise (+)-butaclamol (4 mg/kg) was effective in preventing but not reversing the effects of bromocryptine when employed with a similar time course. The inactive isomer, (-)-butaclamol, was without effect. Similar results were obtained with bromocryptine and haloperidol or (+)-butaclamol when the olfactory tubercle was studied.

These results suggest a non-competitive interaction of bromocryptine with the DA receptor. If bromocryptine is very tightly bound, a decreased amount of binding of ^{3}H-spiroperidol to DA receptors might be expected. In ^{3}H-spiroperidol binding studies, bromocryptine (10 mg/kg) administered 2 hours before sacrifice caused a 30% decrease in Bmax (number of receptors labeled) with no change in Kd. No similar effect was seen with a shorter (30 minute) pretreatment period, paralleling the data from the GBL model. This decreased Bmax was also not evident 2 days after bromocryptine treatment. Together, the data from the GBL and binding studies suggest that following bromocryptine administration there is a critical time period, after which bromocryptine is no longer easily displaced from the DA receptor, yet retains its receptor agonist properties.

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1085 EM AUTORADIOGRAPHIC STUDIES OF THE ADRENERGIC-SEROTONERGIC INTERACTION IN THE DORSAL RAPHE. Jay M. Baraban* and George K. Aghajanian, Depts. of Psychiatry and Pharmacology, Yale Univ. Sch. Med., New Haven, CT 06508

The dorsal raphe and the adjacent central grey receive a dense adrenergic input as demonstrated by biochemical, histofluorescence and immunocytochemical techniques. Previous pharmacological studies have suggested that adrenergic terminals located near serotonin (5-HT)-containing cells exert a tonic activating influence which maintains 5-HT cell firing activity (Baraban et al. Eur. J. Pharmacol., 52:27, 1978). Recent studies have demonstrated both non-5-HT as well as 5-HT neurons within the dorsal raphe nucleus (Aghajanian et al., Brain Res., 153:169, 1978). To determine the relationship of the adrenergic input to these neuronal types, we have undertaken the localization of adrenergic terminals at the ultrastructural level by EM (electron microscopic) autoradiography.

H^3-l-Norepinephrine (NE, 200 μCi, 25-35 Ci/mM) was injected intraventricularly in chloral hydrate anesthetized rats. Animals had been pretreated with the 5-HT uptake inhibitor fluoxetine (10 mg/kg, i.p.) and the monoamine oxidase inhibitor pargyline (100 mg/kg, i.p.). Some animals had previously received intraventricular injections of the 5-HT neurotoxin 5,7-dihydroxytryptamine (5,7-DHT, 100-200 μg free base).

Examination of ultrathin sections of the dorsal raphe prepared for EM autoradiography showed highly selective localization of radioactivity within nerve terminals. Labelled terminals contained both small agranular vesicles and larger dense core vesicles, characteristic of central adrenergic terminals. These terminals frequently participated in classical synaptic junctions. In contrast to the pattern of labelling observed with H^3-NE, nerve terminals forming specialized junctions were not labelled in similar autoradiographic studies with H^3-5-HT, even though prominent labelling of cell bodies, dendrites and axons was present. Hence, it is unlikely that H^3-NE produces non-specific labelling of 5-HT synaptic junctions. Furthermore, in animals whose 5-HT neurons had been destroyed by 5,7-DHT, H^3-NE was localized to intact axo-dendritic terminals of the type observed in controls. In addition, in the 5,7-DHT pretreated animals the dendritic elements post-synaptic to labelled terminals did not show degenerative changes. These studies suggest that adrenergic terminals innervate non-5-HT neurons in the dorsal raphe. Therefore, the adrenergic-serotonergic interaction in the dorsal raphe may be mediated indirectly via non-5-HT interneurons.

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1086 DOPAMINE (DA) AUTORECEPTORS: RAPID DECREASE IN THE RESPONSE TO DA FOLLOWING AGONIST TREATMENT. M.D. Baring, J.R. Walters, NIH, NINCDS, Bethesda, MD 20205

Single unit recording studies have demonstrated that spontaneously firing DA cells in the substantia nigra pars compacta (SNpc) recovering from inhibition induced by a DA agonist such as apomorphine (APO) generally show a biphasic recovery pattern different from, for example, the response of the serotonergic raphe cells to LSD. After an i.v. dose of APO, 0.1 mg/kg, DA cells tend to be completely inhibited for up to 15 min and then recover rapidly to a plateau rate ≈ 40% of baseline, from which further recovery is gradual (Bunney et al, Nature 245:123, 1973). Each subsequent dose of APO is followed by an increasingly attenuated period of inhibition and recovery to the plateau rate until the cells are completely resistant to further injections of APO. At this point the cells are also resistant to the normally induced inhibition of other dopamimetic agents such as piribedil, amphetamine (AMP), and L-dopa (Walters et al, Adv. Neurol. 9:273, 1975). We have more recently observed that cells resistant to the effects of APO are also no longer inhibited by either lergotrile (.5-2.5 mg/kg) or lisuride (1.6 mg/kg), two dopamimetic ergot derivatives.

Previous studies showing that DA cells have normal responses to APO in rats with the striatonigral feedback pathway lesioned with kainic acid (Baring et al, Neurosci. Abst. 4:267, 1978) suggest that the events responsible for this rapid development of resistance might be occurring at the level of the DA autoreceptors on the DA cell body. To investigate this, the iontophoretic effects of DA on the single unit activity of DA cells in the SNpc of chloral hydrate-anesthetized rats were determined before, during and after a series of injections of i.v. APO at increasing doses. It was found that DA cells initially inhibited by iontophoresis of DA became resistant to the effects of this transmitter as the cells developed resistance to the effects of APO (cumulative dose, 0.4-0.8 mg/kg).

This agonist-induced resistance of DA autoreceptors might be a function of the partial antagonist properties of the drug. However, we have observed that cells recovering from i.v. administration of AMP, a drug which acts by releasing DA, (cumulative dose, 12 mg/kg) show diminished responses to APO, injected i.v. 15-45 min after max. inhibition by AMP, suggesting that DA itself may also induce changes in autoreceptor sensitivity. This observation together with the fact, as described above, that APO can initially inhibit DA cells completely before resistance develops, suggests that a rapid change may be occurring in sensitivity to the agonists or transmitter as a normal consequence of DA autoreceptor stimulation and/or subsequent membrane events leading to a change in firing rate.

1087 QUANTITATIVE IMMUNOCYTOCHEMICAL ANALYSIS OF THE INDUCTION OF TYROSINE HYDROXYLASE IN CATECHOLAMINE NEURONS IN RAT BRAIN FOLLOWING RESERPINE. R.H. Benno*, T.H. Joh, L.W. Tucker*, and D.J. Reis. (SPON: A.B. Judd). Lab. of Neurobiol., Dept. of Neurol., Cornell Univ. Med. Coll., New York, NY 10021.

We sought to determine by quantitative immunocytochemistry using the peroxidase-antiperoxidase (PAP) method: (a) if the enzyme tyrosine hydroxylase (TH) is uniformly distributed among all neurons within the nucleus locus ceruleus (LC) of rat brain; (b) if the increase in activity and accumulation of the enzyme (induction) elicited by reserpine (JPET 193:775, 1975) occurs in all or only a sub-population of LC neurons; (c) if reserpine will alter the amount of immunoreactive TH in dopaminergic neurons of the substantia nigra (SN) despite the absence of changes in TH activity (ibid). We first sought to determine the reaction conditions producing linearity of staining intensity (first order kinetics) with respect to the amount of immunoreactive protein; conditions necessary for quantitation. Untreated rats were perfused with 4% paraformaldehyde. Tissues were postfixed in picric acid - paraformaldehyde and embedded in paraffin. Five micron adjacent sagittal sections were taken through the LC and SN and immunostained. Staining intensity was measured by a TV image analysis system (B & L Omnicon) measuring integrated optical density in neurons of the LC and SN. Linearity of the reaction (pseudo first-order kinetics) was primarily dependent upon: (a) the concentration of the substrate diaminobenzidine (DAB); (b) the duration of incubation with DAB; and (c) the dilution of primary antibody. Incubation of sections with primary antibody and reaction with 0.01% w/v DAB and 0.003% v/v H_2O_2 resulted in increased staining directly proportional to incubation time up to 8 minutes. The optimal time for detecting differences in staining intensities was 3 min. In untreated rats, cells of the LC are heterogeneous with respect to TH content. Two cell groups differing in staining intensity are seen: large TH-positive cells located rostrally, ventrally, and dorsally, forming a shell around the nucleus and a central group with less enzyme. Both stain more intensely than the neuropil. Reserpine (10 mg/kg) resulted 3d later in an approximately 2-fold increase of TH staining in individual neurons of both cell groups and the neuropil. No change in the staining intensity for TH occurred in the SN. We conclude: (a) the PAP method can, under rigorous conditions, be utilized for quantitation of tissue antigens; (b) that neurons of the LC are heterogeneous with respect to the amount of immunoreactive enzyme; (c) the induction of TH elicited by reserpine occurs in all neurons in the LC; and (d) reserpine fails to increase TH enzyme protein in dopaminergic neurons of the SN.

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1088 EFFECTS OF FREQUENCY AND PATTERN OF ELECTRICAL STIMULATION OF THE CERVICAL SYMPATHETIC TRUNK ON THE ACTIVITY OF THE ENZYME SEROTONIN:N-ACETYLTRANSFERASE IN THE RAT PINEAL GLAND. C.W. Bowers* and R.E. Zigmond (SPON: V. Chiappinelli). Dept. of Pharmacol., Harvard Med. Sch., Boston, MA 02115

Bilateral stimulation of the cervical sympathetic trunks (CST) of the rat at 10 Hz produces an increase in the activity of serotonin:N-acetyltransferase (NAT) in the pineal gland. The rate and magnitude of this increase are comparable to those observed during the normal nighttime rise in NAT activity in intact rats (Bowers and Zigmond, Soc. Neurosci. Abst. 4:268, 1978).

We have now studied the increase in NAT activity following stimulation at a number of different frequencies and with several different patterns of stimulation. In all experiments male albino rats were exposed to light during the dark part of their day/night cycle, and the CST were stimulated bilaterally at current levels twice those required to produce maximal exophthalmos of the ipsilateral eye. Pineals were removed and frozen immediately after the cessation of stimulation. Enzyme values are given in units of pmoles of product formed per μgm protein in 20 min ± S.E.M.

5 Hz stimulation for 0.5, 1.0 and 2.0 hours produced as large an increase in NAT activity as did stimulation at 10 Hz. For example, after one hour of stimulation at 10 Hz, the NAT activity was 27.2 ± 2.1 while after stimulation for the same length of time at 5 Hz the activity was 26.0 ± 2.1. The NAT activity of unstimulated animals was 2.0 ± 0.1. This suggests that stimulation of the CST at 5 Hz is sufficient to produce a maximal increase in NAT activity in the rat pineal gland. Stimulation at 2.5 Hz produces about 50% of this maximum response while stimulation at 1 Hz produces 10% of the maximum response.

In addition to its sensitivity to the frequency of stimulation of the CST, the NAT activity of the pineal gland is sensitive to the pattern of stimulation. For instance, stimulation of the CST with a repeating pattern of 5 Hz for 2 seconds followed by no stimulation for 8 seconds produced a 3-fold greater increase in NAT activity after one hour than did stimulation continuously at 1 Hz. This difference occurred in spite of the fact that both conditions of stimulation consisted of exactly the same number of stimuli. In contrast, stimulation at 5 Hz for 1 out of every 5 seconds for an hour produced an increase of the same magnitude as did continuous stimulation at 1 Hz. Thus, relatively subtle changes in the pattern of firing of the CST can produce large changes in the biochemical response of the pineal gland. (Supported by a Grant-in-Aid from the American Heart Association. CWB is a fellow of the Scottish Rite Schizophrenia Research Program.)

1089 EXAMINATION OF BLOOD PRESSURE CHANGES FOLLOWING DESTRUCTION OF ASCENDING SEROTONERGIC NEURONS IN SPONTANEOUSLY HYPERTENSIVE (SH) AND NORMOTENSIVE RATS. D. G. Bramlet,* R. A. Browning and J. H. Myers.* Southern Illinois University, School of Medicine, Carbondale, Ill. 62901.

Several laboratories have provided evidence that central serotonergic neurons play a role in the maintenance of hypertension in the SH rat. Recently, Smits et al. (Life Sci 23, 173-178, 1978) reported that electrical stimulation of the dorsal and median raphe nuclei produced an elevation in the blood pressure of normotensive rats. We have now investigated the role of ascending serotonergic neurons in the maintenance of hypertension in the SH and normotensive, Wistar-Kyoto (WKY) rat. The ascending serotonergic pathways of 11-12 week old SH and WKY rats were destroyed by electrolytic lesions of the dorsal and median raphe nuclei. Lesioned animals received a 2mA anodal current for 5 sec. In sham-operated animals, the electrode was lowered to a depth of 1mm above the raphe dorsalis and no current was passed. Nine blood pressure measurements (3 recordings per day for 3 days) were obtained on each rat before and after lesioning using the indirect tail cuff method. All animals were allowed 4 weeks to recover from surgery before obtaining post-lesion blood pressures. Rats were sacrificed 39-40 days post-lesion, at which time their brains were removed for both histological and neurochemical evaluation. As shown below, lesions of the ascending serotonergic pathways had no significant effect on the blood pressure of WKY rats despite an 80% reduction in forebrain serotonin (5-HT). Similarly, a raphe lesion in the SH rats did not significantly alter the further development of hypertension in spite of an 81% depletion of forebrain 5-HT. These findings suggest that the ascending serotonergic fibers emanating from the dorsal and median raphe nuclei are not involved in the maintenance of hypertension in the SH rat and play little role in the regulation of blood pressure in WKY rats.

Blood Presure ± SEM (mm Hg)

	Pre-Lesion		Post-Lesion	
	WKY	SHR*	WKY	SHR*
Sham Group	130 ± 3	168 ± 4	141 ± 4	199 ± 7
Lesion Group	131 ± 4	166 ± 2	136 ± 3	191 ± 4

*Values were significantly higher compared to WKY rats ($p < 0.001$).

(Supported by a grant from the Illinois Heart Association).

1090 EFFECT OF NEONATAL 6-HYDROXYDOPA TREATMENT ON α- AND β-ADRENERGIC RECEPTOR BINDING AND ON BEHAVIOR. David B. Bylund and Walid O. Shekim, Depts. of Pharmacology and Psychiatry, Sch. Med., Univ. Missouri, Columbia, MO 65212.

Treatment of neonatal rats with the neurotoxin 6-hydroxydopa is known to decrease adrenergic innervation and norepinephrine levels in the cerebral cortex while increasing innervation and norepinephrine levels in the cerebellum. Since the number of central β-adrenergic receptors, and perhaps α-adrenergic receptors, appear to be inversely related to changes in norepinephine levels (Bylund, Adv. Exp. Med. Biol. 116,133-162,1979),we studied the effects of 6-hydroxydopa on β-,α_1- and α_2- adrenergic receptor binding. In addition, we studied the effects of the treatment on behavior in order to evaluate it as an animal model of the childhood hyperactivity syndrome.

Neonatal rats were treated (s.c.)on days 1,3 and 5 with either 80 µg/g 6-hydroxydopa, or vehicle (ascorbic acid-saline).At 24-26 days their locomotor activity was measured. Subsequently, the adrenergic receptors were assayed in crude particulate fractions of cerebral cortex, cerebellum, midbrain and medulla-pons using ^{3}H-dihydroalprenolol, ^{3}H-WB-4101 and ^{3}H-clonidine. Norepinephine levels were determined using HPLC with electrochemical detection.

The 6-hydroxydopa treated animals were significantly hyperactive as compared to controls using both the activity box ($F(1,32)=5.4, p=0.027$) and the open field ($F(1,30)=12.4, p<0.001$) methods. As expected, the apparent number of β-adrenergic receptors (B_{max}) was significantly increased in the cerebral cortex (42%,See Table) with no change in the affinity (K_D).

Effect of Neonatal 6-Hydroxydopa Treatment on Adrenergic Receptor Binding in Rat Cerebral Cortex

	n	% of Control B_{max}	% of Control K_D
^{3}H-dihydroalprenolol	4	142± 9*	110±16
^{3}H-WB-4101	6	104±10	96± 9

Values given are means ±S.E.M. Control values (B_{max},K_D): for ^{3}H-DHA,9.1 pmol/g tissue, 0.88 nM; for ^{3}H-WB-4101,13.2pmol/g tissue; 0.80 nM. *$p<0.025$ by paired t test

However, no change was found in the number of cortical α-adrenergic receptors as measured by ^{3}H-WB-4101 binding. Similarly, β-receptor but not α-receptor binding was decreased in the cerebellum. Thus, at least in this model of hyperactivity, the β-adrenergic receptors,but not the α-adrenergic receptors appear to be regulated by the endogenous levels of norepinephrine.

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1091 EVIDENCE OF CATECHOLAMINERGIC AND SEROTONINERGIC INNERVATION OF THE INTRAVENTRICULAR NEURONAL CLUSTER IN THE HAMSTER. J.P. Card*[1] and J.A. Mitchell. Department of Anatomy, Wayne State University School of Medicine, Detroit, Michigan 48201

Previous studies in our laboratory (JCN 180: 43, 1978) demonstrated the presence of a ganglion-like cluster of neurons and neuronal processes on the ependyma overlying the median eminence (ME) of the hamster. In the present study, the selective neurotoxic actions of 6-hydroxydopamine (6-OH-DA) and 5,7-dihydroxytryptamine (5,7-DHT) were employed to establish the catecholaminergic (CA) and serotoninergic (5-HT) character of the axons of extrinsic origin innervating the cluster.

Hamsters were assigned to 2 experimental and 2 control groups (5/group). Experimentals received 2 iv injections of 6-OH-DA (150 mg/kg bw, 24 hrs apart) or an intraventricular infusion of 5,7-DHT (100 ug; following blockade of noradrenergic terminals with desmethylimipramine). Controls received 2 iv injections or an intraventricular infusion of saline, respectively. At 48 hrs after initiation of treatments the ME-3rd ventricle region containing the neuronal cluster was prepared for transmission electron microscopy.

In controls, normal ultrastructure was exhibited by all neuronal perikarya, processes and glial-like supportive cells of the cluster. In contrast, administration of 6-OH-DA resulted in ultrastructural damage to a small number of axons within the cluster. Both early and advanced degenerative changes were evident: axons contained either dense accumulations of cellular debris and swollen vesicles with enhanced osmophilia or increased cytoplasmic density and swollen terminals, respectively. Supportive cells evidenced increased phagocytosis. Administration of 5,7-DHT also elicited axonal degeneration within the cluster. However, the number of terminals affected far exceeded that seen following 6-OH-DA treatment and the degenerative changes were accompanied by disruption of the cytoarchitectural organization of the cluster and adjacent periventricular neurophil. Degenerating axonal profiles were also apparent within the ventricle and ME. Phagocytosis was increased in supportive cells. In addition, perikarya of supportive cells were observed on the periphery of the cluster, an area normally occupied exclusively by nerve cell bodies in controls.

The results indicate that both catecholaminergic and serotoninergic systems are involved in the modulation of the neuronal activity of the supraependymal cluster. Studies are in progress to determine the location of the cell bodies from which the CA and 5-HT axons originate. (Supported by NIH RR-05387)

[1]C. DeVlieg Fellow.

1092 DIFFERENTIAL RECOVERY OF REINFORCEMENT AND MOTORIC FUNCTION FOLLOWING UNILATERAL LOSS OF BRAIN DOPAMINE. Robert J. Carey. VAMC at Syracuse, Syracuse, NY 13210.

Rats with bilateral medial forebrain bundle electrodes which generated comparable rate-intensity functions for self-stimulation were administered intracerebral unilateral injections of 6-hydroxydopamine (4 µl of a 2 µg/µl sol.) into the substantia nigra. The nigral injections produced a virtual complete loss of forebrain dopamine in the injected hemisphere. Initially, this dopamine depletion was manifested behaviorally by a selective and severe decrease in self-stimulation in the dopamine deficient hemisphere as well as in ipsiversive circling and diminished locomotor activity. Over the course of a two month postoperative test period, however, self-stimulation obtained from the dopamine depleted hemisphere gradually recovered and became more sensitive to stimulation than the intact hemisphere. In contrast, the ipsiversive circling and reduced activity level persisted throughout testing. Furthermore, the motoric responses elicited by stimulation of the depleted and intact hemispheres became different. While the typical locomotor exploratory behavior was observed to accompany stimulation in the intact hemisphere stimulation of the depleted hemisphere produced ipsiversive circling. Additionally, giving the rats 2 mg/kg d-amphetamine produced reliable ipsiversive turning but at the same time facilitated self-stimulation equally for electrodes in the intact and dopamine depleted hemispheres. These studies show that dopamine depletion produces persistent motor but not reinforcement deficits.

1093 CONDITIONING OF STRESS-INDUCED ALTERATIONS OF NOREPINEPHRINE METABOLISM IN RAT BRAIN: STUDIES IN PROGRESS. Geraldine Cassens, Alvin Kuruc*, Paul J. Orsulak*, and Joseph J. Schildkraut*. Dept. Psychiat., Harvard Med. Sch., Boston, Mass. 02115.

The administration of single or multiple sessions of footshock increases levels of the sulfate conjugate of 3-methoxy-4-hydroxyphenylglycol (MHPG-SO_4), a major metabolite of norepinephrine in rat brain. In the present studies, we sought to determine whether these stress-induced increases in levels of MHPG-SO_4 in rat brain (UCR) could be elicited by environmental stimuli (CS) that had been previously paired with inescapable footshock (UCS). Male Sprague Dawley rats (200-220 g) were placed in an experimental chamber daily on Days 1-3 and one group received 80 trials of inescapable footshock (1.05 mA presented on a VI 60 schedule), while another group received no footshock. On Day 4, 24 hours after the last session, half of the shocked and half of the unshocked animals were again placed in the experimental chamber (CS), but received no footshock. The remaining halves of the shocked and unshocked groups were not placed in the chamber, but remained in their home cages (No CS). Immediately after the session on Day 4, animals were removed from the experimental chamber or home cage, sacrificed, and brains were assayed for levels of MHPG-SO_4.

Animals that had received footshock on Days 1-3 and were exposed to the CS on Day 4 showed significant increases in brain levels of MHPG-SO_4 ($p<.001$) when compared to: 1) unshocked animals that remained in their home cage on Day 4, 2) unshocked animals that were exposed to the CS on Day 4, or 3) previously shocked animals that remained in their home cage and were not exposed to the CS on Day 4. Further experiments have shown that increases in MHPG-SO_4 levels could be elicited by the CS after only a single pairing of the CS and UCS. Preliminary data suggest that these increases in MHPG-SO_4 are associated with increased defecation and crouching behavior, but not with increased locomotor activity. While these experiments have not differentiated between neuronal "sensitization" or "conditioning," we believe these studies are the first to show that increases in a naturally-occurring catecholamine metabolite in rat brain can be elicited by environmental stimuli that have been previously paired with stress. These findings will be discussed in relation to the deficits in escape behavior observed after exposure to inescapable shock ("learned helplessness").

1094 GUANOSINE 5'-TRIPHOSPHATE IS AN ENDOGENOUS COMPOUND IN THE RABBIT CEREBELLAR CORTEX WHICH "COUPLES" THE BETA-ADRENOCEPTOR TO ADENYLATE CYCLASE. Thomas E. Cote*, Tai C. Chen*, and John W. Kebabian
Experimental Therapeutics Branch, NINCDS, NIH, Bethesda, MD 20205.

A beta-adrenoceptor regulates adenylate cyclase activity in cell-free homogenates of the rabbit cerebellum. The demonstration in vitro of the "coupling" between the beta-adrenoceptor and adenylate cyclase activity requires the presence of either guanosine 5'-triphosphate (GTP) or guanosine 5'-diphosphate (GDP). Thus, repeated washing of the particulate material in homogenates of the rabbit cerebellum abolishes the sensitivity of the adenylate cyclase activity to beta-adrenergic agonists. The addition to the particulate cerebellar material of either the soluble constituents of the cerebellar homogenate or the exogenous guanyl nucleotides, GTP or GDP, restores the sensitivity to beta-adrenergic agonists. Utilizing high pressure liquid chromatography (HPLC) the amount of GTP and GDP in the soluble components of the cerebellar homogenate can be measured; these two guanyl nucleotides can account for the restoration of the sensitivity to beta-adrenergic agonists. The endogenous nucleotides in the cerebellum were isolated with HPLC. Only the endogenous GTP and GDP were capable of restoring the coupling between the beta-adrenoceptor and adenylate cyclase activity; none of the other compounds isolated with HPLC were active. The effectiveness of GDP may reflect its conversion to GTP during the assay of adenylate cyclase activity. If ATP is used as substrate, approximately 75% of the exogenous [14-C]-GDP is recovered as [14-C]-GTP at the end of the assay of adenylate cyclase activity. However, if AMP-P(NH)P is the substrate such conversion is negligible; under these latter conditions GTP, but not GDP, can restore sensitivity to beta-adrenergic agonists. The beta-adrenergic antagonist, [3-H]-dihydroalprenolol (DHA) identifies specific binding sites similar to the beta-adrenoceptor which regulates adenylate cyclase activity. Exogenous GTP does not affect either the number of DHA binding sites or the affinity of these sites for l-isoproterenol. Furthermore, GTP does not cause a shift in the activation affinity of the adenylate cyclase activity for l-isoproterenol. In conclusion, the guanyl nucleotides GTP and GDP are endogenous constituents of the rabbit cerebellum which are essential for the functional "coupling" of the beta-adrenoceptor and adenylate cyclase but which do not affect the receptor per se: the activity of GDP occurs as a consequence of its conversion into GTP; the activity of GTP may reflect a direct effect of this compound.

1095 ALPHA- AND BETA-ADRENERGIC AGONIST ACTIVITIES OF 2-, 5-, AND 6-FLUORINE SUBSTITUTED NOREPINEPHRINES IN THE INTACT DOG. Cyrus R. Creveling, Kenneth L. Kirk*, Daniele Cantacuzene*, Jai D. Kohli*, and Leon I. Goldberg. NIAMDD, Bethesda, MD, 20205 and Dept. Pharmacol., Uni. Chicago, Chicago, IL 60637.

Previous investigations using isolated guinea pig aortic strips and atria demonstrated that substitution of fluorine (F) on the 2-, 5-, or 6-position of the aromatic ring of norepinephrine (NE) results in a marked alteration of the β1- and α-adrenergic agonist properties of NE (Fed. Proc. 38: 543, 1979). Similar agonist specificities with these NE derivatives were also observed in the amine-sensitive cyclic AMP generating system in slices of rat cortex and the displacement of α- and β-specific radioligands from isolated membrane preparations from rat brain (Fed. Proc. 38: 533, 1979).

In the present study we compared the effects of 2-, 5-, and 6-FNE, administered intravenously, on cardiac contractural force (CF) and arterial blood pressure (BP) and, after intraarterial administration, to femoral blood flow (FBF) to that of NE, in pentobarbital anesthesized dogs. 5-FNE was qualitatively similar but more potent than NE on CF, yielding an increase in both diastolic and systolic BP and a decrease in FBF. 2-FNE increased CF, diastolic BP, and FBF whereas 6-FNE had no effect on CF, increased diastolic BP and decreased FBF. Propranolol blocked the increase of CF with 2- and 5-FNE and the increase in FBF by 2-FNE. Thus 2-FNE acts on β1- and β2-adrenergic receptors; 5-FNE on both α- and β1-adrenergic receptors, and 6-FNE only on α-adrenergic receptors, confirming our earlier studies in the guinea pig. The present results, in addition, demonstrate the β2 action of 2-FNE. The marked effect of the site of F substitution on the specificity of these NE derivatives and the lack of effect of similar F substituents on dopamine (Fed. Proc. 38: 601, 1979) presumably reflect fluorine-hydroxyl interactions and/or F induced electronic effects which are manifested only in a chiral molecule. (Supported by NIH GM-22220.)

1096 HORSERADISH PEROXIDASE STUDIES OF AN AUTONOMIC GANGLION. William G. Dail, Celia Barraza*, Suzanne Khoudary*, and Heather Murray. Dept. Anat., Sch. Med., Univ. New Mexico, Albuquerque, NM 87131

To further characterize the organization of the superior cervical ganglion (SCG), target organs and an efferent nerve of the rabbit SCG have been treated with horseradish peroxidase (HRP). The superior cervical ganglion was studied by light microscopy 48 hrs after injection of the iris with HRP. Labeled ganglion cells seem to be evenly distributed throughout the SCG as seen with the o-dianisidine procedure for HRP. In contrast, injection of HRP into multiple sites in the submandibular salivary gland resulted in labeled neurons predominantly located in the caudal half of the SCG, near and below the origin of the external carotid nerve. With exposure of the salivary glands or the external carotid nerve to HRP, labeled neurons were found in the rostral portion of the cervical sympathetic trunk, 4cm from the SCG at the level of the accessory cervical sympathetic ganglion and in the stellate ganglion. Tissue was also treated with diaminobenzidine for study of the distribution of horseradish peroxidase with the electron microscope. Lysosomes filled with HRP were found in the soma of ganglion cells as well as in neurites at the periphery of labeled cells. Some of the HRP labeled neurites received adrenergic terminals (shown by 5-hydroxydopamine loading) and terminals containing small clear vesicles. In some cases nerve processes marked with HRP also contained scattered small dense core vesicles, although typical adrenergic terminals in the rabbit SCG have not been found to contain HRP.

These studies suggest that the superior cervical ganglion is not a homogeneous collection of neurons. Instead there is an apparent grouping within the SCG of neurons destined for a particular target organ. Neurons supplying the iris appear to be an exception to this pattern. The adrenergic fibers which are known to enter the caudal margin of the superior cervical ganglion arise from as far caudal as the stellate ganglion. The recognized target organs of the superior cervical ganglion are the destination of some of these fibers.

1097 EFFECTS OF SUPERIOR-COLLICULUS LESIONS ON APOMORPHINE-INDUCED ACTIVITY AND STEREOTYPY IN RATS. Paul Dean*, Peter Redgrave* and Sian G. Pope* (SPON: C. D. Jarvis). Dept. Psychology, Sheffield Univ., Sheffield, S10 2TN, U.K.

The efferent pathways by which alterations in striatal dopamine influence behaviour are poorly understood. Recent evidence has implicated the projection from caudate nucleus to substantia nigra (Marshall and Ungerstedt, Science, 198:62, 1977) and thence to superior colliculus (Wirtshafter et al., Neurosci. Abstr., 4:287, 1978; but see Crossman and Sambrook, Brain Res., 159:211, 1978) in the circling produced by apomorphine in rats with unilateral nigral lesions. The present experiment examined the effects of superior colliculus lesions on the stereotypy and activity changes produced by apomorphine in normal rats.

Rats with large bilateral electrolytic lesions of the superior colliculus (SC) were compared with non-lesioned controls in an open-field and a Skinner box following systemic injections of apomorphine (4-6 mg/kg). In the open-field, apomorphine produced a greater increase in locomotor activity in the SC than the control animals. The SC rats, however, showed much less stereotyped behaviour. All animals showed pronounced stereotypy when confined in the Skinner box, but in the SC group this was confined to sniffing and rearing; the intense stereotyped gnawing and licking that characterised the control group was not observed in rats with SC lesions.

These differences are consistent with the effects of high doses of amphetamine (12-24 mg/kg) on the behaviour of rats with SC lesions. Such doses produce extremely large increases in open-field activity, with little stereotypy (Pope, Dean and Redgrave, in press).

It appears that the stereotyped behaviour produced by systemic injection of dopamine agonists, which is thought to be associated with increased transmission within the nigro-striatal dopamine system, requires the integrity of the superior colliculus for its full expression, and may therefore be mediated in part by the striato-nigral and nigro-tectal pathways. In contrast, the increase in locomotor activity produced by such drugs, which is thought to involve the mesolimbic dopamine system, is probably mediated by pathways that do not pass through the superior colliculus.

1098 STANDARDIZATION OF THE SPG HISTOFLUORESCENCE METHOD FOR MONOAMINE TRANSMITTERS. J.C. de la Torre, Dept. of Neurosurgery, Univ. of Miami School of Medicine, Miami, Florida 33101.

The SPG histofluorescence method is a rapid, highly sensitive microscopic technique used to visualize tissue monoamine transmitters in cryostat sections. The SPG method differs from other histofluorescent microscopic procedures in 4 ways: 1.The reacting SPG solution sits at room temperature. 2.The tissue amine solution reaction takes 3 seconds. 3.The procedure from frozen tissue to microscopic examination is done in under 10 minutes. 4.No elaborate equipment, animal perfusion or personnel trained in histochemistry is required. Moreover, animal or human tissue biopsy can be wrapped in aluminum foil and buried in dry ice for transportation to the cryostat. One disadvantage in the original method (de la Torre & Surgeon, Histochemistry 49: 81, 1976) was the occasional variability in fluorescence intensity encountered after heating the tissue preparations on a hot plate during the final step in the procedure. The hot plate step has now been eliminated with the resulting effect of obtaining very consistent fluorescent preparations while still maintaining the high sensitivity of this procedure. The 7-step recommended method is as follows: (a) Prepare the SPG solution: 10.2 g sucrose, 4.8 g monobasic KH_2PO_4 and 1.5 g glyoxylic acid monohydride in 100 ml distilled water. (b) Stir until clear. (c) Titrate solution to pH 7.4 with 1 N NaOH and (d) top off with distilled water for a final volume of 150 ml. Prepare the tissue as follows: (1) Cut fresh tissue from body (brain, gut, muscle, etc.) into 5-10 mm slabs and immediately freeze on a pre-cooled chuck in cryostat at -30°C. (2) After tissue is frozen (about 10 min.), cut cryostat sections 16-32 μm thick, pick up section by pressing glass slide against cryostat knife. (3) Dip slide 3 times (1 sec./dip) in SPG solution. Quickly wipe off excess solution from bottom and edges of slide and immediately start drying step. (4) Slides are placed between 2 strong hairblowers set at maximum cool air for several minutes. Tissue must be completely dried (ground glass appearance). (5) After drying, place 1-2 drops of Light USP Mineral Oil to cover entire tissue on slide. (6) Place slides in pre-warmed 95°C oven for 2½ min. We recommend leaving a flat copper plate of any size or thickness in oven to hold slides so heat is more evenly distributed to tissue section. When placing slides in oven, do not keep oven door open longer than a few seconds since temperature will quickly drop from 95°C to below 90°C. Quick placement of slides in oven should not drop oven temperature below 90°C so that heat exposure time of 2½ minutes is satisfactory. (7) After oven exposure, excess or running oil is wiped off around tissue section and 1-2 fresh drops of mineral oil are placed on tissue, section is cover-slipped and examined in fluorescent microscope.

1099 ROLE OF BIOGENIC AMINES IN CEREBRAL ISCHEMIA OF THE MONGOLIAN GERBIL. G. Delbarre* and B. Delbarre (SPON: M. Cohn), Lab. Chir. Exp., Fac. de Med., 37032 Tours, France.

Studies in different animal models have established that cerebral ischemia profoundly alters the cerebral neurotransmitter functions of the monoamines. In our present investigation, our model for ischemia consisted of mongolian gerbils subjected to a unilateral surgical occlusion of the common carotid artery. Since many gerbils of this species lack a posterior communicating artery, the percentage of successfully induced ischemic subjects is directly related to the number of gerbils with this deficiency. Using a pharmacological approach, we studied here the effects of drugs known to interfere with monoaminergic metabolism. One hour before one of the common carotid arteries was ligated in the gerbils anesthetized with ketamine 40 mg kg^{-1}, imipramine 20 mg kg^{-1}, niamid 100 or 200 mg kg^{-1}, L.Dopa 300 mg kg^{-1}, HTP (DL) 37.5 or 75 mg kg^{-1} or saline was administered intraperitoneally. Neurological status was evaluated with the stroke index reported by McGraw et al (Stroke 7: 485-488, 1976) starting one hour after ligation and again at 4, 24, 48, 72 and 96 hours. Like saline, imipramine, niamid and L.Dopa did not affect the neurological status of the gerbils (Table 1). In contrast, HTP significantly improved the mean stroke index (Table 1). Our findings agree with those of Lhermitte and Degos (Neuropharmacol. 1: 666-672, 1978) who reported that 5 HTP improves the post anoxic syndrome of intention or action myoclonus described by Lance and Adams (Brain 86: 111-136, 1963).

TABLE 1

Saline 3.7 ± 1.3	Imipramine (20 mg/kg) 4.3 ± 1.8	
Saline 4.7 ± 1.8	Niamid (100 mg/kg) 9.8 ± 2.2	
Saline 2.8 ± 1.3	Niamid (200 mg/kg) 16.6 ± 2.6	
Saline 12.8 ± 2.5	L.Dopa (300 mg/kg) 12.8 ± 2.5	
Saline 11.9 ± 2.8	HTP (DL) (37.5 mg/kg) 3.7 ± 1.4*	HTP (DL) 75 mg/kg 3.3 ± 1.4*

Mean Stroke Index at 96 hours.
*Significantly different from saline control.

1100 MICROIONTOPHORETIC STUDIES ON DENERVATION SUPERSENSITIVITY TO MONOAMINES IN THE RAT HIPPOCAMPUS. C. de Montigny, R. Wang, T.A. Reader and G.K. Aghajanian. Centre de recherche en sciences neurologiques, Université de Montréal and Departments of Psychiatry and Pharmacology, Yale University.

The dorsal hippocampus receives serotonin (5HT) and norepinephrine (NE) inputs from the median raphé and locus coeruleus respectively. The present study was undertaken to assess the responsiveness of hippocampal pyramidal cells of the CA_3 region to putative neurotransmitters and analogs applied iontophoretically following 5HT and NE denervation. Sprague-Dawley rats received intraventricular injections (20 μl) of one of the following substances: 1) 200 μg (free base) of 5,7-dihydroxytryptamine (57DHT) one hour after desipramine administration (25 mg/kg, i.p.); 2) 200 μg (free base) of 6-hydroxydopamine (6OHDA); 3) NaCl 0.9%. In a fourth group of animals the locus coeruleus was destroyed bilaterally by electrolytic lesion. Iontophoretic experiments and biochemical determinations were performed two weeks to two months after these pretreatments. Hippocampal 5HT and NE content were determined with the radioenzymatic methods of Saavedra, and Coyle and Henry. 5HT 0.02 M in NaCl 0.02 M (pH 3.6), NE 0.1 M (pH 4.0), γ-aminobutyric acid (GABA) 0.05 M in NaCl 0.05 M (pH 4.0) and isoproterenol (ISO) 0.1 M (pH 4.0) were applied iontophoretically onto extracellularly recorded pyramidal cells.

Hippocampal 5HT and NE contents dropped to 11% and 37% of control values following 57DHT and 6OHDA respectively. In 57DHT pretreated animals, the responsiveness of pyramidal cells to all iontophoresized substances remained unchanged as compared with controls. In contrast, following 6OHDA pretreatment and locus coeruleus lesion responses to NE were markedly prolonged. However, the initial time course of the response to NE was not modified. Responses to ISO,5HT and GABA were not altered by 6OHDA pretreatment.

The prolongation of the effect of NE without any modification of initial responsiveness after both types of NE denervations suggests the presynaptic nature of this supersensitivity. This is corroborated by the unaltered responsiveness to ISO which is not a substrate for NE reuptake system. Furthermore, acute pharmacological blockade of NE reuptake with desipramine (5 mg/kg, i.p.) in intact animals induced the same phenomenon. The absence of a similar presynaptic type of supersensitivity to 5HT following 5,7-DHT may reflect the relatively minor role of reuptake in terminating the action of iontophorized 5HT in this region. The unmodified initial responsiveness to both 5HT and NE after denervation suggests that NE and 5HT receptors of pyramidal cell somata, in contrast to that of lateral geniculate and amygdala neurons (Wang et al., Brain Res., 1979), fail to develop postsynaptic type of supersensitivity.

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1101 ALCOHOLIC METABOLITES OF CATECHOLAMINES AND RELATED AMINES IN BRAIN AND URINE: ANALYSIS BY GAS CHROMATOGRAPHY/MASS SPECTROMETRY AND THE EFFECTS OF L-DOPA. David J. Edwards and Marguerite Rizk*. Univ.of Pittsburgh Sch.Med.,Western Psychiatric Inst.&Clin., Pgh.PA.15261.

Although 3-methoxy-4-hydroxyphenylglycol(MHPG)is the major metabolite of norepinephrine(NE)in the CNS and its assay is often used as an index of NE metabolism, less attention has been paid to the alcoholic metabolites of other monoamines. They nevertheless appear to be quantitatively important metabolites of the parent amines,particularly the β-hydroxylated ones. We have developed a method using gas chromatography/mass spectrometry which permits the simultaneous assay of phenylethylene glycol, m- & p-hydroxyphenylethanol(mHPE & pHPE),p-hydroxyphenylglycol(pHPG), and 3-methoxy-4-hydroxyphenylethanol(MHPE),metabolites of phenylethanolamine,m- & p-tyramine, octopamine and dopamine(DA),respectively, and MHPG and 3,4-dihydroxyphenylglycol(DHPG),both metabolites of NE. Results obtained for the urinary excretion of free and total metabolites in control and L-DOPA(150mg/kg,i.p.) treated Sprague-Dawley rats (300-350g) were as follows:

	μg/24 hr (mean±S.E.)			
	Control (n=4)		+L-DOPA (n=5)	
Metabolite	free	total	free	total
mHPE	.53±.26	12±1	.36±.15	70±2**
pHPE	8.3±3.4	58±6	1.9±1.0*	37±9
pHPG	.12±.03	4.8±.9	.12±.06	4.9±1.8
MHPE	.18±.04	8.3±2.4	.96±.47	107±15**
MHPG	.95±.12	55±9	1.3±.17	80±6*

*p<.05, **p<.001: comparison vs controls, one-tailed t-tests.

Thus, this dose of L-DOPA produced 6- and 13-fold increases in total mHPE and MHPE,respectively, and a 47% increase in total MHPG. In a separate experiment,a high dose of L-DOPA(500 mg/kg) produced 15-,59- and 4-fold increases in the 24-hr excretion of total mHPE,MHPE, and MHPG,respectively. Whole brain concentrations of total MHPG and pHPG in control rats(160-200g)were 61±3 and 3.1 ±0.6ng/gm,respectively. MHPE was detected in only trace amounts (<2ng/g)in control rat brains and could not be quantitated. One hour following the i.p. injection of 100mg/kg of L-DOPA, brain levels of total MHPG,pHPG and MHPE were 93±4(an increase of 53% compared to controls,p<.001), 2.5±.3,and 14.3±2.1ng/gm. mHPE was not detected in either control or L-DOPA treated animals, and if present was <0.6ng/g. Our results suggest that L-DOPA is significantly metabolized in addition to DA to both NE and m-tyramine. These latter amines may therefore account for some of the effects of L-DOPA. However, since mHPE could not be detected in brain in either control or L-DOPA treated rats, it is unlikely that the formation of m-tyramine contributes to any CNS effects of L-DOPA.

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1102 SEROTONERGIC NEURONS IN THE GANGLION OF REMAK OF THE CHICK. Miles L. Epstein, Linda Hegstrand and Michael D. Gershon. Depts. of Anatomy and Psychiatry, Univ. of Wisconsin, Madison, WI 53706 and Dept. of Anatomy, Columbia Univ., Coll. Phys. and Surg., New York, NY 10032.

The ganglion of Remak is a neural structure, unique to avians, that extends from the cloaca to the bile duct on the dorsal surface of the gut. In recent studies, we have found this neural structure to contain axons that readily take up ^{3}H-serotonin (5HT). Pieces of hindgut from chick embryos of various ages were removed, incubated with ^{3}H-5HT (0.5μM), fixed, and processed for radioautography. Examination of the rectum from 7 and 8 day embryos showed the presence of some labeled cell bodies and axons inside Remak's ganglion. In older embryos the labeling of cell bodies is no longer seen and label is found only over axons. Biochemical analysis, using the method of Savedra et al. (J. Pharm, Exp. Ther. 186:508), of Remak's ganglion from newly hatched and from 4 week old chickens indicates the presence of endogenous serotonin. These observations suggest that there are serotonergic neurons in Remak's ganglion.

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1103 FOURTH VENTRICULAR TANYCYTES IN THE RABBIT BRAIN STEM: A RELATIONSHIP WITH MONOAMINERGIC NUCLEI. David L. Felten, John P. Cummings, Suzanne Y. Felten* and Bruce Burnett*. Dept. Anat., Indiana Univ. Sch. Med., Indianapolis, IND 46227.

The ependyma of the fourth ventricle of adult and developing rabbit brains was examined with a Golgi-Cox method. Tanycytes with shafts extending into the rhombencephalon were found on the floor of the fourth ventricle. The somas of the tanycytes varied widely in shape, including cuboidal, polygonal, oval, and fusiform. Cilia frequently extended from the apical surface of the tanycyte somas into the fourth ventricle. Tanycyte necks tapered to both smooth and spiny shafts of varying lengths and widths. The widths ranged from less than 1 μm to more than 6 μm. The shafts extended into the brain stem to terminate in only 3 places: (1)Midline tanycytes on the floor of the fourth ventricle sent shafts into the midline region of the medullary raphe. These shafts entered a massive dendrite bundle and closely apposed cell bodies and dendrites of serotonergic nuclei raphe obscurus and pallidus, dendrites of the adjacent reticular formation which entered the bundle, and blood vessels of the midline. The shafts ran for 3000 μm or more in the adult rabbit medulla in a vertical orientation; (2) Paramedian tanycytes sent shafts into noradrenergic group A2 in nucleus intercalatus. The shafts abutted neurons in this region. A few shafts extended into the dorsal nucleus of X, just medial to group A2; and (3) Tanycytes at the lateral edge of the floor of the fourth ventricle sent shafts into the locus coeruleus, where they ended in apposition to cell bodies and dendrites of locus coeruleus, and local blood vessels. The shafts of the tanycytes traversed a zig-zag course through the rhombencephalon as they extended towards the monoaminergic nuclei. The tanycyte shafts extended into these nuclei as early as 18 days of fetal gestation. As the shafts reached the midline medullary raphe nuclei and locus coeruleus, the neurons showed a rapid expansion and maturation of dendritic arborizations. The tanycyte somas exhibited a very dull yellow fluorescence with the formaldehyde condensation technique. We suggest that (1) the tanycytes on the floor of the fourth ventricle send shafts almost exclusively into monoaminergic nuclei; (2) the tanycytes might serve as an anatomical communication channel between the fourth ventricle and the monoaminergic cells; and (3) the early appearance of these tanycytes in ontogeny may serve a trophic or permissive role on the dendritic sprouting of the monoaminergic nuclei in which they terminate, either directly or through a transported substance. Supported by an Alfred P. Sloan Foundation Fellowship.

1104 REGULATION OF TYROSINE HYDROXYLASE ACTIVITY IN THE DOPAMINE NEURON AFTER SEVERE DOPAMINE DEPLETION. D.C. German, B.A. McMillen, M. Dalsass, and P.A. Shore. Univ. of Texas Health Science Center, Dallas, TX 75235.

Tyrosine hydroxylase (TH) in dopamine (DA) neurons is activated not only by enhancement of impulse flow, but also by cessation of impulse flow. Enhanced impulse flow is believed to result in TH activation via removal of end product (DA) inhibition of the enzyme as well as by kinetic changes in TH. Cessation of impulse flow is believed to result in TH activation because of the absence of released DA to act on presynaptic DA receptors.

To assist in an understanding of the relative contribution of enhancement or cessation of impulse flow to TH activity, we examined the consequences of severe DA depletion on in vivo TH activity in the rat corpus striatum (by 30 min. accumulation of striatal DOPA after decarboxylase blockade with NSD-1015). Accordingly, striatal TH activity was measured after a large dose of reserpine (2.5 mg/kg s.c.). One day later, TH activity had increased by about 3 fold. With no DA in the neuron to be released, it might be expected that the neuronal firing rate in nigro-striatal neurons would be compensatorily increased, yet no DA could be released to act on DA presynaptic receptors. Thus TH activation after severe DA depletion could arise by enhanced impulse flow, by a presynaptic mechanism, or by a combination of these. γ-Butyrolactone (GBL), which has been shown to cause a cessation of nigro-striatal impulse flow and to increase TH activity by a presynaptic mechanism, enhanced striatal TH activity about 3 fold. If reserpine's effect on TH were due solely to a lack of release of DA to act on presynaptic receptors, then GBL should not affect this action of reserpine. However, we found that GBL inhibited by over 50 percent the elevation of TH activity seen in one day reserpine rats. A similar degree of TH activation was observed in rats pretreated for 30 min. with the potent DA depleter, Ro 4-1284 (2.5 mg/kg i.p.), and GBL similarly inhibited this activation.

The marked inhibition by GBL of the elevation of TH activity seen after reserpine or Ro 4-1284 suggests that a significant portion of the enzyme activation is associated with enhancement of DA neuronal impulse flow. After the combination of reserpine-GBL (or Ro 4-1284-GBL), TH activity is actually lower than that seen following GBL administration to normal rats. This observation may be explained by the removal (by reserpine or Ro 4-1284) of DA storage function modulation of presynaptic regulation of TH activity as discussed elsewhere at this meeting (McMillen and Shore). Electrophysiological experiments are in progress studying the effects of DA depletion on DA neuronal impulse flow. (Supported by USPHS Grants MH-27574, MH-30546 and MH-05831).

1105 PROLIFERATION AND LACK OF SPECIES AND ORGAN SPECIFICITY IN THE TRANSIENT CATECHOLAMINERGIC CELLS OF DEVELOPING MAMMALS. Michael D. Gershon, Gladys Teitelman, Taube P. Rothman, Tong H. Joh and Donald J. Reis. Dept. of Anatomy, Columbia Univ. Coll. of P&S, New York, New York, 10032 and Dept. Neurol. Cornell Med. Coll., New York, New York, 10021.

Transient catecholaminergic (TCA) cells have been found in the mesenchyme of the developing rat gut (Cochard, Goldstein and Black, P.N.A.S. 75:2986, 1978; Teitelman, Joh and Reis, Brain Res. 156:229, 1978). These cells appear at 11 days' gestation and disappear before day 15. The TCA cells contain catecholamine and the biosynthetic enzymes, tyrosine hydroxylase (TH) and dopamine beta hydroxylase. In order to further evaluate the TCA cells we sought to determine if their appearance was specific for the rat gut or if they also appear in other species and in other organs. We also wanted to know if the cells are immature and still proliferating, or mature and postmitotic. The immunocytochemical demonstration of tyrosine hydroxylase (TH) with the unlabeled antibody, peroxidase-antiperoxidase method was used as a marker for TCA cells. A single injection of ^{3}H-thymidine (^{3}H-TdR), with a 2 hour survival period, was used to assess their proliferative ability. TCA cells were not limited to rat gut. They were also found in mouse gut where they were present by 10 days' gestation and disappeared before day 13. In addition, TCA cells appeared in rat kidney, sacral spinal cord, and dorsal mesentery. Several cells in the rat gut and other locations simultaneously displayed both markers. That is, they contained TH and were labeled by ^{3}H-TdR. We conclude that the TCA cells do not only occur in rats but occur in other mammalian species as well. Some of these ectopic and evanescent cells also colonize organs other than the gut. At least some of the TCA cells divide. These data suggest that migrating catecholaminergic precursor cells reach a variety of sites that do not, in adult mammals, contain adrenergic neuronal perikarya. It remains to be determined whether these immature dividing cells die or acquire non-adrenergic characteristics. Supported by NIH grants NS12969, HL18974, MH2485, NASA grant, NSG-2259, and a Basil O'Connor starter research grant of the National Foundation - March of Dimes.

1106 SLOW BURSTING NEURONS IN THE PREOPTIC/ANTERIOR HYPOTHALAMUS OF UNANESTHETIZED RABBITS: EFFECT OF NOREPINEPHRINE, SEROTONIN, AND THERMAL STIMULATION. C. J. Gordon* and J. E. Heath. Dept. of Physiology and Biophysics, Univ. of Illinois, Urbana, IL 61801.

Rabbits were stereotaxically implanted with a guide tube to direct a microelectrode into the preoptic/anterior hypothalamus (POAH), a thermode and thermocouple reentrant tube to change and measure POAH temperature, respectively, and a cannula in the lateral ventricle to inject norepinephrine (NE) and serotonin (5-HT). A microdrive was secured to the head for lowering a tungsten microelectrode into the medial POAH. Firing rate of single units was recorded while the temperature of the POAH was clamped near 41°C and 34°C. When a neuron could be thermally classified, NE or 5-HT (30-100 µg) was infused into the lateral ventricle in concentrations of 1-3 µg/µl. Occasionally the single units were thermally stimulated both before and after the application of a neurotransmitter.

A total of 55 single units were isolated in the POAH with 30% displaying a rhythmic bursting activity. The time of bursting ranged from 9 to 67 seconds with a mean of 23±4 seconds. Three types of bursting neurons were isolated: (a) tonically firing cells induced into bursting by POAH thermal stimulation, (b) normally bursting units not influenced by POAH temperature but inhibited by NE (12% of bursting units) or 5-HT (19%), and (c) tonically firing cells induced into bursting by 5-HT (18% of bursting units) or NE (6%). In category "a" thermally induced bursting was inhibited by NE (12%) and 5-HT (19%).

Past investigations dealing with the measurement of thermally mediated motor responses have revealed rhythmic motor responses with frequencies similar to the neural bursting rhythms reported in this study. These monoamine and thermally sensitive bursting neurons may be important in the mediation of thermoregulatory reflexes.

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1107 EXCITATORY EFFECTS OF GABA AGONISTS ON NIGRAL DOPAMINE CELLS: MEDIATION BY ACTION ON RETICULATA INHIBITORY NEURONS. Anthony A. Grace* and Benjamin S. Bunney. Depts. Pharmacol. & Psychiatry, Yale Univ. Sch. Med., New Haven, CT 06510.

A number of investigators using biochemical and electrophysiological techniques have reported the apparent excitatory effects of indirectly applied GABA agonists on dopamine (DA) cells of the substantia nigra. We further investigated the effects of GABA on substantia nigra neurons using single unit recording techniques and microiontophoresis. Both DA neurons and non-DA zona reticulata neurons were found to be inhibited by microiontophoretically applied GABA. However, a population of non-DA cells in the zona reticulata (ZR) was found to be 20 times more sensitive to the inhibitory effects of GABA than were DA neurons. GABA applied microiontophoretically in the vicinity of the ZR neuron caused an increase in activity of nigral DA cells recorded simultaneously. This effect was blocked by i.v. picrotoxin. Conversely, the excitatory substance glutamic acid, when iontophoretically ejected into the ZR, caused an inhibition of DA neurons which was also blocked by i.v. picrotoxin. Dose response studies with the GABA agonist muscimol, administered parenterally (i.v.), revealed that incremental doses of muscimol increased the firing rate of DA neurons in a stepwise fashion which was directly proportional to the parallel inhibition this treatment induced in non-DA cells. Thus, GABA agonists appear to cause an increase in nigral DA cell firing rate by preferentially inhibiting GABA sensitive GABAergic reticulata neurons (i.e. through a process of disinhibition).

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1108

Withdrawn by Author

1109 THE SPINAL PROJECTION OF THE LOCUS COERULEUS (LC) = AN ELECTROPHYSIOLOGICAL STUDY. P. G. Guyenet, University of Virginia School of Medicine, Charlottesville, VA 22908.

It has been reported in one recent histofluorescence study that all the noradrenaline (NE)-containing neurons of the rat LC may send a collateral into the spinal cord (SC); using retrograde labelling techniques, others have suggested that only a fraction of LC cells, located in the posterior and deeper portions of the nucleus, send axons into the SC. In the present study, we confirm that even after multiple and massive pressure injections of HRP in the rat cervical SC (C_5 - C_7) only a subpopulation of LC cells, located as previously described, show evidence for the presence of the marker (2 - 3 µl total of 50% HRP, Hanker-Yates reaction). This result prompted us to perform a comparative electrophysiological study of SC and other LC projections. Single barrel or five barrel electrodes were used for recording single unit activity in rats anesthetized with chloral hydrate and bipolar concentric electrodes to stimulate the SC (C_6 - C_7) or the dorsal NE bundle (DB).

A small minority of LC cells only could be driven antidromically (AD) from the SC with latencies of 25 - 50 msec corresponding to conduction velocities of 0.5 to 1 m/sec. Prominent A-B break was observed with paired stimuli. The refractory periods of the A and B segments were respectively 2 - 2.5 msec and 12 - 100 msec. These cells were spontaneously firing at 0.2 to 2.5 spike/sec and could be totally inhibited by iontophoretic morphine and GABA. Their localization in the LC, as determined by the site of a spot of fast green iontophoresed after completion of recording, matched closely the topography of HRP labelled cells observed after SC injections.

Essentially in agreement with previous studies, most LC neurons could be AD-activated by stimulating the DB with latencies corresponding to conduction velocities of .6 to 0.8 m/sec. The refractory periods of the A and B components of the spikes were identical with that of neurons AD-activated from the SC. Three of these neurons (in 5) could be AD-activated both from the SC and the DB, although the current required to drive them from the DB was larger than that required to drive the majority of other LC cells.

It is concluded that the LC projection to the SC is composed of a subpopulation of neurons. These cells have an anteriorly directed collateral which presumably does not travel in the DB. Their electrophysiological and pharmacological properties are similar to those of the other cells in the nucleus.

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1110 ALTERATIONS IN [^{3}H]CLONIDINE BINDING DUE TO 6-HYDROXYDOPAMINE AND MAO INHIBITORS. Margaret A. Hamburg*, Dorothy W. Gallager, Iain Campbell*, and John F. Tallman (SPON: W. E. Bunney, Jr.). Biological Psychiatry Br. and Clinical Neuropharmacology Br., NIMH, Bethesda, MD 20205

[^{3}H]Clonidine, an α-adrenergic agent, binds specifically to membranes prepared from rat cerebral cortex. Binding is rapid, reversible and saturable. Initial studies suggested two binding sites for clonidine; however, a ten-fold dilution of membranes led to the appearance of a single set of sites with properties like the higher affinity site. In addition, when incubations are carried out in the presence of guanyl nucleotides, GTP and analogues decrease steady-state binding by enhancing the dissociation rate ten fold due to the elimination of a slowly dissociating component of binding. Thus, only in the presence of GTP does both rapid and complete dissociation of bound clonidine occur. These experiments indicate that several factors might be responsible for the appearance of the two sites. The resolution of two sites into a single site depending upon assay conditions is supported by data showing the pharmacological profile of the apparent two sites to be identical: epinephrine > clonidine > norepinephrine > piperoxane > yohimbine > WB4101 > prazosin. This profile is different from that of the WB4101 binding site: prazosin > WB4101 > epinephrine > piperoxane > yohimbine > clonidine.

Lesions induced by intraventricular injection of 300 µg of 6-hydroxydopamine (6-OHDA) resulted in increases in the binding of [^{3}H]clonidine in cortex. Scatchard analysis over a full range of concentrations (0.25-40 nM) indicated a similar increase in binding at all points. WB4101 binding was also enhanced by 6-OHDA treatment.

Chronic (but not acute) treatment with the MAO inhibitor clorgyline (4 mg/kg, s.c. daily) resulted in a 25% decrease in the number of clonidine binding sites with no change in affinity. No changes in WB4101 binding sites were observed. These experiments indicate that [^{3}H]clonidine binds to a single population of high-affinity binding sites in brain which are distinct from binding sites for WB4101.

1111 LOCUS COERULEUS LESIONS: EFFECTS ON CEREBRAL OXIDATIVE METABOLISM IN VIVO: III-ACUTE AND CHRONIC CHANGES IN RELATION TO ADRENERGIC RECEPTORS. Sami I. Harik, Joseph C. LaManna, Myron Rosenthal and Shailesh P. Banerjee, Dept. Neurol., U. Miami Med. Sch., Miami, FL 33101 and Dept. Pharmacol., U. Rochester Med. Sch., Rochester, N.Y. 14642

Two weeks following unilateral locus coeruleus (LC) lesion produced by the local injection of 6-OH dopamine norepinephrine (NE) is depleted in the ipsilateral cerebral cortex of rats. NE levels in the cortical hemisphere contralateral to LC lesion are not different from those of control (sham lesioned) rats. NE depletion is associated with an increase in the density of β-NE receptors as measured by the specific binding of ^{3}H-dihydroalprenolol. No changes, however, in α-NE receptors are noted as measured by the specific binding of ^{3}H-WB-4101. NE depletion is also associated with loss of the normal blood volume increase when the cortical surface is stimulated to increased activity by electrical pulses. Also, re-reduction of cytochrome a,a_3, oxidized during the increased energy demand is slowed. The latter measurements were made from cortical tissues in situ by dual wavelength reflection spectrophotometry.

In order to determine if compensatory changes occur in NE-depleted hemispheres and to study the natural history of denervation supersensitivity, similar measurements were made 6 weeks following unilateral LC lesions. In these rats, marked NE depletion was again demonstrated in the cortical hemisphere ipsilateral to LC lesion. Despite the marked differences in NE levels between the two hemispheres, no differences were detected in the binding characteristics of α and β-NE receptors between the two sides. Likewise, increased energy demand produced by electrical stimulation was accompanied in both hemispheres by increased blood volume and transient oxidations and re-reduction of cytochrome a,a_3. The rates of each of these reactions did not differ in the two hemispheres.

The return of the density of β-NE receptor binding sites, as well as the metabolic events, to conditions similar to those of the control hemispheres, may be causally related or simply epi-phenomana of another event. In either case, it appears that the metabolic reactions described above may serve as an in vivo physiological index of β-NE receptor denervation supersensitivity. (Supported by Jack Wechter Memorial Fund)

1112 REGIONAL CHANGES IN CENTRAL NERVOUS SYSTEM CATECHOLAMINE METABOLISM DURING PERFORMANCE ON FIXED RATIO OR VARIABLE INTERVAL OPERANT SCHEDULES. Thomas G. Heffner, Daniel Luttinger*, John A. Hartman* and Lewis S. Seiden. University of Chicago, Chicago, IL 60637.

Previous studies have indicated that the performance of operant behavior is accompanied by changes in the metabolism of both norepinephrine (NE) and dopamine(DA) in the brains of rats. These experiments were designed to determine: 1) which central catecholamine (CA) projections are involved in this phenomenon and 2) if performance maintained by different operant schedule requirements is associated with differential alterations in CA metabolism. CA turnover rates in various brain regions were estimated from the decline of NE or DA levels from brain tissue following administration of the CA synthesis inhibitor α-methyltyrosine (αMT , 250 mg/kg, i.p.). Initial studies established that the decline of NE and DA from each brain region examined conformed to a single exponential function during a period of 1 hr after αMT injection. Water-deprived rats trained on either a fixed ratio 5 (FR-5) or a variable interval 5 second (VI-5) schedule of water reinforcement were placed in operant testing chambers after αMT injection. Water-deprived control rats were returned to single housing cages following αMT. All rats given αMT were sacrificed 37 min after injection. In rats performing on the FR or VI schedules, the apparent turnover rate of DA was increased relative to that in water-deprived control rats in the anterior striatum, the anterior hypothalamus, the amygdala and the spinal cord. No behavior-related changes in DA turnover rates were detected in the posterior striatum, posterior hypothalamus, nucleus accumbens, olfactory tubercle, septum, frontal cortex, ventral tegmental area or substantia nigra. Animals performing on the FR or VI schedules also showed an increased turnover of NE in the anterior hypothalamus relative to controls but showed no apparent changes in the relative rates of NE turnover in the posterior hypothalamus, nucleus accumbens, olfactory tubercle, amygdala, septum, substantia nigra, ventral tegmental area, hippocampus, cerebellum, frontal cortex or spinal cord. No schedule-dependent differences in the turnover rates of CA were observed between rats performing on the FR and VI schedules. These results indicate that the performance of operant behavior is associated with highly specific regional changes in the metabolism of catecholamines in brain. As such, these findings suggest that particular groups of central DA and NE neurons are involved in the mediation of conditioned behavior. (Supported by USPHS MH-11191-, MH-14274, MH-10562, and GM-07151).

1113 DECARBOXYLATION OF DOPA IN THE RAT STRIATUM AFTER COMPLETE AND PARTIAL UNILATERAL LESIONS OF THE NIGRO-STRIATAL SYSTEM. F. Hefti, E. Melamed, J. Liebman* and R.J. Wurtman. Lab. Neuroendocrine Regulation, MIT 56-245, Cambridge, Mass. 02139, USA.

The effects of L-DOPA were studied in animal models for early and end stage Parkinsonism using rats with partial and complete unilateral nigro-striatal lesions. 0.5 to 8ug 6-OH-DA injected in the substantia nigra (SN) produced a gradual decrease of striatal dopamine (DA) content. Complete lesions were obtained by injecting 8ug 6-OH-DA in the SN and additional 4ug in the medial forebrain bundle. After 2 weeks, the animals were injected with DOPA (100 mg/kg, 45min) and tyrosine hydroxylase (TH) and DOPA decarboxylase (DDC) activities were measured, as well as DA and its metabolites DOPAC and HVA (using HPLC with electrochemical detection).

In untreated animals with partial lesions, there was a linear correlation between TH activity and DA content in the lesioned striatum, indicating that TH activity can be used as a marker for the extent of the lesion. The DOPAC/DA and HVA/DA ratios were increased when the lesion reduced TH to more than 50%, suggesting increased DA release from the remaining neurons. Administration of DOPA produced increases in the striatal content of DA, DOPAC and HVA. In the lesioned side, DA levels did not reach the control side values whereas DOPAC and HVA were higher. After complete lesions (TH activity reduced to 5%, DDC to 20%, DA to 2%), DOPA administration increased DA concentration 4-fold to 8% in the lesioned striatum and 2-fold to 200% on the unlesioned side as compared to untreated and unlesioned controls. DOPAC and HVA levels were elevated far above control values on both sides. DOPA induced strong turning behavior away from the lesioned side, suggesting that the DA formed is functionally significant.

Animals with complete unilateral lesions and additional electrolytic lesions in the medial and dorsal raphe nuclei, which reduced striatal serotonin to 15%, also turned after DOPA and showed similar increases in striatal DA, DOPAC and HVA as rats with unilateral 6-OH-DA lesions alone after DOPA. In an additional experiment, different raphe lesions, which reduced striatal serotonin content ranging from 0 to 90%, failed to decrease striatal DDC activity. In order to test whether DOPA is decarboxylated in striatal blood vessels, animals with a complete cerebral hemisection were injected with DOPA and a peripheral decarboxylase inhibitor (which should inhibit DDC in blood vessel walls); DOPA still increased the very low DA content and markedly elevated DOPAC and HVA. In isolated bovine striatal microvessels, only a very low DDC activity was observed.

The data do not favor a major role of DDC contained in serotoninergic terminals or blood vessels in the decarboxylation of DOPA in the parkinsonian brain. This process may occur in residual DA neurons, but a role of other striatal cells is not excluded.

1114 CHANGES IN [H^3]-5-HYDROXYTRYPTAMINE RECEPTOR BINDING IN POST-MORTEM TISSUES: REGIONAL DISTRIBUTION OF [H^3]-5-HYDROXYTRYPTAMINE BINDING IN HUMAN BRAIN. J. A. Heltzel and W. H. Vogel, Dept. Pharmacology, Thomas Jefferson University, Philadelphia, PA 19107.

Membrane binding of [H^3]-5-hydroxytryptamine (5HT) was determined in crude homogenate preparations of whole rat brain minus cerebellum using the procedure of Bennett and Snyder (Molecular Pharmacol. 12: 373, 1976). Binding parameters of freshly dissected tissue and tissue frozen immediately following decapitation were evaluated by tissue linearity studies in the presence of 7 nM 5HT and by varying the ligand concentration in the incubation medium. 100 μM unlabelled 5HT served as a blank for non-specific binding.

	pmoles 5HT bound per gram tissue	K_D	B_{max}
Fresh Control	4.90 ± 1.34 (8)	1.4 nM	14.9
Frozen Control	4.34 ± 1.24 (4)	2.1 nM	17.4

No significant changes were noted in pmoles 5HT bound/g using 7 nM 5HT ligand, in the equilibrium dissociation constant (K_D), or in the number of receptor sites (B_{max}) between fresh and frozen tissue. Because specific 5HT binding represented a higher fraction of total binding at 3.5 nM ligand concentration, this level was used in the remaining studies. To simulate human post-mortem conditions, rats were killed by cervical dislocation, allowed to remain at room temperature for 3 hours, and refrigerated for 16 - 18 hours; brain tissue was removed on ice and stored at -70°C for 24 hours or longer.

	pmoles 5HT bound per gram tissue	K_D	B_{max}
Frozen Control	1.73 ± 0.67 (29)	2.1 nM	17.4
Frozen Post-mortem	2.76 ± 0.90 (31)	3.7 nM	10.6

Significant increases ($p < .001$) were observed in specific 5HT binding in post-mortem samples. An increase in the K_D was also seen in tissues from rats treated to reproduce human post-mortem conditions. 5HT receptor density appeared to be reduced.

Membrane receptor binding of 5HT in homogenates of human post-mortem brain samples showed the following preliminary profile of regional 5HT binding: pallidum > precentral cortex, hypothalamus > postcentral cortex, thalamus > substantia nigra, putamen. Regional distribution was estimated as a function of the pmoles 5HT specifically bound per gram tissue. Regional distribution determined by Scatchard analysis of subcellular fractions of human post-mortem brain samples are in progress.

1115 MULTIPLE ELECTROCONVULSIVE SHOCKS INCREASE AMPHETAMINE-INDUCED ROTATIONAL BEHAVIOR BUT REVERSE ITS PREFERRED DIRECTION IN RATS. Gordon K. Hodge and Thomas L. Hall*. Department of Psychology, University of New Mexico, Albuquerque, NM 87131.

Although the mechanism responsible for the therapeutic effect of electroconvulsive shock (ECS) remains obscure, biochemical, pharmacological, and some behavioral data suggest partial involvement of catecholamine (CA) systems. Manipulations of CA systems in rats affect a number of behaviors; if ECS treatment alters CA function, then it might be expected that ECS would produce commensurate behavioral changes in these animals.

To evaluate this possibility we compared effects of multiple ECS treatments (50 ma for 350 msec delivered across the ears; 1 treatment per day for 8 consecutive days; n = 14) with sham treatments (electrodes connected but no current; n = 14). 48 h before ECS treatments, all animals were administered d-amphetamine sulfate (2.0 mg/kg, i.p.), and rotational behavior was recorded for the following 3 h. 48 h after the last ECS treatment, all rats were again given amphetamine, and their rotational activity recorded for 3 h. Body weights were measured on days of amphetamine administration.

Amphetamine-induced rotational behavior increased following ECS treatments ($p < .05$). Moreover, the preferred sides of rotation, as assessed by pretreatment directions, were reversed by ECS ($p < .01$). Posttreatment body weights of ECS-treated rats were lower than those of sham controls ($p < .01$).

ECS treatment undoubtedly creates profound alterations in many neural systems. But because it is therapeutically efficacious in reducing depression, and since drugs which facilitate CA function are also effective in this regard, it is possible that at least one mechanism mediating ECS effects involves enhancement of CA systems. Our results are consistent with this interpretation. Insofar as increased rotational behavior is indicative of increased activity, the increased rotations of the ECS animals may have been due to higher activity of CA systems; this is consistent with pharmacological potentiation of these systems which results in hyperactivity and increased rotations. The cause of reversals in preferred direction of rotation is unknown, but such reversals may reflect a disparate effect of ECS on different systems or perhaps ECS exerts differential effects on functional components of the same system. While a number of factors may have accounted for the observed weight reductions in the ECS rats, it is known that facilitation of CA systems also promotes weight loss over the short term.

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1116 LONG-TERM EFFECTS OF MULTIPLE DOSES OF METHAMPHETAMINE ON STRIATAL AND HIPPOCAMPAL TRYPTOPHAN HYDROXYLASE IN THE RAT. A.J. Hotchkiss* and J.W. Gibb, Dept. Biochem. Pharmacol. and Toxicol., University of Utah, Salt Lake City, Utah 84112.

Previous studies have shown that multiple sequential doses of methamphetamine (METH) cause a decrease in nigrostriatal tyrosine hydroxylase (TH) activity (Koda, L.Y. and Gibb, J.W., Pharmacologist 13 [1971] 253). Since there are many anatomical and biochemical similarities between the extrapyramidal dopaminergic system and the serotonergic projections from the raphe nuclei to the telencephalon, the activity of tryptophan hydroxylase (TPH), considered to be a marker for serotonergic neurons, was measured in the neostriatum and the hippocampus as well as in the pineal and midline raphe nuclei following multiple doses of METH.

Neostriatal TPH was reduced to 20% of control (35 mm/g/hr) at 24 hours following 4 doses of METH (15 mg/kg, s.c., every 6 hrs). In animals treated with a 5-dose regimen, TPH remained about 20% of control at 36 hours, 3.5 days and 8.5 days. At each time TH was also significantly reduced (40 to 50% of control).

The dose-effect of METH was evaluated using 4 sequential doses at 6-hour intervals and rats were sacrificed at midday, 5 days after the first METH dose. The doses studied were 5, 10, and 15 mg/kg. At this time, neostriatal TPH was 104%, 68% and 30% while TH was 96%, 91% and 56% respectively. A similar dosage schedule and time of sacrifice was used to evaluate three drugs for their ability to block METH effects on TPH; these drugs have been shown to block METH-induced depression of TH. Haloperidol (3 mg/kg i.p.), α-methyltyrosine methylester, (60 mg/kg, i.p.) or aminooxyacetic acid (30 mg/kg, i.p.) was given 4 times, concurrently with METH (15 mg/kg); in every case both neostriatal TH and TPH activities were not depressed when each of these drugs were coadministered with METH. This suggests that the mechanisms of action of METH and the drugs blocking the METH effect may be similar in both the dopaminergic and the serotonergic systems.

Other nuclei were also studied. Five days after 4 METH doses (15 mg/kg), hippocampal TPH was decreased to 28% of control while pineal TPH was not changed significantly. Midline raphe nuclei taken from a 1 mm slice (A 0.5 to P 0.5 mm, according to König and Klippel) also showed no change in TPH 5 days after 4 doses of METH. (Supported by USPHS grant 00869.)

1117 A SIMPLE TECHNIQUE TO DEMONSTRATE MONOAMINE CONNECTIONS UTILIZING AXONAL TRANSPORT OF A FLUORESCENT DYE IN COMBINATION WITH THE GLYOXYLIC ACID METHOD. Albert O. Humbertson, Jr. and Walter R. Buck, Dept. Anat., Sch. Med., The Ohio State University, Columbus, O., 43210.

Recently several investigators have combined the HRP technique with the glyoxylic acid method in order to determine if any of the neurons contributing to specific projections contain monoamines. This combined technique is useful, but somewhat laborious and time consuming. We have overcome some of the difficulty by utilizing a fluorescent dye, 4,6-diamino-2-phenylindole (DAPI), in place of HRP as a retrograde marker. This dye is retrogradely transported the same as HRP, but has the advantage that unfixed frozen sections can be used from the animal. The labelled neurons can be identified on slides with a fluorescence microscope equipped with a 360nm. filter system. We tested the feasibility of using this system with the glyoxylic acid method by injecting the fluorescent dye into the cervical spinal cord. Two days following the injection the animals were sacrificed and the brainstems and spinal cords were processed by the glyoxylic acid method. The sections were viewed with a 490nm. filter system in order to identify the monoamine containing neurons. The filter system was changed to 360nm. to identify the neurons containing the retrogradely transported DAPI. By merely switching the filter system back and forth, doubly labelled neurons can be identified. Details of the technique will be presented. (Supported by U.S.P.H.S. Grant NS-07410.)

1118 REGULATION OF RETINAL TYROSINE HYDROXYLASE: LONG-TERM EXPOSURE TO LIGHT INCREASED THE APPARENT Vmax WITHOUT A CONCOMITANT INCREASE OF ENZYME MOLECULES. P.M. Iuvone* and N.H. Neff. Dept. Pharmacol., Emory Univ. Sch. Med., Atlanta, GA 30322 and Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hosp., Washington, D.C. 20032

Continuous exposure of rats to light increased the rate of dopamine turnover in retinal amacrine neurons and gradually elevated the apparent Vmax of retinal tyrosine hydroxylase (TH) over a 4 day period. Three different immunotitration procedures were performed on retinal homogenates to determine if the change in apparent Vmax was caused by a change in the amount of enzyme protein or a change in the specific activity of the enzyme. Antibody directed against rat adrenal TH (donated by T.H. Joh) effectively inhibited retinal TH activity. All three immunotitration procedures indicate that the change in apparent Vmax was due to a change in TH specific activity rather than to a change in the amount of enzyme molecules. In addition, the change in apparent Vmax was found to be pH dependent, further supporting the notion of a change in specific activity.

Retinal amacrine neurons have dendritic processes but not axons. Our results suggest that changes in the specific activity of TH may be one of the mechanisms responsible for regulating catecholamine synthesis in dendrites.

1119 COMPARATIVE EFFECTS OF HALLUCINOGENIC DRUGS ON BEHAVIOR AND RAPHE UNIT ACTIVITY IN FREELY-MOVING CATS. Barry L. Jacobs and Michael E. Trulson. Program in Neurosci., Dept. Psychol., Princeton Univ. Princeton, NJ 08544.

The effect of hallucinogenic drugs is hypothesized to be mediated primarily through a depression of the activity of serotonin-containing neurons. This hypothesis was tested by recording single unit activity from the dorsal raphe nucleus of freely moving cats (see complete methodology in Brain Res. 163: 135-150, 1979), while concomitantly scoring the occurrence of hallucinogen-specific behaviors (e.g. limb flicking and abortive grooming). The following drugs were administed i.p.

Drug	Dose (µg/kg)	Raphe unit activity (change from baseline)	Limb flicks/hr
LSD	10	-18.0%	12.7
	50	-48.0%	25.8
DOM	50	- 9.5%	4.8
	250	-41.8%	32.2
Psilocin	100	-19.3%	5.0
	750	-54.5%	1.6
5-MeODMT	10	-14.2%	1.0
	50	-80.8%	5.8
	100	-87.6%	11.7
Amphetamine	5000	+64.0%	0

In general, these data confirm previous studies of the action of these drugs on raphe neurons in immobilized and/or anesthetized rats. These results also provide general support for the serotonin hypothesis of hallucinogenic drug action, since the four hallucinogens produced significant decreases in raphe unit activity, whereas amphetamine did not. However, since the potency of these various drugs in producing hallucinations in humans, and limb flicks in cats, i.e., LSD>DOM>5-MeODMT≅psilocin, does not correspond to their efficacy in depressing raphe unit activity, the latter effect apparently does not account for all of the important actions of hallucinogenic drugs. Aghajanian has shown that these drugs differ in their preferential action on raphe neurons as compared to their postsynaptic target neurons. Furthermore, several recent studies have shown that LSD and DOM have potent dopamine agonist effects, in addition to their effects on the brain serotonin system. Psilocin and 5-MeODMT, on the other hand, have little or no effect on the dopamine system. Therefore, we propose that inactivation of serotonergic neurotransmission is necessary and sufficient for hallucinogenesis, and that an additional dopamine agonist action greatly potentiates these effects.

1120 CHANGES IN CATECHOLAMINE CONCENTRATION-TIME PROFILES IN HEART. BRAIN AND PLASMA AFTER ACUTE ISCHEMIC STROKE. NADER S. JALLAD*, DONALD J. WEIDLER. Division Of Clinical Pharmacology Department of Pharmacology, University of Miami, Miami, Florida.

We have demonstrated previously that the onset of acute cerebral ischemia causes myocardial damage and cardiac arrhythmias. Since the alteration of tissue and plasma catecholamine concentrations may be important in effecting these cardiac changes, the concentrations of norepinephrine, epinephrine and dopamine in heart, brain, and plasma were studied as a function of time for 24 hr after the onset of acute ischemia stroke. Twenty-four anesthetized cats underwent ligation of the left middle cerebral artery, after which cats were permitted to awaken. Plasma samples were taken hourly and 4 cats each were killed at 4, 8, 12, 16, 20 and 24 hr. In brain tissue, norepinephrine and dopamine concentrations became significantly depleted in the ischemic cerebral hemisphere; but not in the non-ischemic hemisphere. The epinephrine concentrations in both ischemic and non-ischemic hemispheres did not change over the 24 hr. Plasma norepinephrine concentrations manifested two major peaks one at 8 hr and one at 20 hr. Plasma dopamine had a major peak at 8 hr and a minor peak at 18 hr, while plasma epinephrine peaked at 8 hr and 16 hr. In myocardial (left ventricular) tissue, the norepinephrine concentration was significantly elevated only in the 16-20 hr range; dopamine was elevated in the 4-8 hr range and peaked again at 16 hr. Myocardial epinephrine concentration had only one major peak, which occurred at 8 hr. The peak norepinephrine concentration in the heart (at 16 hr) was approximately 10 times the peak concentrations attained by epinephrine and dopamine. Based on these findings, we conclude that a period of increased susceptibility to cardiac arrhythmias may occur in the 16 -20 hr period after the onset of acute cerebral ischemia.

1121 CIRCADIAN RHYTHMS IN α- and β-ADRENERGIC RECEPTOR BINDING IN RAT BRAIN. Marian S. Kafka, Anna Wirz-Justice*, Dieter Naber*, and Alfred J. Lewy. National Institute of Mental Health, Bethesda, MD 20205

Psychoactive drugs such as antidepressants and neuroleptics alter specific binding to brain adrenergic receptors. In the pineal gland, a marked circadian rhythm in β-adrenergic sensitivity has been found in the absence of drug treatment. This study was designed to investigate whether under physiological conditions a circadian rhythm in α- and β-receptor binding was present in the brain.

Male Sprague-Dawley rats maintained for 3 weeks on a controlled light/dark cycle (lights on from 7 a.m. to 7 p.m.) were sacrificed in groups at 4-hour intervals, plasma prepared from the pooled blood, and hypothalami and forebrains (anterior to the cerebellum) dissected and frozen. Alpha- and β-adrenergic receptors in rat brain membranes were measured by the specific binding of [^{3}H]-WB4101 and [^{3}H]dihydroalprenolol, respectively. Plasma melatonin was measured by gas chromatography-mass spectrometry.

Melatonin showed the expected nocturnal rise, indicating the adequacy of the entraining light/dark cycle. Forebrain α-adrenergic receptor binding increased in the afternoon. The difference between minimum and maximum binding was 37% (analysis of variance, $F = 7.18$, $p < 0.001$). β-Adrenergic receptor binding increased in the morning. The difference between minimum and maximum binding was 39% (analysis of variance, $F = 5.48$, $p < 0.001$). Binding to α- and β-adrenergic receptors in the hypothalamus followed the same patterns as in the forebrain.

The circadian variation in α- and β-receptor binding suggests that circadian changes in the occupancy of adrenergic receptors by the neurotransmitters norepinephrine and epinephrine may modulate adrenergic function in the brain. The short-term physiological changes in adrenergic receptor binding are of the same order of magnitude as the changes observed after long-term drug treatments. While the time of day at which the rats are sacrificed (e.g., morning or afternoon) alters the magnitude of the receptor binding, it remains to be seen whether drug-induced changes in the number of adrenergic receptors are dependent on changes in the number of receptor sites, a shift in the timing (phase) of the circadian rhythm, or on both of these factors.

1122 RELEASE OF NOREPINEPHRINE AND DOPAMINE IN VITRO FROM BRAIN REGIONS OF AMYGDALOID KINDLED RATS. G. Jean Kant, James L. Meyerhoff, and Michael E. Corcoran. Dept. Medical Neurosciences, Walter Reed Army Institute of Research, Washington, D.C. 20012, and Dept. Psychology, Univ. of Victoria, Victoria, B.C., Canada.

Goddard et al. reported that brief bursts of subconvulsive non-polarizing electrical brain stimulation presented chronically to certain brain structures resulted eventually in a permanent change in the response of the animal to include localized seizure discharge, behavioral automatisms and generalized convulsions. This "kindling effect" has been shown to be trans-synaptic in nature, but the mechanism and specific neurons involved have not been identified. Considerable data indicate that brain catecholamine systems suppress various types of seizure susceptibility. It has been shown that drugs that potentiate norepinephrine and dopamine function suppress seizures while drugs that interfere with these monoamines can increase seizure susceptibility.

In our laboratory we have developed a sensitive model technique to measure small absolute amounts of endogenous norepinephrine and dopamine released in vitro. We decided to use this method to determine whether amygdaloid kindling involves permanent changes in the releasability of NE and/or DA from various brain regions.

Thirty-four male Long-Evans rats were stereotaxically implanted with bipolar electrodes into the basolateral amygdala. Seventeen animals were kindled beginning 10 days after surgery. Each day the rats received 1 sec of constant current 60 Hz square wave stimulation. The remaining rats served as yoked controls, receiving electrical stimulation at 3 Hz (a non-kindling frequency) for 1 sec. daily.

Three weeks after the last seizure, the animals were sacrificed by decapitation; the brain was removed and dissected into: amygdala-pyriform, frontal cortex, parietal cortex, caudate, accumbens-tubercle, thalamus, hippocampus, hypothalamus, and septal region. The brain tissue pieces were weighed, chopped (0.3mm x 0.3mm), and washed twice with cold Krebs-Ringer bicarbonate buffer. Each sample was then resuspended in 1.0 ml of 37°C buffer and incubated at 37°C for 10 minutes with or without added KCl to bring the final KCl concentration to 5,15 or 45 mM. Release was terminated by centrifugation and the supernatants were analyzed for NE and DA by radioenzymatic assay.

Spontaneous release of NE and DA was measurable in all regions studied. Added Potassium stimulated an increase in release of both transmitters with 45mM KCl having a greater effect than 15mM KCl.

Both spontaneous and KCl-stimulated release of NE and DA were similar for both kindled and control tissue in all tested regions.

1123 PHENYLACETIC ACID EXCRETION IN SCHIZOPHRENIA. F. Karoum, L. Chuang*, S. Potkin* and R. J. Wyatt. Lab. of Clinical Psychopharmacology and Lab. of Preclinical Pharmacology, DSMHR, Division of Special Mental Health Research, NIMH, Saint Elizabeths Hospital, Washington, D. C. 20032

Phenylethylamine (PEA) is hypothesized to be elevated in some schizophrenic patients. To further assess the role of PEA in this disorder, we measured the excretion of phenylacetic acid (PAA), the major metabolite of PEA, in 31 normal subjects and, 29 chronic schizophrenics.

The total and glutamine conjugate of PAA excretion was significantly ($p<0.01$) reduced in the chronic schizophrenic patient. It was unaltered in depressed patients. The total excretion of PAA (mean ± SEM) in controls, and schizophrenics was respectively, 161.87 ± 19.1 and 100.38 ± 12.5 mg/24 hr.

Further studies are needed to determine whether or not the altered PAA excretion is related to reduced monoamine oxidase activities in schizophrenics or to abnormal phenylalanine metabolism.

1124 STRESS-INDUCED INCREASE IN DIHYDROXYPHENYLACETIC ACID IN RAT FRONTAL CORTEX: MODIFICATION BY CHRONIC EXPOSURE TO COLD. Linda Kennedy*, Charles Saller*, and Michael Zigmond (SPON: B. N. Dixit). Department of Pharmacology, School of Pharmacy, and Departments of Psychology and Biological Sciences, University of Pittsburgh, Pittsburgh, PA. 15260.

Rats subjected to restraint showed a significant elevation in plasma catecholamines 15 minutes after the onset of the stress (6-8 ng/ml peak vs. basal level of less than 2 ng/ml). This was accompanied by a 41% increase in dopamine (DA) (control,.060 µg/g; restrained, .084 µg/g; $p<.05$), and a 29% increase in dihydroxyphenylacetic acid (DOPAC) (control, .047 µg/g; restrained, .060 µg/g; $p<.05$) in frontal cortex, suggesting an increase in DA turnover in that structure. No such change was observed in the striatum. The DOPAC concentration of frontal cortex was also increased 45% by a second stressor, 2-deoxy-D-glucose (750 mg/kg, i.p., administered 30 minutes prior to sacrifice). This apparent increase in DA turnover in frontal cortex was not accompanied by a change in cAMP content, although d-amphetamine (1.5 mg/kg, i.p.) did produce a 41% increase in cAMP which could be blocked by prior administration of fluphenazine (0.5 mg/kg, s.c.).

We next examined the ability of restraint to elevate DOPAC in animals subjected to chronic stress. Rats were maintained at 4°C for 15 days prior to sacrifice. Such treatment induced a 25% increase in tyrosine hydroxylase activity in frontal cortex (controls, 8.47 pmole/mg protein/min; chronic cold, 10.38 pmole/mg protein/min; $p<.05$), but no increase in DOPAC level. However, in cold-stressed rats, 15 minutes of restraint produced a 94% increase in DOPAC, a three-fold larger increase than that observed following restraint in naive animals.

These data thus support previous suggestions that stress increases DA turnover in rat frontal cortex. They further suggest that chronic stress enhances the biochemical response to subsequent acute stress.

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1125 DIFFERENTIAL PROJECTION INTO THE DORSAL RAPHE COMPLEX BY BRAIN STEM AFFERENTS. R. E. Kingsley*, J. Strahlendorf, H. Strahlendorf and C. D. Barnes, Dept. Physiol., Texas Tech University School of Med., Lubbock, TX 79430, *Dept. Physiol. Indiana University School of Med., South Bend IN. 46556.

The Dorsal Raphe complex (DR) is a particularly complicated region of the reticular formation which receives afferent fibers from widely scattered and functionally dissimilar regions of the CNS (1). Because of the great deversity of these inputs, it is particularly appropriate to determine the degree of physiological convergence and divergence of the afferents within the Raphe.

In response to single shock stimuli delivered to the Locus Caeruleus (LC), Substantia Nigra (SN) and the Inferior Olive (IO), we recorded field potentials from within the DR. These recordings were made along a series of points from a set of electrode tracks which passed thru portions of the nucleus. After recording from a large number of points, we constructed a 4th dimensional matrix of data values which represented the rostral/caudal vs dorsal/ventral position vs voltage vs time response of the neurons within the DR to the corresponding stimulus. The voltage measurements were converted to relative current according to the method of Howland et. al. (2). Their algorithm eliminates the dominating effect which large somas and axon bundles have on ordinary field potential recordings. Finally, these data were plotted as a time-sequenced series of topographical contour maps with the peaks and valleys representing the sources and sinks of current respectively. Current sources are areas of net CNS depolarization.

Earch of the three nuclei which were stimulated evoked rapidly developing (<0.5 msec.) current sinks which persisted as long as 7 msec. after the stimulus. One of these sinks, in the center of the nuclear complex, was common to all three stimuli. Other current sinks developed later (1.5 to 5.0 msec.) for each of the stimuli. In general, the LC evoked these later sinks in the caudal region of the DR followed by a very late developing, but intense sink in the rostral portion. The SN evoked additional sinks in the caudal, dorsal and rostral portions of the DR. The dorsal and rostral sinks overlapped the large rostral LC sink, but was activated earlier by the SN. Late sinks associated with IO stimuli were very complex, but were in general confined to the rostral part of the DR.

1. Sakai, K., et. al. Afferent Connections of the Nucleus Raphe Dorsalis in the Cat as Visualized by the Horseradish Peroxidase Technique, Brain Res 137: 11-35 (1977).
2. Howland, B., et. al. Reflex Inhibition by Dorsal Root Interaction, J. Neurophysl. 18: 1-17 (1955).

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1126 BLOCKADE OF DORSAL CENTRAL GRAY AREA FEARLIKE AVERSION BY DORSAL RAPHE NUCLEUS STIMULATION. R. Sanford Kiser, Cora A. Brown, Manjit K. Sanghera and Dwight C. German. Depts. Psychiat. and Physiol., Univ. Tex. Health Sci. Ctr., Dallas, TX 75235.

Stimulation of the dorsal central gray area (DCG) and adjacent tectum elicits aversive affective sensations of fear and apprehension in humans and "fearlike" behavior in animals. Previous pharmacological studies have suggested that a serotonin (5HT) mechanism inhibits DCG fearlike behavior. The present study tested whether the nearby 5HT-containing dorsal raphe nucleus (DRN) is involved.

Rats were implanted with two bipolar stimulating electrodes, in the DCG and the DRN. The animals were trained to escape DCG stimulation (100 msec trains of capacity coupled cathodal square wave pulses, 0.5 msec pulse duration, 60 Hz, 5 trains per second) by decremental bar pressing. In this paradigm, each bar press during a DCG stimulation period decrements the DCG current by 5% of its initial level. The animals received a baseline test run, consisting of twenty-five 120 second DCG stimulation trials (average initial DCG current = 20.4 ± 3.0 μA RMS) separated by 120 second time-out periods (with no brain stimulation). During the baseline test run, both the average number of decremental bar presses and the average latency to the first decremental bar press remained relatively constant. The following day, the animals received an identical test run, except that DRN stimulation (100 msec trains of capacity coupled cathodal square wave pulses, 0.5 msec pulse duration, 2 Hz, 5 trains per second) at 50.0 μA RMS was administered during the time-out periods preceding the first 15 DCG trials.

DRN stimulation caused a decrease in the average number of decremental bar presses, an increase in the average first bar press latency, and a profound reduction in the DCG fearlike behavior, yet did not cause somnolence or other signs of behavioral impairment. This "anti-aversive" effect had a long latency to onset and persisted long after the cessation of DRN stimulation. Six days later, a test run identical to the baseline test run revealed that bar pressing had returned to baseline.

These data indicate that stimulation of the DRN area reduces DCG fearlike behavior, possibly by influencing the DRN itself (or nearby endorphin-containing neurons). (Research supported by NIMH grant MH-26032.)

1127 DEVELOPMENT OF MONOAMINERGIC CIRCUITRY IN SOMATOSENSORY CORTEX OF NEONATAL RAT. Donald A. Kristt and James D. Silverman*. Dept. Path., Johns Hopkins Univ. Sch. Med., Baltimore, Md. 21205.

In the present study three aspects of developing MA synaptic organization in postnatal neocortex were evaluated: (i) the distribution and numerical density of MA synapses as a function of age and cortical depth, (ii) the structure of early formed MA synapses as compared to non-MA synapses, and (iii) differences among MA synapses themselves as a function of cortical depth. Forty Sprague-Dawley rat pups, ranging in age from newborn to 16 days of age were studied. MA synapses were identified using an ultrastructural cytochemical marker, 5-hydroxydopamine (5-OHDA), which results in the appearance of small granular vesicles (SGV) in their pre-synaptic terminals. From birth to 7 days of age, 20-30% of all synapses, sampled in somatosensory cortex, contain SGV's. However, few SGV synapses are seen in 8 day old cortex, and by 12 days of age, SGV's are no longer detectable in cortex. A specific distribution for these SGV synapses -- which is distinct from the overall synaptic distribution -- is first seen at 3 days of age and is essentially unchanged until 7 days postnatally. During this entire period, the SGV synapses predominate in the primordium of layer IV, where they account for 50-70% of all synapses. Morphometric analysis of SGV synapses indicates that there are differences in junctional symmetry, vesicle shape and configuration of the contact zone between SGV and non-SGV synapses, as well as between SGV synapses themselves in the various cortical layers. The laminar distribution and morphological characteristics of SGV synapses suggest that the MA projection to neocortex exhibits a high degree of spatial specificity during its ingrowth. Also, the relatively high proportion of SGV synapses in the first postnatal week may reflect a potent influence exerted by the MA inputs on immature neocortex. The decreased numerical density of SGV synapses after 7 days of age is probably due to the development of the blood-brain barrier to 5-OHDA.

Although the origins of the pre-synaptic elements are not definitely established there is evidence that they are derived from MA nuclei in brainstem, and that they arrive in cortex before birth. Since this has not been directly tested, somatosensory cortex of several neonatal rats have been injected with HRP. It was found that in the locus coeruleus numerous cells ipsilateral to the injection are retrogradely labeled before the SGV synapses become concentrated in primordial layer IV. No neurons in the raphe nuclei in midbrain were labeled. A few neurons located in the region of C.N. VII were retrogradely labeled and may represent catecholaminergic neurons from cell group A5.

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1128 LOCUS COERULEUS LESIONS: EFFECTS ON CEREBRAL OXIDATIVE METABOLISM IN VIVO. II THE COUPLING BETWEEN OXYGEN DELIVERY AND ENERGY DEMAND. Joseph C. LaManna, Andrew I. Light,* Sami I. Harik and Myron Rosenthal, Dept. Neurol., U. Miami Med. Sch., Miami, FL 33101

When the cerebral cortex of rats is stimulated focally by trains of electrical square-wave pulses (2 sec trains, 20 Hz, 0.5 msec duration), there is a negative shift of extracellular potential, an increase in extracellular potassium ions and a proportional increase in local metabolic demands. The latter is measured in situ by dual wavelength reflection spectrophotometry of cytochrome a,a_3 or by NADH microfluorometry. The stimulus-induced transient oxidation of cytochrome a,a_3 is accompanied by an increase in local blood volume, as indicated by increased absorption at 590 nm. Re-reduction of cytochrome a,a_3, which occurs within approximately 5-10 sec following termination of stimulation, is accompanied by a decrease in blood volume back to baseline levels. Similar responses occur in the rat cerebral cortex contralateral to 6-OH-dopamine lesion of the nucleus locus coeruleus (LC). However, in the norepinephrine (NE) depleted hemisphere ipsilateral to LC lesion, blood volume responses to electrical stimulation are greatly diminished or absent and the rate of re-reduction of oxidized cytochrome a,a_3 is markedly slowed. The time to peak oxidation of cytochrome a,a_3 is amplitude-independent in normal control rats and this is unaltered in either cortical hemisphere of unilateral LC lesioned rats. The time to half recovery of the oxidized cytochrome is only slightly dependent upon amplitude. Ipsilateral to LC lesion, the recovery time is not only increased, but becomes strikingly amplitude-dependent; i.e. the effect of NE depletion becomes more pronounced at higher stimulus intensities or greater energy demands. Preliminary results indicate that the rate of NADH oxidation as well as the rate of NAD^+ re-reduction with stimulation is slowed in the cortical NE depleted hemisphere. The significance of the time to peak oxidation of NADH is that it is controlled by the rate of ADP availability and is ouabain sensitive. Thus, these results suggest that NA-K -ATPase is slowed in the cortical hemisphere depleted of NE by LC lesion and also indicate that the dynamic coupling between oxygen delivery and energy demand is altered when NE is unavailable. (Supported by PHS grants NS 14319 and NS 14325.)

1129 IN VIVO VOLTAMMETRIC RECORDING OF DOPAMINE AND 5-HYDROXYTRYPTAMINE RELEASE AND METABOLISM FROM DIFFERENT BRAIN REGIONS OF THE RAT. Ross F. Lane*, Arthur T. Hubbard* and Charles D. Blaha*. (SPON: M.L.Laudenslager, Jr.). Dept. Chem., Univ. Calif. Santa Barbara, CA 93106.

Voltammetric methods have been developed which allow continuous, simultaneous in vivo monitoring of the extracellular levels of dopamine (DA), homovanillic acid (HVA) and 5-hydroxytryptamine (5-HT) in brain tissue of the unanesthetized, unrestrained rat. A potential is applied to a carbon electrode (5-175 μm) stereotaxically implanted in a specific brain region and changes in current attending oxidation of DA, 5-HT and HVA are recorded. The potential at which oxidation occurs is indicative of which molecule is being oxidized. The methods are currently being used to study drug-induced release of DA and 5-HT from rat caudate nucleus (CN) and substantia nigra (SN). In the CN, amphetamine (AMP) administration evoked a rapid, temporary and dose-related release of DA, time-correlated with characteristic AMP-induced behavioral effects. Conversely, α-methyltyrosine (AMT, 250 mg/kg) induced a gradual and long lasting decrease in DA release and HVA formation. 5 hr after AMT treatment, release of DA elicited by AMP was completely prevented. Treatment with the DA receptor agonist apomorphine (2 mg/kg) caused a rapid and transitory decrease in DA release with time course in correlation with drug-induced stereotyped behavior. DA release and HVA synthesis were also stimulated by the antipsychotic neuroleptic DA antagonists pimozide, spiroperidol and haloperidol. Both DA and 5-HT were found to be rapidly released in CN by p-chloroamphetamine (PCA, 5 mg/kg). PCA-induced release of 5-HT was selectively prevented by pretreatment with p-chlorophenylalanine (300 mg/kg) and blocked by the 5-HT uptake inhibitor fluoxetine (20 mg/kg)(Lilly). Fluoxetine alone (20 mg/kg) produced a moderate increase in 5-HT, presumably due to inhibition of 5-HT reuptake.

AMP, AMT and apomorphine were also used to investigate release of DA from SN. Release of DA was stimulated by AMP (5 mg/kg) and decreased both by AMT (250 mg/kg) and apomorphine (2 mg/kg). Pretreatment with AMT (250 mg/kg) prevented AMP-stimulated release of DA.

These data indicate that in vivo voltammetry can be succussfully employed to probe the functional dynamics of DA and 5-HT neuronal systems. They provide support for currently held views that dendritic release of DA in SN may serve as an additional mechanism regulating the activity of the DA nigrostriatal pathway. (Supported by USPHS NIH Grant NS 13556).

1130 GENESIS OF CENTRAL MONOAMINE (MA) NEURONS IN THE RHESUS MONKEY. Pat Levitt and Pasko Rakic. Section of Neuroanatomy, Yale Univ. School of Medicine, New Haven, Ct.

Neurogenesis of the locus coeruleus (LC), substantia nigra (SN) and raphe complex (RC) was analyzed in autoradiograms from twenty-two 2-3 month old monkeys, each of which had been exposed to a pulse of ^{3}H-thymidine on selected embryonic (E) days. Heavily labeled neurons, representing cells that had undergone final division on the day of the ^{3}H-thymidine injection, were noted only in 8 monkeys exposed to the isotope between E27-43. LC neurons are generated between E27-36 with the major proliferation occurring between E30-33 and peaking around E32 (13% labeling index; heavily labeled neurons/total neurons). Neurogenesis in the LC occurs in a prominent mediolateral spatiotemporal gradient, such that the majority of neurons generated on E30 eventually become situated in the medial part of the nucleus, whereas most cells in the lateral portion are generated on E32 and E33. A sharp peak of proliferation of neurons destined for the compact portion of the LC occurs around E32. Cells that comprise the more diffuse areas of the LC are generated more evenly throughout the E30-33 period. SN neurons are generated between E36-43, with peak labeling in both pars compacta (12%) and pars reticulata (9%) occuring in specimens injected at E38 or E40. No appreciable spatiotemporal gradient was noted in either portion of the SN. Neurons of the ventral tegmental area are also generated between E38-43. Neurogenesis of the RC occurs between E28-43 with only a moderate rostrocaudal spatiotemporal gradient: Neurons of raphe dorsalis and centralis superior undergo final mitosis between E28-36, with the largest fraction of heavily labeled neurons (6 & 15% respectively) at E30, whereas cells of raphe magnus, pontis, obscurus and pallidus are generated between E28-43, with the highest labeling indices (27, 23, 19 & 17% respectively) in cases injected at E38 and E40. Thus, brainstem MA nuclei of rhesus monkey are generated during the first quarter of prenatal life. Histochemical studies in human embryos also indicate that MA neurons are present during the first quarter of gestation (Olson, et al., Z. Anat. Ent.-Gesch., 139, 1973). Thus, the timing of neurogenesis of MA systems in primates differs significantly from that in rodents, where genesis of these nuclei begins around midgestion and extends well into the last half of fetal life. (Lauder, et al., J. Comp. Neur., 155, 1974; Pierce, Prog. Br. Res., 40, 1973). However, although gestation in the rhesus monkey (165 days) is nearly 8 times longer than that in rodents (21 days in rat), and the number of MA cells several times greater, the majority of neurons of any single nucleus is generated within a similarly short period in the two species (3-4 days in rodent; 3-5 days in macaque), indicating that MA neuron systems in primates develop rapidly from a larger precursor pool. (Supported by NS 14841).

1131 NEOCORTICAL DEVELOPMENT AFTER PRENATAL LESIONS OF NORADRENERGIC PROJECTIONS. Hart G.W. Lidov and Mark E. Molliver, Departments of Cell Biology/Anatomy and Neurology, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

Monoaminergic axons are amongst the earliest extrinsic afferents to invade the developing telencephalon. Axons that synthesize catecholamines are present in the rat telencephalon as early as 13-14 days of gestation, while neurons of the cerebral neocortex are born later, between day 14 of gestation and the first post-natal day. Furthermore, in perinatal cerebral cortex, presumably monoaminergic synapses constitute a large proportion of the total synaptic population (70% in layer IV of SI). Combined with supporting biochemical data, these facts have led to the proposition that monoaminergic projections may exert a trophic influence on other elements of the neuropil in the immature nervous system. We are attempting to test this hypothesis by chemical destruction of the cortical noradrenergic afferents in fetal rats.

Fetuses were given intraperitoneal injections of the neurotoxin, 6-hydroxydopamine (200 mg/kg) on day 17 of gestation. The effectiveness of the lesions was confirmed in adults by glyoxylic acid histofluorescence and dopamine-β-hydroxylase immunofluorescence; vehicle injected animals were used as controls. In addition examination of the brains by Nissl and Golgi methods revealed the following findings: 1) The gross morphology, lamination, and cytoarchitecture of cortex appear normal. Dendritic arbors and spines are qualitatively normal. 2) The structure of the barrel field was examined in tangential sections as a measure of the thalamic influence on cortex. The barrels appeared normal in both form and size. 3) Histofluorescence reveals that the dopamine axons retain their normal distribution in frontal, cingulate, and perirhinal cortex and do not sprout into layers or regions normally occupied by noradrenergic fibers. 4) A large number (6/24) of animals had foci of ectopic neurons. These cells were found either adjacent to the ependymal layer or at the brain surface between the pia and the molecular layer. These results indicate that while cortex organization proceeds normally in the absence of noradrenergic afferents, these axons may influence either neuronal proliferation or migration. Further studies are in progress to assess the effects of similar lesions placed on days 13 and 15 of gestation. We conclude that no evidence for a trophic effect of noradrenergic axons upon the development of cortical neurons or layers has been demonstrated. {Antiserum to DBH was generously provided by Reinhard Grzanna. Support: USPHS NS08153, NS10290; H.G.W.L. supported by training grant GM-7309.}

1132 DOPAMINE MODULATORY ACTIONS AT NEUROMUSCULAR JUNCTIONS OF LOBSTER STOMATOGASTRIC SYSTEM. C. Lingle. (SPON: D.L. Barker). Dept. Biol., U.O., Eugene, OR 97403.

Striated muscles of the stomatogastric system of many decapod crustacea receive excitatory innervation from either cholinergic or glutamatergic-type motor neurons. The effects of dopamine on muscles and neuromuscular junctions of the spiny lobster, Panulirus interruptus, foregut were examined.

Dopamine produces prolonged enhancement of nerve-evoked contractions down to at least 50 nM at both types of junctions. In two muscles receiving cholinergic innervation, enhancement occurs at 5 nM or less. In these two muscles dopamine also produces a contracture and frequently elicits spontaneous rhythmic contractions that occur asynchronously in different fibers. The presence of such contractions just after removal of appropriate muscles from the animal suggests exposure to an endogenous agent able to elicit such activity, perhaps dopamine. That the mechanisms underlying rhythmic contraction are endogenous to the muscles, but require tonic activation, is suggested by 1.) asynchrony among adjacent fibers, 2.) lack of effect of cholinergic receptor blockade or TTX, and 3.) failure of 1/10 Ca^{+2} to reduce spontaneous activity while evoked contractions are reduced. Octopamine and serotonin do not elicit these contractions, but reduce and increase, respectively, the frequency of their occurrence.

Intracellular recording from sensitive muscles during dopamine application reveals a slow depolarization and a conductance decrease, consistent with the slow contracture. A potassium conductance decrease is likely. Additionally, during dopamine application spontaneous rhythmic conductance increases are observed which may be either hyperpolarizing, when contractions do not occur, or depolarizing. A chloride conductance increase is likely. In muscles in which dopamine only produces enhancement of evoked contractions, effects on membrane potential are less clear. Generally, small depolarizations are observed. Although effects on hyperpolarizing current pulses are small, dopamine produces an apparent conductance decrease when measured by depolarizing current pulses. The enhancement of ejps produced by dopamine can not be accounted for entirely by effects on membrane resistance, suggesting that both pre- and post-synaptic sites of action are likely. In addition, since the rate of relaxation of evoked contractions is increased during dopamine, a direct non-electrical effect on muscle is indicated. These effects of dopamine appear to be unique to neuromuscular junctions of the stomatogastric system, since no effects of dopamine on Panulirus opener muscle evoked contractions were observed at 10 uM or below.

1133 DISRUPTION OF THE CIRCADIAN RHYTHM OF EXCITABILITY IN THE MESOLIMBIC CATECHOLAMINE SYSTEM BY PERIPHERAL DEPRIVATION OF LATERAL EYE MOVEMENTS AND REM. A. Livermore, Jr.* and J. R. Stevens, Depts. Neurol. & Psychiat., Univ. Oregon Health Sci. Center, Portland, OR 97201, USA.

The rapid eye movements (REMs) of paradoxical sleep (PS) evoke light modulated potentials and multiple unit activity in the suprachiasmatic nucleus and in the ventral tegmentum of the mesencephalon, junction of dopamine cells of origin of the mesolimbic and nigrostriatal systems with the medial terminal nucleus of the accessory optic tract (Stevens & Livermore, Exp. Neurol. 60:541, 1978). Relationships observed between PS, photoperiod, maturation, and reproductive activities, led to the hypothesis that REMs may serve to transmit photic information to regions of the brain which modulate cerebromonoamine transmission in relation to daily and seasonal light changes.

To test this hypothesis, mesolimbic dopamine system excitability was examined in rats deprived of eye movements by peripheral muscle section. Injection of low-dose amphetamine in the rat induces a marked increase in locomotor activity which depends on the integrity of the mesolimbic dopamine system. This response displays a circadian cycle reaching a zenith around midnight and a nadir around noon in rats on a 12:12 light:dark cycle. Twenty-two Long Evans rats, 11 eye muscle resected and 11 sham controls, were injected with 1.2 mg/kg d-amphetamine i.p. at 0100, 0700, 1100, 1500, and 1900 hours at intervals of not less than 28 hours and placed in activity cylinders equipped with photocells which recorded locomotor activity. Twelve weeks postoperatively, the normal relationship of the amphetamine-induced hyperactivity response to the daily light:dark cycle of operated rats was grossly disrupted compared with sham-operated controls. In two animals inadvertantly blinded by the surgery, the circadian rhythm of the response was abolished. The data lend support to the hypothesis that lateral eye movements and REMs provide photic modulation to cerebral centers which regulate biologic rhythms of mesolimbic catecholamine activity.

1134 EVIDENCE THAT SEROTONIN IS A CIRCULATING NEUROHORMONE IN THE LOBSTER. Margaret S. Livingstone* and Edward A. Kravitz. Dept. Neurobiol., Harvard Med. Sch., Boston, MA 02115.

We have previously reported that serotonin is synthesized and stored in two locations in the lobster nervous system: the second thoracic roots and the pericardial organs. Both these structures are characterized by a cortex of nerve ending-like varicosities with an appearance typical of neurosecretory tissues. In this communication we report that serotonin can be released from these structures by depolarization and that physiologically effective levels of endogenous circulating serotonin are found in the hemolymph.

When second thoracic roots are incubated in ^{3}H-tryptophan they synthesize and store ^{3}H-serotonin. This serotonin can be released in a calcium dependent manner by potassium induced depolarization. A five minute depolarization with 100 mM potassium will release about two-thirds of the newly synthesized serotonin in the root (1-2 pmoles of radioactive serotonin). When roots are incubated in the presence of the decarboxylase inhibitor NSD-1055, 5-hydroxytryptophan, the precursor of serotonin, accumulates instead of serotonin. 5-Hydroxytryptophan is not released from these tissues with depolarization.

Serotonin levels in lobster hemolymph were measured using high-performance liquid chromatography with electrochemical detection. A peak in the hemolymph samples was observed that eluted with the same retention time as authentic serotonin. This peak was further identified as serotonin by measuring the peak height as a function of the applied voltage of the electrochemical detector. We thereby determined that the compound in the hemolymph eluting at the same time as serotonin had the same redox potential as authentic serotonin. The basal hemolymph levels of serotonin in lobsters maintained in circulating sea water in holding tanks or in seminatural environments ranged from 10^{-9} to 10^{-8} M. In lobster neuromuscular junction preparations, serotonin causes a facilitation of transmitter release from nerve endings and a direct contracture of muscle fiber. The threshold concentration of serotonin for both of these effects is about $5x10^{-9}$ M. Thus serotonin is present in lobster hemolymph at high enough concentrations to exert physiological actions on target tissues. We are currently measuring the serotonin levels of animals in different behavioral and environmental states. (Supported by NIH).

1135 FOREBRAIN NOREPINEPHRINE DEPLETION ATTENUATES THE BLOCKING EFFECT. Joan F. Lorden, Edward J. Rickert*, Ralph Dawson, Jr., and MaryAnn Pelleymounter*. Dept. Psychol., University of Alabama in Birmingham, Birmingham, AL 35294.

Lesions of the dorsal noradrenergic bundle (DB) retard the extinction of both runway and lever-press responses (Mason & Iverson, JCPP, 1977, 91, 165-73). DB lesions also impair the acquisition of a successive light-dark discrimination due to a tendency of DB rats to perseverate in responding to the negative stimulus. Rats with DB lesions are not hyperactive but do appear to be more distractable than normal animals. (Mason & Fibiger, Brain Res., 1978, 159, 421-6). On the base of this and related research, it has been proposed that a failure to filter out irrelevant stimuli could account for the behavioral effects of DB lesions. We have tested this hypothesis using Kamin's two-stage blocking paradigm.

Rats were pretrained to suppress operant responding in the presence of a light or tone (CS_A) that predicted footshock. This was followed by conditioning with a compound cue (CS_{AB}: light + tone) formed from the original cue (A) and a new stimulus (B). In normal rats pretraining to A blocks conditioning to B. That is, normal animals do not suppress operant responding when tested in the presence of only the redundant cue (B). Rats with 6-hydroxydopamine DB lesions were compared to rats with control lesions (5,7-dihydroxytryptamine lesions of the dorsal and median raphe nuclei) and vehicle-treated rats in the blocking paradigm. When vehicle or control lesion groups were tested for response suppression in the presence of the redundant stimulus, the blocking effect was observed. Rats with DB lesions showed an attenuation of the blocking effect and suppressed lever-press responding in the presence of the irrelevant cue. DB rats differed significantly from both the vehicle and raphe lesion groups. No significant differences were found among the three groups during either the pretraining or the conditioning to the compound cue. Nor were any differences obtained among DB, raphe or vehicle groups when pretraining was omitted and all animals were trained only on the compound cue.

Fluorometric assays of hippocampal norepinephrine (NE) and serotonin (5-HT) indicated that DB lesions reduced mean hippocampal NE by 83% and 5-HT by 3% in comparison with vehicle-treated controls. Rats with raphe lesions showed a 9% depletion of NE and a 78% depletion of 5-HT. Similar results were obtained in the remaining telencephalon. Telencephalic dopamine levels were unaffected by the lesions. The results provide support for the idea that depletion of forebrain NE impairs the ability of rats to exclude irrelevant stimulus information. (Supported by NSF grant BNS 77-15251 and a University Faculty Research Grant).

1136 TOPOGRAPHICAL ORGANIZATION OF LOCUS COERULEUS: EFFERENT PROJECTIONS OF CONSTITUENT NEURONS. S.E. Loughlin*, S.L. Foote, and F.E. Bloom. A.V. Davis Ctr. for Behav. Neurobiology, Salk Inst., La Jolla, CA 92037.

The nucleus locus coeruleus (LC) is known to have widely divergent efferent projections. The question of whether those neurons which project to a given terminal area are located within a defineable portion of LC has been answered for only a few cases (e.g., neurons projecting to spinal cord, Satoh et al., Exp.Br.Res.,30,'77, and others). In order to address this question in a systematic, quantified way, we have constructed computerized 3-dimensional maps of the spatial distribution of LC neurons in rat and used them to determine whether, and to what extent, these neurons are topographically organized with respect to their efferent projections. A computer-linked microscope is used to digitize LC cell positions and landmarks from serial sections. A computer graphics system is then used to realign these sections and re-create a 3-dimensional LC. Initially, 12μ, sagittally oriented, Nissl stained sections from a paraffin embedded brain were used to reconstruct a "normal" LC. The total numer of neurons digitized, approximately 1700, was in agreement with previous counts of the nucleus (Swanson, Br.Res.,110,'76). In addition, computerized "sectioning" of this digitized LC in the frontal and horizontal planes yielded LC profiles like those observed in similarly oriented histological sections from other brains. The accuracy of this reconstruction is also being quantitatively assessed using statistical analyses which compare the number and distribution of cells in 2 or more normal nuclei. In experimental animals, injections (.02-.2μl) of 30% Sigma Type VI HRP were placed stereotaxically in LC terminal areas and 40μ sections through LC and the injection site were reacted with TMB (Mesulam, J.Histo.Cyto.,26,'78). Following injections into hippocampus and spinal cord, relatively compact, identifiable subpopulations of cells within LC were preferentially labelled. While injections into hippocampus predominantly labelled dorsal LC, spinal cord injections yielded labelled cells only in the ventral third of LC. By contrast, injections into other terminal areas, such as hypothalamus, cerebellum, amygdala, and neocortex, produced more complex patterns of labelling. For example, amygdala injections resulted in several large, densely labelled cells scattered throughout LC and a greater number of lightly labelled neurons. The locations of labelled cells within LC following these HRP injections are also being mapped on the computer system. Distributions of labelled cells will be compared statistically with the normal cell distribution and with each other to determine possible topographical and density differences among these populations. USPHS Grant #AA03504.

1137 THE RAPHE NUCLEI IN THE 13-LINED GROUND SQUIRREL, A CYTO-ARCHITECTONIC STUDY. Cecilia Machin*, Carmen Rua*, Elizabeth Taber-Pierce and Charles P. Lyman*. Dept. Anat., Harvard Med. Sch., Boston, MA 02115

A detailed study of the cytoarchitecture of the raphe nuclei in the 13-lined ground squirrel, Citellus Tridecemlineatus, a hibernating animal, has been completed using classical neuroanatomical techniques at the light level. The eight semicontinuous, caudal to rostral, 5-HT nuclear groups are characterized by a distinctive cellular structure. (The interpeduncular nucleus, although present within the raphe was not included in the study, since it is believed to be functionally different from the 5-HT containing nuclei.) Data collected from four adult animals form the basis for the study. Sections were stained by the iron-hemotoxylin method of Loyez, by the Kluver-Barrera method and with cresyl violet. The cytoarchitecture of these nuclei was compared with previous descriptions of these nuclei in the cat, an animal upon which much of the sleep literature is based. A close relationship has been shown between 5-HT and slow-wave-sleep (SWS). Some research supports the conclusion that SWS and hibernation are physiologically homologous.

1138 INTERRELATIONSHIP BETWEEN CENTRAL NERVOUS SYSTEM β- AND α_2-ADRENERGIC RECEPTORS. A. Maggi*, D.C. U'Prichard and S.J. Enna.(SPON: S.J. Strada). Univ. Texas Med. Sch., Houston, Tx. and Northwestern Univ. Med. Sch., Chicago, Ill.

Dibner and Molinoff have reported that in vitro exposure of rat brain cortical slices to isoproterenol (ISO) causes a rapid and reversible decrease in β-receptor binding and activity. To further characterize this phenomenon, rat brain cortical slices were incubated at 37° in oxygenated Rall's buffer in the presence of 100μM ISO. Following incubation, the slices were thoroughly rinsed, then disrupted and membranes prepared to study neurotransmitter receptor binding using ^{3}H-ligand binding procedures. β-adrenergic receptors were assayed using ^{3}H-dihydroalprenolol as a ligand; α_1-adrenergic receptor binding was studies using ^{3}H-WB4104 as a ligand; and α_2-adrenergic receptor binding was studied using ^{3}H-clonidine as a ligand. As reported previously, in slices incubated with ISO there was a 50-60% decrease in the number of β-adrenergic receptor binding sites occuring within 30 min, with no change in receptor affinity. However, in the present study, a 50-60% increase in α_2-receptor binding was also found to occur in these membranes. The time-course of the α_2-receptor increase paralleled the time-course of the β-receptor decrease. No change was noted in ^{3}H-WB4101 receptor binding under these conditions. Co-incubation with sotalol, (20μM) a specific β-receptor antagonist, completely blocked both the β- and α_2-receptor changes induced by ISO. In contrast, incubation with clonidine, a specific α_2-receptor agonist, resulted in a decrease in α_2-receptor binding, with no change in α_1- or β-receptor binding. This clonidine-induced decrease in α_2-receptor binding was completely blocked by co-incubation with tolazoline (50μM), a specific α_2-receptor antagonist.

These results suggest that there may be an interrelationship between brain β- and α_2-adrenergic receptors such that overactivation of the former leads to desensitization of the β- and supersensitivity of the α_2-receptor. This increase in α_2-receptors may be a neuronal compensatory mechanism for overcoming excessive β-receptor discharge. (Supported in part by USPHS grants NS-13803, an RCDA NS-00335 (S.J.E.) and a Salk Institute-Texas Research Fnd. Award).

1139 NORADRENALINE AND SELECTIVE ATTENTION. Stephen T. Mason and Hans C. Fibiger. Div. Neurological Sciences, Dept. Psychiatry, Univ.British Columbia, Vancouver, B.C., Canada V6T 1W5

Depletion of forebrain noradrenaline (NA) to less than 10% of normal by intracerebral injection of 4μg of the selective neurotoxin 6-hydroxydopamine (6-OHDA) dissolved in 2μl saline produces resistance to extinction of a number of appetitively and aversively motivated tasks (for review see Mason, 1979). The most powerful explanation of this effect appears to be an alteration in the stimulus sampling of the animal as a result of the lesion-induced loss of a brain mechanism normally involved in the filtering out of irrelevant environmental stimuli (Mason and Iversen, 1978; Mason and Fibiger, 1979). This role for NA in selective attention was tested directly by examining the behaviour of NA-depleted rats in a latent inhibition paradigm and on a non-reversal shift. Latent inhibition involves pre-exposing animals in the absence of reinforcement to stimuli which will later be used as the discriminative stimuli in a successive discrimination operant task. Normal animals come to ignore these stimuli during the pre-exposure and so take longer to learn to use them in the discrimination task than do non-pre-exposed rats. 6-OHDA depletion of forebrain NA blocked the latent inhibition effect, suggesting an inability of the lesioned rats to learn to ignore stimuli during the pre-exposure phase. A further prediction of the attentional model is that the transfer from one stimulus dimension to a previously irrelevant one (non-reversal shift) should proceed more quickly in the lesioned rats since they, unlike controls, have not learned to ignore this initially irrelevant dimension. Such was observed in the non-reversal shift from a brightness relevant, spatial irrelevant to a brightness irrelevant, spatial relevant T-maze discrimination task. These two findings are interpreted as providing strong evidence in favour of a role for forebrain NA in stimulus sampling and selective attention.

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1140 CHANGES IN TYROSINE HYDROXYLASE ACTIVITY IN THE BRAIN AND ADRENAL GLAND OF RATS FOLLOWING ACUTE AND CHRONIC ELECTROCONVULSIVE SHOCK. J. Masserano, G. Takimoto and N. Weiner. Dept. Pharmacology, Univ. Colorado Sch. Med., 4200 E. 9th Ave., Denver, CO. 80262.

In previous studies, we have demonstrated that various stresses (decapitation, pain, immobilization and cold) are associated with activation of rat adrenal tyrosine hydroxylase (TH). In the present study we have examined the effects of electroconvulsive shock (ECS) on adrenal and brain TH following both single and multiple (1 ECS/day for 7 days) applications. Rats were shocked with 300 mA applied transorbitally for 0.2 seconds. Five or sixty minutes following the single application, and 24 hours following the multiple administrations, of ECS the animals were injected with pentobarbital (60 mg/kg i.p.) and the adrenal glands were removed surgically. The animals were then decapitated and the following brain areas were removed; frontal cortex, caudate, hippocampus, hypothalamus, substantia nigra, locus coeruleus and nucleus solitarius. Five minutes following the single application of ECS, TH activity was significantly increased above control values in both the adrenal and caudate. Sixty minutes following acute ECS, adrenal and caudate TH activity had returned to control values. TH activity in the remaining 6 brain areas was unaffected by acute ECS treatment. Following repeated ECS treatment, TH activity was significantly increased in the adrenal gland, locus coeruleus and nucleus solitarius. Chronic ECS produced no change in TH activity in the remaining 5 brain areas.

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1141 HALLUCINOGENS SENSITIZE SEROTONIN AND NOREPINEPHRINE RECEPTORS IN THE FACIAL MOTOR NUCLEUS. R.B. McCall & G.K. Aghajanian, Depts. Psychiat. & Pharmacol., Yale Univ. Sch. Med., New Haven, CT 06508

Our previous results indicate that serotonin (5-HT) and norepinephrine (NE) markedly facilitate excitatory inputs to the facial nucleus without directly exciting facial motoneurons (Brain Res., in press, 1979). In the present study the effects of hallucinogens on responses to 5-HT and NE in the facial nucleus were investigated by single-cell recording. Intravenous d-lysergic acid diethylamide (LSD, 10-100 μg/kg) had no effect by itself on glutamate-induced excitation of facial motoneurons. In contrast, the facilitation of facial neuron excitation by iontophoretic 5-HT and NE was greatly enhanced by low doses of LSD (5-10 μg/kg, iv.). The LSD-enhanced response continued for at least two hours. The iontophoresis of LSD at low currents which by themselves had no effect, also enhanced 5-HT facilitation; at higher currents LSD temporarily antagonized the response. Thus in small amounts LSD appears to sensitize 5-HT and NE receptors in the facial nucleus. The effects of two simple indoleamine hallucinogens, psilocin and N,N-dimethyltryptamine (DMT), were also tested. Like LSD, psilocin (0.5-2 mg/kg, iv.) markedly potentiated the effect of 5-HT and NE on motoneurons, but had no direct action when given alone. In contrast, DMT (0.5-2 mg/kg, iv.) facilitated glutamate-induced excitation of facial motoneurons by itself. This effect was blocked by the 5-HT antagonist metergoline indicating that DMT can act as a 5-HT agonist in the facial nucleus. In animals pretreated with metergoline, DMT markedly potentiated the facilitation of motoneuron excitation by NE. Thus DMT can also act to sensitize NE receptors in the facial nucleus. Like DMT, the hallucinogen mescaline (0.5-2 mg/kg, iv.) acted as a 5-HT agonist and potentiated the effect of NE. In contrast, a non-hallucinogenic ergot derivative, lisuride, had no effect on glutamate-induced excitation of motoneurons or on the facilitating effect of 5-HT and NE. In addition, the peripheral 5-HT antagonists methysergide, metergoline, cyproheptadine, and cinanserin blocked the action of 5-HT and failed to potentiate the effect of NE. These data suggest that enhancement of certain spinal reflexes by LSD and mescaline (Anden et al., Br. J. Pharmacol., 34, 1968; Maj et al., J. Pharm. Pharmacol., 29, 1977) may result from a sensitization of 5-HT and/or NE receptors in motor nuclei. In addition, if the sensitizing effects of hallucinogenic drugs observed in the facial nucleus occurs in other areas of the central nervous system, then the mechanism of receptor sensitization might contribute to the psychedelic effects of these drugs.

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1142 THE TIME COURSE OF DISCHARGE ACTIVITY OF LOCUS COERULEUS AND MIDBRAIN RAPHE REM-OFF NEURONS DURING ENTIRE SLEEP-WAKING CYCLES. Robert W. McCarley, Andrew Strassman* and J. Allan Hobson. Laboratory of Neurophysiology, Department of Psychiatry, Harvard Medical School, Boston, MA 02115.

There is increasing evidence that neurons in locus coeruleus (LC) and midbrain raphe may play a regulatory role in mammalian behavioral state control. To test the hypothesis that these neurons participate in control of the cyclically occurring events of the sleep cycle it is essential to know the time course of their discharge activity over entire sleep cycles. Although previous studies had obtained samples of neuronal discharge activity during various states, none had obtained entire cycle recordings. Therefore we decided to examine extracellularly recorded discharge activity during unperturbed, entire sleep cycles in unanesthetized, head-restrained cats. A particular aim was to look at evidence that REM-off cells (waking/REM sleep discharge ratios < 0.5) in these biogenic-amine contining areas showed discharge time courses compatible with their playing an inhibitory role to REM-on pontine reticular cells, as postulated by the reciprocal interaction model of sleep cycle control. The time course of REM-off activity predicted by the mathematical equations of this model has four main characteristics: 1) A discharge peak as REM ends and waking begins; the model postulates that REM is terminated by increased inhibition of executive, excitatory neurons in the pontine reticular gigantocellular tegmental field (FTG). 2) A gradual, slow decline as REM-off activity is reduced by inhibitory self-feedback loops. 3) A low point of activity at REM onset corresponding to maximum disinhibition of REM executive cells. And 4) resumption of REM-off activity in the latter part of the REM period, as REM-off activity is recruited by the FTG activity.

We have recorded over 50 REM-off neurons in LC and raphe and thus far have examined unperturbed sleep-waking cycles in 6. Compared with the theoretical curve, the actual data show a slower decline from the peak at cycle beginning (associated with waking) and a shorter lead time of discharge activity increase anticipating REM-end (which marks the cycle end). Overall, however, there is agreement with the major features of the predicted curve: a peak at cycle onset, a long slow decline in activity associated with the behavioral state of slow wave sleep, a low point at REM onset, and a rise at cycle end. In particular the agreement between theory and fact on the characteristic slow decline in discharge activity during slow wave sleep deserves emphasis; the REM-off cells begin turning off long before REM onset. These findings are compatible with the hypothesis that REM-off cells may themselves be reponsible for some manifestations of the waking state and that their decrease in activity may disinhibit REM executive cells and so allow generation of a REM sleep episode.

1143 CORRELATION OF THE EFFECTS OF PARACHLOROAMPHETAMINE BY LIGHT AND HISTOFLUORESCENT MICROSCOPY. Robert E. McClung and Joe Wood. Dept. Neurobiology and Anatomy, University of Texas Medical School at Houston, Houston, Texas 77025.

Neurochemical and histofluorescent techniques have suggested that parachloroamphetamine (PCA) interacts with neurons to alter serotonergic mechanisms. The present study presents a correlation between light and fluorescent microscopic observations of the effects of PCA, aimed at clarifying the cellular and subcellular effects of the drug on a selected serotonergic neuronal population. Male rats were injected with saline or PCA (20 mg/kg I.P.) 12 hours prior to sacrifice. Behaviorally, at 12 hours post-injection, the animals demonstrated a hyper-reactivity as would be expected following administration of an amphetamine-like drug. Following decapitation, the brain was removed, frozen on dry ice, and 10-20 μ frozen sections were cut on a cryostat. Sequential sections were alternately treated with: 1) glyoxylic acid or 2) glutaraldehyde-dichromate-chromate followed by cresyl violet. In this manner the changes in histofluorescence could be correlated with the chrome-amine deposits present within the neurons. Neurons in the dorsal and medial raphe system showed decreased fluorescence and fewer chrome-amine granules which supports the concept that PCA is taken up in brainstem serotonergic neurons and alters their functional characteristics. At 12 hours post injection, some damage was apparent in the large serotonergic neurons of the brainstem. Histofluorescent sections also revealed a loss of serotonin in the forebrain projections of the raphe system. This suggests a dysfunction in the terminals of raphe neurons which have been reported previously to not show cell body alterations following PCA treatment. In contrast, catecholamine fluorescence and chrome deposits in nearby nuclei did not appear to differ between control and PCA treated animals. Thus, in the present study, PCA initially effects serotonergic neurons and terminals resulting in decreased levels of the indolamine while not visibly altering catecholamine stores.
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1144 A NOVEL FLUORESCENT MARKER OF CNS VASCULATURE USED IN COMBINATION WITH MONOAMINE HISTOFLUORESCENCE. Jacqueline F. McGinty*, Leonard Y. Koda*, and Floyd E. Bloom. A.V. Davis Ctr. for Behav. Neurobiol., Salk Inst., La Jolla, CA 92037.

Pontamine sky blue (PSB) is routinely used to localize the placement of microelectrodes in brain tissue. One disadvantage of the technique is the difficulty of detecting small amounts of this dye with bright field microscopy. We have found that iontophoresed PSB fluoresces bright red under a rhodamine filter. This characteristic has enabled us to localize discrete microelectrode placements which are not detectable with bright field illumination. In addition, iontophoresed PSB is compatible with monoamine (MA) histofluorescence and fluorescein immunocytochemistry, and thus, provides an easy and sensitive method for the localization of electrode positions in histochemically-characterized brain regions (McGinty, et al., in preparation). PSB can also be used to fluoresce brain vasculature. In the present study, we combined PSB and glyoxylic acid-induced MA fluorescence to quantify capillary density in several monoamine-rich brain areas.

Six male Sprague-Dawley rats (200-300 g), with or without pargyline pretreatment (4 hours after 50mg/kg) were anesthetized and perfused with a chilled 0.5% paraformaldehyde-2% glyoxylic acid solution. The rats were then injected transcardially with 3-4 ml of chilled 0.2% PSB (Searle Diagnostic, Bucks, England) in Ringer's solution or 3-4 ml of India ink (Higgins, #4466). Twenty micron serial sections were then processed for MA histofluorescence (Bloom and Battenberg, J. Histochem. Cytochem. 74:561, 1976). Areas of interest were photographed with a 10x objective under epi-illuminated rhodamine and/or catecholamine filters. Since India ink was not compatible with histofluorescence, ink-injected tissues were Nissl-stained and photographed under bright field illumination. Blood vessels were counted on photographic enlargements (final mag. 225x). Data were compiled from 10-12 micrographs of each area per animal. Contrary to previous indications that the norepinephrine-rich locus coeruleus is highly vascularized (monkey-Finley and Cobb, J. Comp. Neurol. 73:49, 1940; rat-Shimizu and Imamoto, Arch. Histol. Jap. 31:229, 1970), our preliminary observations suggest that rat locus coeruleus is not highly vascular (3.3 ± 0.3 vessels/100μ^2) as compared with adjacent central grey (6.3 ± 0.5 vessels/100μ^2). Quantification and illustration of other brain regions will also be presented. PSB and India ink appear to be equally sensitive in revealing capillary networks. However, the advantage of using PSB is its compatibility with histofluorescence methods.

1145 SPINAL PROJECTIONS OF NOREPINEPHRINE-CONTAINING NEURONS IN THE RAT. S. McKellar and A.D. Loewy(SPON: S. Goldring). Dept. Anat. & Neurobiol., Wash. Univ. Sch. Med., St. Louis, MO 63110.

Although the noradrenergic innervation of the spinal cord has been well studied, the cells of origin of these pathways have not been fully identified. In order to study this problem, we have used the technique of Blessing et al. (Neurosci. Lett. 9:311, 1978) to demonstrate catecholamine fluorescence and horseradish peroxidase (HRP) in the same cells. Multiple 1 μl injections of a 50% HRP solution were made in the T1-T2 levels of the spinal cord of rats and after 2-3 days, the rats were perfused and serial sections of their brainstem were cut on a Vibratome and the sections examined with a fluorescence microscope. After the fluorescent cells were photographed, the sections were reacted with tetramethybenzidine, remounted, examined microscopically, and photographed.

Fluorescent cells were retrogradely labeled by HRP in the A7 group, which lies ventrolateral to the brachium conjunctivum, A6 group or locus coeruleus (especially its ventral portion), the subcoeruleus group, and the A5 group, which lies lateral and dorsal to the superior olive. No fluorescent cells appeared to be labeled by the HRP in the A1 cell group of the ventrolateral medulla or in the A2 group of the solitary complex. This lack of evidence may indicate that the HRP technique is not sufficiently sensitive. Alternatively, the fluorescence technique used here may demonstrate norepinephrine but not epinephrine due to the mild reaction conditions employed. We tentatively suggest that the spinal projections from these cell groups (Dahlström and Fuxe, 1965) arise from epinephrine-containing cells rather than from norepinephrine-containing cells.

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1146 ROLE OF DOPAMINE STORAGE FUNCTION IN THE CONTROL OF TYROSINE HYDROXYLASE ACTIVITY IN THE DOPAMINE NEURON. B.A. McMillen and P.A. Shore. Dept. of Pharmacol., Univ. of Texas Health Science Center, Dallas, Tx. 75235.

Cessation of nigro-striatal dopaminergic neuronal impulse flow by axotomy or by administration of γ-butyrolactone (GBL) causes a marked increase in striatal tyrosine hydroxylase (TH) activity and in striatal dopamine (DA) content. Activation of TH is thought to arise as a consequence of the lack of DA release which normally allows stimulation of presynaptic DA receptors (autoreceptors) which inhibit TH activity. However, TH activation following cessation of impulse flow is short-lived compared with blockade of impulse flow or the elevation of DA. Thus there appears to be a mechanism which serves to modulate TH activity despite the continued absence of impulse flow. This could result from saturation of available intraneuronal DA storage sites, leading to an excess of intraneuronal DA free to bind to TH enzyme and inhibit its activity. DA storage function would thus play a role in the regulation of TH activity.

If this hypothesis is true, then reserpine might be expected to inhibit not only the usual GBL-induced rise in striatal DA, but should also inhibit GBL-induced activation of TH. Accordingly, the effect of reserpine (2.5 mg/kg s.c.) was tested in rats. Striatal TH activity (measured in vivo by 30 min. striatal DOPA accumulation after decarboxylase inhibition with NSD-1015) was greatly elevated one day after reserpine, but had returned to normal by 3 days despite continued depression of DA levels. GBL enhancement of striatal DOPA accumulation after NSD-1015 was markedly reduced in 3 day reserpine rats compared with normal rats (1.2 vs. 3.7 μg/g above NSD-1015 alone). This effect appeared to be due to a shorter-lived effect of GBL on TH activity. Analysis of DA after GBL alone showed that GBL-induced increase in DA levels peaked at a shorter time and at a lower content than in controls (4 vs. 11 μg/g DA increase).

Haloperidol also activates TH in part, at least, via DA autoreceptors. In 3 day reserpine rats, haloperidol enhancement of TH activity was also greatly depressed (1.6 vs. 4.4 μg/g DOPA above NSD-1015 alone) and the usual elevation of striatal DA metabolites seen after haloperidol was almost abolished. Thus inhibition of DA storage function by reserpine alters the coupling of DA autoreceptor activity with TH activity. It is suggested that DA storage function modulates TH activity by controlling the amount of DA available for attachment to and inhibition of TH enzyme. This hypothesis is consistent with recent immunocytochemical observations which suggest an association of TH enzyme with synaptic vesicles in DA neuronal terminal areas. (Supported by USPHS Grant MH-05831).

1147 **REGIONAL VARIATIONS IN RAT BRAIN EPINEPHRINE TURNOVER.** **Ivan Mefford* and Jack D. Barchas (SPON: R. N. Adams). Dept. Psychiatry, Sch. Med., Stanford Univ., Stanford, CA 94305.**

The turnover of epinephrine (E) and the major catecholamines, norepinephrine (NE) and dopamine (DA) were studied in the rat brain hypothalamus, midbrain, locus coeruleus and lower brainstem nuclei, nucleus tractus solitarii and vagal nuclei. Catecholamines were measured in these regions following pharmacological manipulation with the monoamine oxidase inhibitor, pargyline. Further studies are in progress using α-methyl-p-tyrosine and an inhibitor of phenylethanolamine N-methyl transferase, SK&F 64139. Animals were sacrificed at varying intervals after treatment, brains removed, dissected and analyzed for catecholamines by high pressure liquid chromatography (HPLC) with electrochemical detection. Preliminary evidence suggests turnover rates were highest in nucleus tractus solitarii and lower brainstem followed by locus coeruleus and hypothalamus respectively. On a relative basis, E turnover appears to be much greater than NE and/or DA in these regions. This high turnover rate in these brainstem areas suggests a possibly important role for E in the mechanism of a central response to stress.

1148 POTENTIATION OF MONOSYNAPTIC PURKINJE CELL EXCITATION AND INHIBITION FOLLOWING LOCUS COERULEUS ACTIVATION. Hylan C. Moises, Barry D. Waterhouse and Donald J. Woodward. Dept. Cell Biology, Univ. Tx. Health Sci. Ctr., Dallas, Tx. 75235.

Previous studies from our laboratory have demonstrated in the rat that iontophoretically applied norepinephrine (NE) exerts potent modulatory effects on the cerebellar synaptic circuitry, in addition to its well-known depressant influence on Purkinje (P) cell firing. Moreover, we recently reported that stimulation of the noradrenergic pathway from the locus coeruleus (LC) to cerebellum can enhance both excitatory and inhibitory responses of the P cell evoked by activation of multisynaptic afferent pathways from the sensorimotor cortex (Moises et al., 1978, Neurosci. Abst. 4:279). In this study, we examined whether LC conditioning stimulation could similarly facilitate responses of the P cell evoked by local stimulation of its monosynaptic afferent inputs.

Single shocks (0.2 msec duration at 0.5/sec) were applied to the cerebellar surface through bipolar electrodes, and the responses of P cells both on and off the local (LOC) beam of parallel fiber (PF) activation were recorded extracellularly in halothane-anesthetized rats. Poststimulus time histograms were used to quantitate responses to LOC stimulation when tested before and at various time intervals (50-1000 msec) after preconditioning LC stimulation, which in all cases was at current intensities subthreshold for directly affecting P cell discharge.

Excitatory spike responses and pure off-beam inhibitions were tested in 20 neurons following LOC stimulation. In 9 of 12 cells, inhibitory responses to LOC stimulation, mediated by basket-stellate cell input, were greatly enhanced in both magnitude and duration when preceded by LC conditioning stimulation (3 shocks of 0.1 msec duration at 50-100 Hz). In 3 of 4 neurons tested, excitatory spike responses evoked by direct PF input to the P cell were increased (from 0.55 to 0.83 spikes/stimulus) following LC activation. Post-excitatory inhibitions observed after the evoked spike excitation were also augmented by subthreshold LC stimulation in those 3 cells. Typically, this facilitatory noradrenergic influence on all synaptic inputs was most readily observed when LC conditioning was applied 100-500 msec prior to local PF stimulation. Stimulation in areas outside LC did not alter (3 cells) or reduced the off-beam inhibitory response (1 cell). These data suggest that tonic noradrenergic input may act to facilitate the transmission of afferent information to the cerebellar cortex by augmenting directly the actions of conventional monosynaptic afferent inputs that converge on the P neuron. (Supported by grants from NSF BNS77-00174, NIDA DA-02338 and the Biological humanics Foundation to DJW).

1149 THE EFFERENT PROJECTIONS OF THE DORSOLATERAL PONTINE TEGMENTUM IN THE CAT. Michele S. Moss* and Allan I. Basbaum. Dept. Anat., Sch. Med., UCSF, San Francisco, Ca. 94143.

The dorsolateral pontine tegmentum (DLPT) is a major catecholamine containing area which has been implicated in stimulation-produced and opiate analgesia. With a view towards determining the relationship of this region with other areas involved in pain control, we examined the efferent projections of the DLPT in the cat. Injections of 3H-leucine (0.1-0.3 ul) were made in the area of the locus coeruleus (LC), sub-coeruleus (SC) and parabrachial (PB) nuclei. After appropriate survival times, the brain and spinal cord were processed for autoradiography.

Ascending axons of the DLPT travel in a prominent bundle located just ventrolateral to periaqueductal grey. In the midbrain, the major terminal fields are located in the dorsal raphe and the ventrolateral periaqueductal grey. Projections are also seen in the rostral dorsolateral periaqueductal grey, the nucleus of Edinger Westphal and, via a descending branch of the ascending bundle, in the ventral tegmental area of Tsai and in the interpeduncular nucleus. In the diencephalon, the densest projections are located in the paraventricular thalamus and in the intralaminar thalamic nuclei (centralis medialis, paracentralis and centralis lateralis). Projections to the CM-PF complex resulted from injections into the reticular formation (RF) just medial to the LC/SC. A prominent terminal field in VPM was attributed to amino acid transport via "taste afferents" from the medial PB nucleus. The lateral hypothalamus, the zona incerta and the Fields of Forel are also densely innervated. Moderate projections were recorded in the paraventricular, periventricular and dorsomedial nuclei of the hypothalamus. Rostrally, there are dense projections to limbic structures including the bed nuclei of the stria terminalis, the diagonal band of Broca and the region in and around the central nuclei of the amygdala.

The descending projections seen to the dorsal motor nucleus of X, the solitary nucleus, the spinal trigeminal nucleus, the RF and the spinal cord are less pronounced than the ascending DLPT projections described above.

The projections of the DLPT show considerable overlap with both the distribution of 5HT and immunoreactive enkephalin in the cat. The functional significance of this overlap remains to be determined.

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1150 CATECHOLAMINES IN DOG AND MOUSE OLFACTORY BULB: LAMINAR DISTRIBUTION AND RESPONSE TO DEAFFERENTATION. N. S. Nadi*, R. Head* and F.L. Margolis, Dept. Phys. Chem. & Pharm., Roche Institute of Molecular Biology, Nutley, NJ 07110.

Histochemical and immunocytochemical evidence in the literature has qualitatively indicated the presence and location of dopamine (DA) and norepinephrine (NE) in the mammalian olfactory bulb. The laminated structure of the olfactory bulb makes it possible to dissect it into layers enriched in known neuron types. Combining this with a very sensitive radioenzymatic assay for DA, NE and epinephrine (E) [a modification from da Prada & Zürcher, *Life Sci.* 19, 1161 (1976)], we have examined the distribution of these three catecholamines in the following layers of the dog olfactory bulb: the fiber layer (F) containing incoming olfactory nerve fibers; the glomerular layer (G) containing mitral cell dendrites, olfactory nerve endings and periglomerular cells; the mitral cell-granule cell layer (M-G) containing mitral cell perikarya, granule cells and tufted cells; and the white matter (W) containing afferent and efferent fibers of the olfactory bulb. The DA and NE exhibited distinctive patterns in the four layers of the dog bulb as shown below.

		Layer			
Catecholamine	Whole bulb	F	G	M-G	W
DA (ng/g wet wt.)	52	42	120	68	29
NE (ng/g wet wt.)	38	32	33	58	65

The levels of E were very low in the dog olfactory bulb (0.09 ng/wet wt.) and showed a uniform distribution across the four layers analyzed. In whole olfactory bulbs from mice, we have obtained the following preliminary results: DA, 73 ng/g wet wt.; NE, 117 ng/g wet wt.; and E, 1 ng/g wet wt. The effects of peripheral deafferentation by intranasal irrigation with zinc sulfate (Zn) [0.17 M] or vinblastine (Vb) [10 mM] on the levels of catecholamines in the mouse olfactory bulbs have also been investigated. At 3 weeks after treatment, when the majority of the peripheral input to the bulb had degenerated, the levels of DA in the mice treated with Zn or Vb were significantly lower (11 ng/g wet wt.) than those in saline-treated controls (73 ng/g wet wt.). In contrast, the levels of NE and E in the bulbs of Zn- and Vb-treated animals were not significantly different from the saline-treated controls. Since DA levels in the olfactory epithelium are below the limits of sensitivity of the assay, it is possible to conclude that the origin of the DA which is lost is intrabulbar. The cellular origin of the DA lost as a result of peripheral deafferentation and the influence of the peripheral neuronal input on its regulation will be discussed.

1151 OCTOPAMINE-SENSITIVE ADENYLATE CYCLASE: PROPERTIES AND PHARMACOLOGICAL CHARACTERIZATION. James A. Nathanson and Edw. J. Hunnicutt*. Dept. Neurol., Yale Medical School, New Haven, Ct. 06510.

A potent and highly specific octopamine (OCT)-sensitive adenylate cyclase has recently been identified in the firefly light organ (Nathanson, Science 203, 65). The characteristics of this enzyme support the hypothesis that OCT (or synephrine) is the neurotransmitter effecting neural control of lantern flashing, and that cyclic AMP (or pyrophosphate) mediates the intracellular action of the transmitter on light production. We now report some of the properties of OCT-sensitive adenylate cyclase. These data allow for the first time a detailed pharmacological characterization of an octopamine receptor.

Enzymatic activity was measured in washed particulate fractions prepared from isolated Photinus light organs. At optimal concentrations, OCT stimulated basal activity 50-fold. The pH optimum for OCT activation was between 7.4 and 7.9, and maximal stimulation occurred at 30-40°C. Hormone stimulation was supported by Mn^{++}, Co^{++} and Mg^{++} but not by Co^{++}, Ni^{++}, Fe^{++}, Ba^{++}, Cu^{++}, Zn^{++} or Cd^{++}. Calcium had complex effects on enzyme activity. The optimal Mg^{++}/ATP concentration was 8/2mM. GTP doubled maximal enzyme activity and slightly increased the K_a of the enzyme for OCT but was not essential for hormone activity.

Hormone activation was stereo-selective, D(-)OCT being 20-fold more potent than L(+)OCT. Among positional isomers, p-OCT was more than 30 times more potent than either m-OCT or o-OCT. Among beta-hydroxylated phenylethylamines, OCT was more potent than either norepinephrine (NE) or beta-hydroxy-phenylethylamine. Among the non-beta-hydroxylated amines, the OCT precursor, tyramine, was much more active than dopamine or phenylethylamine. Synephrine, the N-methylated derivative of OCT, was considerably more potent than N-methyl-NE (epinephrine). Both the alpha-adrenergic agonist, phenylephrine and the beta-adrenergic agonist, isoproterenol, were much less active than OCT, and the indolamine, serotonin, showed no activity. A number of additional compounds were tested for their ability to block the activation of OCT-sensitive adenylate cyclase.

These results support the presence of a phenylethylamine receptor distinct from those receptors known to be activated by the catecholamines, dopamine, norepinephrine and epinephrine.

1152 EFFECTS OF INTRA-HIPPOCAMPAL INJECTION OF KAINIC ACID ON BRAIN NOREPINEPHRINE. M.F. Nelson, R. Zaczek*, L. Oshida* and J.T. Coyle. Dept. Pharmacology, Johns Hopkins Univ. Balto, MD 21205

Our previous studies have demonstrated that injection of kainic acid (KA) into the rat hippocampus (HIP) acutely caused generalized tonic-clonic convulsions in association with degeneration of HIP intrinsic neurons and a profound reduction in HIP norepinephrine (NE) (Eur. J. Pharmacol. 50:209, 1978). Pretreatment of rats with anesthetics and anticonvulsants that block the seizures attenuated the HIP neuronal degeneration (Eur. J. Pharmacol. 52: 323, 1978). In the present study, the factors that mediate the fall in NE levels were examined.

Rats, briefly anesthetized with ether, received a stereotaxic injection of KA (0.5 µg in 0.5 µl) in the dentate gyrus; NE was measured in several brain regions at various times after injection. KA caused a rapid depletion of NE in all regions innervated by the locus coeruleus (LC) including the ipsilateral and contralateral HIP, lateral neocortex and cerebellum; notably, the hypothalamus and pons medulla were unaffected. The fall in NE was accompanied by an 80-110% increase in MHPG-SO_4 bilaterally in cortex and HIP. The reduction in NE was evident by 1 hr and maximal by 3-5 hr after injection (-50 to -70%). NE returned to normal over 3-5 days except in the injected HIP, which recovered slowly over two weeks. Neurochemical and immunocytochemical analysis of the injected HIP revealed the integrity of the NE-fibers in spite of loss of HIP intrinsic neurons.

To determine the dependency of NE depletion on impulse flow in the LC projection, an electrothermic lesion was placed in the midbrain immediately before the intra-HIP KA injection, and the rats were sacrificed 3 hr later for NE assay. The bundle lesion alone in sham injected rats resulted in a slight increase in NE levels in the cortex; however, prior bundle lesion did not prevent NE reduction in any area within 3 hr of injection of 0.5 µg of KA into the HIP.

To evaluate the possibility that KA might act at presynaptic sites on the NE terminal, HIP slices were preincubated with [^{3}H]-NE and the release of [^{3}H]NE was determined in a perfusion chamber. KA stimulated the release of [^{3}H]NE with an EC_{50} at 300 µM; release was blocked in Ca^{++} deficient medium. L-Glutamate was > 30-fold less potent than KA and did not exhibit additivity with KA. Addition of tetrodotoxin to the perfusion medium blocked KA induced release of [^{3}H]NE.

The data suggest that intracerebral injection of KA causes a marked release of NE that is regulated locally at the terminal level and is independent of impulse flow from the LC. This presynaptic mechanism of release becomes diffusely activated throughout the LC projection by injections in areas causing generalized seizures.

1153 MODULATION OF SPINAL MOTONEURON EXCITABILITY BY 5-HYDROXYTRYPTAMINE AND NOREPINEPHRINE. Richard S. Neuman and Susan R. White. Fac. Med., Memorial Univ. Nfld., St. John's, Nfld., A1B 3V6.

5-hydroxytryptamine (5-HT) and norepinephrine (NE), applied near lumbar spinal motoneurons by microiontophoresis, decreased the threshold for glutamate evoked motoneuron action potentials, increased the duration and frequency of motoneuron activity evoked by suprathreshold amounts of glutamate and increased the amplitude of ventral or dorsal root evoked motoneuron field potentials. These facilitatory effects of 5-HT and NE often lasted for several minutes after drug application ceased. Neither 5-HT nor NE directly elicited motoneuron action potentials in the absence of other excitatory input. The 5-HT antagonist, metergoline, prevented 5-HT but not NE facilitation of motoneuron excitability. These results indicate that, as has been reported for brainstem motoneurons (McCall & Aghajanian, Neurosci. Abs. 4 (1978) 447), 5-HT and NE enhance the effects of excitatory inputs to spinal motoneurons by actions on separate receptors.

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1154 SELECTIVITY OF NORADRENERGIC AXON SPROUTING FOLLOWING PERINATAL 6-OHDA TREATMENT. John A. Olschowka*, Hart G.W. Lidov, Reinhard Grzanna, and Mark E. Molliver, Departments of Cell Biology/Anatomy and Neurology, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

The administration of 6-hydroxydopamine (6-OHDA) or 6-hydroxydopa early in development results in a marked elevation of norepinephrine, of (^{3}H) norepinephrine uptake, and of dopamine-beta-hydroxylase (DBH) in brainstem and cerebellum. These neurochemical changes in brainstem and cerebellum have been attributed to an increased outgrowth (i.e. sprouting) of noradrenergic (NA) terminals innervating these areas. There has been no comprehensive detailed morphologic analysis of the altered pattern of NA innervation in the 6-OHDA treated animal. The present study uses an immunohistochemical method for the visualization of DBH to demonstrate the distribution and geometric patterns of NA axon terminals in the diencephalon, cerebellum and brainstem of treated animals.

Rats were given intraperitoneal injections of the neurotoxin, 6-OHDA (200-300 mg/kg) on day 17 of gestation or during the first 24 hr after birth. Vehicle injected animals served as controls. DBH immunofluorescence revelaed a total absence of NA axons in the telencephalon and a marked increase in the NA innervation of selective brainstem areas. The distribution of sprouted fibers was restricted to those areas which normally receive a NA innervation. A marked increase was seen in the paraventricular, anteroventral, and reticular nuclei of the thalamus, in the cerebellar cortex, and in the superior colliculi, motor and sensory nuclei of V, and cochlear nuclei of the brainstem. Within cerebellar cortex, lobules I-VIa are densely innervated in the normal and show the maximum degree of sprouting. The flocculo-nodular lobe, which is sparsely innervated in the normal, reveals very little sprouting. In both cerebellum and in superior colliculus, layers that normally receive a dense NA innervation show a greater increase in NA fiber density than do sparsely innervated layers. In those regions where the density of noradrenergic fibers was increased in 6-OHDA treated animals, the normal geometric pattern of NA axons was maintained. It can be concluded that the sprouting of noradrenergic fibers following 6-OHDA treatment is not random or diffuse, but exhibits some degree of neuronal specificity in that it conforms to the normal pattern of NA innervation. (Support: USPHS NS06117, NS08153, NS10290; H.G.W.L. supported by training grant GM-7309.)

1155 ACETALDEHYDE(AcH) CONDENSATION PRODUCTS OF DOPA OR DOPAMINE(DA): METABOLIC AND BEHAVIORAL ASPECTS. T.C. Origitano*, J. Hannigan*, P.D. Patel*, A. Kahn* and M.A. Collins. Dept. Biochem., Loyola Stritch Sch. Med. and Hines V.A. Hosp., Maywood, IL 60153.

Because of the possibility of their involvement in the acute or chronic aspects of alcoholism, certain tetrahydroisoquinolines (TIQs) derived from catecholamines(CAs) and the AcH produced in ethanol oxidation were examined with respect to their metabolism and effects on amines in brain and effects on alcohol preference after peripheral or central administration in rodents. Sensitive and specific assays--high performance liquid chromatography(HPLC) with electrochemical detection; capillary GC with electron capture--were utilized in the chemical studies, and a simple HPLC method for the separation of TIQ metabolites and endogenous amines was developed which employs Partisil CX columns. Carboxysalsolinol(cSAL), the TIQ from DOPA and AcH, present in rat brain following intraperitoneal injection, was decarboxylated to a limited extent to salsolinol(SAL), but the main metabolite appeared to be O-methylated. Striatal serotonin(5-HT)content was selectively raised by cSAL, but DA levels were decreased only if a decarboxylase (DC)inhibitor was pre-administered. Inclusion of cSAL in a diet containing DC inhibitor resulted in a statistically significant increase in alcohol solution preference after 3-4 wks in alcohol-avoiding mice, which is in accord with the results in rats by M. Hirst(unpubl.) and Myers & Melchior(Pharm. Biochem.Behav.7,381,1977). In further neurochemical studies, SAL, the TIQ from DA, also elevated 5-HT levels (like cSAL)when given intraventricularly in rats(200ng),but it also lowered striatal 5-hydroxyindoleacetate and CA levels; higher SAL doses tended to obscure these decreases. In general agreement with G.Cohen's studies (in press), 7-O-methylation of SAL seemed to be a prominant CNS route. We conclude that the simple TIQs derived from DOPA or CAs, and possibly their O-methylated metabolites, have significant interactions with the serotonergic system(perhaps via inhibition of 5-HT uptake and 5-HT specific monoamine oxidase analogous to β-carboline condensation products) which could be part of the neurochemical basis for their effects on alcohol preference and analgesia. Supported by USPHS AA00266 and V.A. Medical Research Service.

1156 SEROTONIN INNERVATION OF RAT FOREBRAIN: A RADIOAUTOGRAPHIC STUDY. A. Parent, L. Descarries and A. Beaudet*, Laboratoires de neurobiologie, Université Laval, Québec, and Centre de recherche en sciences neurologiques, Université de Montréal, Montréal, Canada.

Light microscope radioautography was used to study the organization of ascending serotonin (5-HT) systems in adult rat brain, following lateral ventricle perfusions of [^{3}H]5-HT. The cell bodies, axonal projections and terminal fields of 5-HT neurons were clearly visualized in paraventricular and basal forebrain areas. Labeled cell groups in midbrain raphe were seen to give rise to two major fiber systems. First, a prominent bundle, apparently originating from nucleus raphe dorsalis, ascends medially within the ventral half of periaqueductal gray, along with the dorsal longitudinal fasciculus. Ramifications of these fibers terminate in inferior and superior colliculi and subcommissural organ. At mesodiencephalic junction, the bulk of this bundle sweeps downward abruptly to innervate the entire periventricular gray of diencephalon. Second, a series of small fascicles, issued from nuclei raphe dorsalis (B-7 and B-6) and medianus (B-8) at various rostro-caudal levels, course directly ventralward through the tegmental and cerebellar peduncle decussations. These fascicles converge toward the medial forebrain bundle (MFB), arching beneath the red nucleus. Thence, some fibers enter fasciculus retroflexus to reach the medial habenula, others cross the midline within the supramammillary commissure, whereas most ascend in the MFB giving off numerous collaterals to hypothalamus. Part of the 5-HT innervation of the mammillary bodies seems to arise from labeled neurons in the medial lemniscus (B-9) whose axons travel in the mammillary peduncle. At thalamic levels, dense 5-HT terminal fields are present in the ventral portion of lateral geniculate nucleus and in midline and intralaminar nuclei. At rostral hypothalamic levels, labeled fibers leave the MFB, and enter the striae medullaris and terminalis to reach the lateral habenula and amygdaloid complex, respectively. Another contingent spreads more laterally to innervate the globus pallidus and neostriatum. At septal levels, many labeled axonal varicosities closely surround the cell bodies of the lateral nucleus, while ongoing fibers ascend through the diagonal band of Broca. Some of these continue dorso-caudally into the fornix and reach hippocampus, whereas others sweep around the corpus callosum, and course within the indusium griseum leaving collaterals to cingulate cortex. At the ventral surface of brain, patches of dense innervation are associated with the islands of Calleja. More rostrally, fascicles of labeled fibers pass through the anterior olfactory nucleus and arborize in the glomerular layer of olfactory bulb. In accordance with earlier data gathered by means of various neuroanatomical methods, this study emphasizes the complex and widespread distribution of 5-HT systems in rat forebrain.(Supported by MRC of Canada)

1157 DIFFERENTIATION OF MULTIPLE SEROTONIN RECEPTORS IN THE CENTRAL NERVOUS SYSTEM. Stephen J. Peroutka, Richard M. Lebovitz* and Solomon H. Snyder. Dept. Pharmacol., Johns Hopkins Univ., Sch. Med., Baltimore, MD 21205

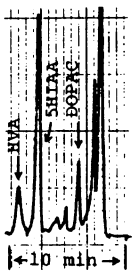
Serotonin receptors in the central nervous system of rats can be labeled by ^{3}H-5-hydroxytryptamine(5-HT), ^{3}H-lysergic acid diethylamide (LSD) and ^{3}H-spiroperidol. Binding data suggest ^{3}H-5-HT and ^{3}H-spiroperidol label distinct, non-interconverting populations of serotonin receptors while ^{3}H-LSD labels both sites. Evidence supporting this conclusion includes: (1) potencies of various drugs differ by factors up to a thousand-fold in competing differentially for ^{3}H-5-HT and ^{3}H-spiroperidol binding; (2) saturating doses of 5-HT do not affect the saturation kinetics of ^{3}H-spiroperidol binding. Conversely, ^{3}H-5-HT saturation is not affected by saturating concentrations of spiroperidol; (3) the number of specific ^{3}H-LSD binding sites is equal to the sum of ^{3}H-5-HT and ^{3}H-spiroperidol binding sites: (4) ^{3}H-LSD binding resembles ^{3}H-5-HT or ^{3}H-spiroperidol binding if the brain membranes are incubated in the presence of saturating amounts of spiroperidol or 5-HT, respectively; (5) guanine nucleotides affect agonist interactions with ^{3}H-5-HT binding but have no effect of ^{3}H-spiroperidol binding. ^{3}H-LSD binding is affected in an intermediate manner; (6) the potencies of antagonists in inhibiting 5-hydroxytryptophan induced head twitches correlate with relative drug potencies for ^{3}H-spiroperidol binding.

We conclude that both ^{3}H-5-HT and ^{3}H-spiroperidol label apparent serotonin receptors in the central nervous system. Under the assay conditions employed in this study, the ^{3}H-ligands label distinct, non-interconverting populations of serotonin receptors. ^{3}H-LSD labels both receptor populations to a similar extent. We suggest that the receptors labeled by ^{3}H-5-HT and ^{3}H-spiroperidol be designated as 5-HT$_1$ and 5-HT$_2$ receptors, respectively.

1158 ANALYSIS OF BIOGENIC AMINE METABOLITES IN RAT BRAIN HPLC WITH ELECTROCHEMICAL DETECTION. Kenneth W. Perry* and Ray W. Fuller. The Lilly Research Laboratories, Indianapolis, IN 46206.

The high sensitivity of electrochemical detection (EC) combined with the specificity of high performance liquid chromatography (HPLC) have provided an important new analytical tool in neurochemistry (R. N. Adams, Anal. Chem. 48, 1128A, 1976). We have developed an HPLC-EC method for determining catecholamine metabolites--homovanillic acid (HVA), 3,4-dihydroxyphenylacetic acid (DOPAC), and 3-methoxy-4-hydroxy-phenylethyleneglycol sulfate (MOPEG-SO_4)--and the serotonin metabolite 5-hydroxy-indoleacetic acid (5HIAA) in whole brain and in brain regions. Measurement of concentration of these metabolites can provide an index of the turnover of the neurotransmitter amines in brain.

Brain tissue is homogenized in acidified n-butanol. The homogenate is centrifuged, and heptane and 0.1 HCl are added. Following shaking and centrifugation, the butanol-heptane phase contains HVA, DOPAC and 5HIAA, and the aqueous phase contains MOPEG-SO_4 (and biogenic amines). The HVA, DOPAC and 5HIAA are then extracted with 0.1 M sodium acetate and are assayed simultaneously by HPLC-EC using a C_8 reverse phase column (4 mm x 25 cm) such as Dupont Zorbax with a mobile phase of 0.1 M Na_2HPO_4-0.05 M citric acid-10% methanol at pH 5. A typical chromatographic tracing is shown. The MOPEG-SO_4 is not electrochemically active and must first be hydrolyzed to free MOPEG by heating for 15 min at 85° in 0.2 N HCl. Free MOPEG is extracted into ethyl acetate, which is then evaporated. The MOPEG is dissolved in 400 µl of 0.1 N formic acid and subjected to HPLC-EC. Absolute sensitivity for these metabolites as pure standards is in the 0.1 pmole range, and the practical sensitivity for their assay in tissue is about 1 pmole per injection.



The metabolites were determined in six brain regions (cerebellum, cortex, corpus striatum, hypothalamus, midbrain and brain stem) to demonstrate the utility of the method and the localization of the metabolites. Typical concentrations (in pmoles/g) in whole brain were 610 ± 20 for DOPAC, 470 ± 20 for HVA, 2460 ± 60 for 5HIAA, and 710 ± 20 for MOPEG-SO_4. Pargyline (20-40 mg/kg i.p.) lowered DOPAC, HVA and 5HIAA at 48 hrs. The lowering of all three was antagonized by co-administration of harmaline (30 mg/kg i.p.). Harmaline had earlier been shown to antagonize selectively the inactivation of type A monoamine oxidase by pargyline. These findings indicate that the formation of all three metabolites occurred by type A monoamine oxidase.

1159 EFFECTS OF BIOGENIC AMINES IN RAT CEREBRAL CORTEX AFTER SEROTONINERGIC DEAFFERENTATION. MICROIONTOPHORETIC STUDIES. T.A. Reader, A. Ferron* and L. Descarries, Centre de recherche en sciences neurologiques (Département de physiologie), Université de Montréal, Montréal, Québec, H3C 3T8, Canada.

Various findings have recently reinforced the hypothesis that serotonin (5-HT), norepinephrine (NE) and dopamine (DA) afferents exert a modulatory influence on relatively large populations of cortical neurons. The present microiontophoretic studies were carried out to assess the responsiveness of cortical neurons to 5-HT, NE, DA and acetylcholine (ACh), after selective lesioning of the serotonin system. In normal adult Sprague-Dawley rats, DA, NE and 5-HT inhibited the majority of spontaneously active cortical neurons recorded in the fronto-parietal cortex. This effect, obtained with ejection currents of 50-100 nA during 20-30 s, was usually of prolonged duration (4-6 min) and often associated with a spike hyperpolarization. ACh-excitatory responses (to 40 nA for 10-15 s), which normally lasted for 60-90 s, were also blocked or reduced by the monoamines, for as long as the inhibition of spontaneous activity induced on the same cells. To produce a selective 5-HT deafferentation, 5,7-dihydroxytryptamine was administered by intraventricular injection after desmethylimipramine pretreatment. Biochemical determinations and iontophoretic experiments were performed two weeks later. The endogenous cortical 5-HT content, assayed with a radioenzymatic method, was reduced to less than 10% of controls. In such 5-HT-denervated animals, the initial phase of inhibitory responses to 5-HT as well as total duration of these responses were significantly enhanced in 70% of the cells recorded (mean duration: 14 min). The inhibition of ACh-excitatory responses by 5-HT was also found to be of longer duration in 75% of the units hypersensitive to 5-HT. Since the afferent serotoninergic component had been eliminated, the absence of reuptake mechanisms could account for prolongation of the responses to 5-HT. However, in a great proportion of neurons hypersensitive to 5-HT, the inhibitory responses to DA or NE were markedly reduced. It therefore seems likely that cortical aminergic systems exert their modulatory effects through distinct receptor sites. The elimination of one afferent component could induce enzymatic changes in the effector cells and/or increase receptor affinity or the number of receptor sites, thus giving rise to enhanced responses to 5-HT and its agonists. Furthermore, an increased number of 5-HT receptors might in turn alter receptor sites for other neuromodulators, thus explaining the lesser responsiveness to DA and NE.

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1160 3-METHOXY 4-HYDROXY PHENETHYLENE GLYCOL (MHPG) IN MONKEY BRAIN, CSF, AND PLASMA DURING NALOXONE PRECIPITATED MORPHINE ABSTINENCE. D.E. Redmond, Jr., R.H. Roth, S.E. Hattox, J.M. Stogin* and J. Baulu* Depts. Psychiatry, Pharmacology, Yale, New Haven, Ct. 06510

The recent demonstration of the ability of clonidine to suppress the abstinence syndrome in humans[1] has focused attention on possible noradrenergic hyperactivity as a partial biological substrate of the syndrome. The recent electrophysiological observations made in rats demonstrating a dramatic increase in the firing rate of locus coeruleus cells during abstinence lend support to this hypothesis.[2] We now present preliminary data showing increases in brain and plasma of the major NE metabolite, MHPG, in morphine-tolerant primates after treatment with the opioid antagonist, naloxone.

Three groups of 3 Cercopithecus aethiops were treated as follows: Group 1 (G1) received morphine sulfate 3 mg/kg 3 x's daily for 10 days, and then 4 mg/kg 4 x's daily for 3 days, Group 2 (G2) received matching volumes of saline with each injection of Group 1. 30 minutes before sacrifice G1 and G2 monkeys each received three injections of 0.2 mg naloxone two hours after the last morphine or saline injection. Group 3 (G3) monkeys received one 4 mg/kg dose of morphine two hours before sacrifice. All animals were anesthetized with ketamine 5-10 mg/kg prior to venous blood and cisternal CSF sample collections and sacrificed by brain removal under deep pentobarbital anesthesia. The hypothalamus was rapidly dissected after immersion of the brain in an ice bath, while plasma was separated and prepared with NA metabisulfite. All specimens were stored immediately in liquid nitrogen prior to analysis of MHPG by the GC-MS method of Maas et al.[3]

Results confirmed increases in MHPG in hypothalamus during abstinence, accompanied by specific abstinence signs. G1 monkeys had 0.332 µg/g ± 0.036 (SEM) in hypothalamus, compared with 0.139 ±0.04 for G2 monkeys, or 0.142±0.016 for G3 monkeys. The plasma MHPG of morphine + naloxone monkeys was significantly greater than that of the two control groups. Plasma concentrations, in the same order, were 29.7±8.1 ng/ml, 14.7±1.6, and 9.7±0.5 ng/ml; and CSF concentrations were 47.3±9.7 ng/ml, 38/4±4.8, and 27.4±2.7.

We conclude that MHPG increases in hypothalamus and plasma following naloxone administration in morphine dependent monkeys. The correlation of hypothalamic and plasma MHPG ($r=0.91$, $p<0.01$) supports the utility of further studies of MHPG changes in the human opioid abstinence syndrome.

[1] *Lancet* 8070:929-930; 8090:599-602,1978; [2] *Nature* 276:186-188, 1978; [3] *Brain Res.* 118:167-173,1976.

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1161 THE NORADRENALINE CONTENT OF RAT BRAIN BLOOD VESSELS John F. Reinhard, Jr., Eldad Melamed, Sherman Elspas* and Michael A. Moskowitz
Laboratory of Neural & Endocrine Regulation, Dept. of Nutrition & Food Science, M.I.T., Cambridge, MA 02139

Brain extraparenchymal (pial) blood vessels and choroid plexus receive a histochemically defined adrenergic innervation from the superior cervical ganglion. Controversy exists as to the innervation of brain parenchymal blood vessels (microvessels), and to the relative distribution of noradrenergic neurons in parenchymal and extraparenchymal blood vessels of the brain. We therefore measured the catecholamine content of blood vessels taken from the leptomeninges, choroid plexus and brain parenchyma.

450-550 g male Sprague-Dawley rats were killed by decapitation and microvessels were isolated by a sucrose density gradient method after removal of the meninges and choroid plexus. Catecholamines were measured using high pressure-liquid chromatography and electrochemical detection after homogenizing the tissues in 0.1 M perchloric acid.

The noradrenaline content of microvessels, leptomeninges and choroid plexus was 528±17, 534± 122 and 277±154 ng/g, respectively; the catecholamines dopamine and adrenaline were not detected in these brain fractions. Right frontal cortical grey matter contained 640 ± 128 ng/g of noradrenaline. To test whether some of this amine was due to blood within the vessel lumen, animals were perfused in situ with isotonic saline. Levels of noradrenaline remained unchanged in microvessels and meninges but decreased by 34% in choroid plexus. Our results suggest that brain blood vessels contain measurable amounts of the catecholamine noradrenaline which does not derive from the luminal contents. Such an approach will be useful to determine the relative contributions of intrinsic (l. coeruleus) or extrinsic (superior cervical ganglia) noradrenaline-containing neurons to the innervation of the brain vasculature.

1162 MODULATION OF BRAIN ADRENERGIC RECEPTORS BY SPECIFIC LESIONS OF THE DORSAL NORADRENERGIC BUNDLE. T.D.Reisine, D.C.U'Prichard[1], S.T.Mason[2], H.C.Fibiger[2] and H.I.Yamamura. Department of Pharmacology, Univ. of Arizona Health Science Center, Tucson, AZ 85724, Department of Pharmacology, Northwestern Univ.[1] and Division of Neurol. Sci., University of British Columbia[2].

It has been suggested that the characteristics of central adrenergic receptors may be regulated by the levels of norepinephrine (NE) at the adrenergic synapse. To test this hypothesis as well as to better define the precise pre- or postsynaptic location of adrenergic receptors in the brain, lesions of the dorsal NE tract from the locus coeruleus were induced by bilateral intracerebral injections of the neurotoxin 6-hydroxydopamine (6-OHDA). In eight brain regions of control and lesioned rats, the levels of NE were measured and the characteristics of α_1, α_2 and β-adrenergic receptors were determined by the binding of the radiolabeled ligands ^{3}H-WB-4101, ^{3}H-clonidine and ^{3}H-dihydroalprenolol, respectively. In seven of the brain regions of the lesioned animals, NE levels were severely depleted. However, in the cerebellum, NE concentrations increased 54% over control values. Beta-receptor density was elevated in the lesioned amygdala, cortex, hippocampus, septum and thalamus but lowered in the cerebellum. Alpha$_1$-receptor binding increased significantly in the lesioned cortex, thalamus and septum, was below control levels in the cerebellum and unaltered in the other brain regions. Alpha$_2$-receptor binding was above control levels in the lesioned cortex but in contrast decreased in the lesioned septum and amygdala. Interestingly, intraventricular 6-OHDA lesions, while lowering cerebellar NE levels, elevated both α_1 and β-receptor binding. The sensitivity of α_1 and β-receptors in the lesioned brain regions changed in an opposing fashion to the levels of NE. These data suggest that α_1 and β-receptors are postsynaptic to NE-containing neurons and are significantly affected by the levels of NE at the adrenergic synapse. Alpha$_2$-receptors are mainly postsynaptic in the cortex. However, a substantial population of these receptors appear to be presynaptic to adrenergic innervation in the amygdala and septum. These presynaptic receptors might be similar to perpherial alpha$_2$-receptors and as such could function to regulate NE release. Supported in part by USPHS grants and RSCA to H.I.Y.

1163 ALCOHOL CONSUMPTION AND ACTIVITY LEVEL IN MALE RATS AFTER DORSAL TEGMENTAL BUNDLE LESIONS. Daniel L. Richardson* and Stanley A. Lorens. Dept. Pharm., Bldg. 135, Loyola U. Med. Center, Maywood, IL 60153.

Central norepinephrine (NE) pathways have been postulated to play a major role in determining the behavioral effects of ethanol (ETOH). Recently, Mason et al. (1979) reported that 6-hydroxydopamine (6-DA) lesions of the dorsal tegmental bundle (DTB) prevented the emergence of 15% v/v ETOH ingestion and blocked the reduction in locomotor behavior produced by 1.0 g/kg ETOH. In the present experiments the highest concentration (w/v) of ETOH preferred (at least 50% of 24 hr fluid intake) was established using the alternate day, free choice method. Beginning with 1%, each rat was exposed 3 times to 2% increments in ETOH concentration until they drank less than 40% of their daily fluid from the ETOH bottle. ETOH concentration then was reduced until a preferred concentration was reached. Between rats these varied from 2-12% resulting in 1.0 - 5.4 g/kg/day ETOH intakes. The animals were exposed to the preferred ETOH concentration for 7 consecutive alternate days before and 9 days after 6-DA (4 µg) DTB lesions (n=7) or control operations (n=6). Subsequently, the rats were exposed to decreasing concentrations (0.5 - 0.125 mg%) of quinine using the same procedure. Finally, open field activity and the effect of 1.0 g/kg ETOH on activity level in a LVE photocell chamber were examined. Regional forebrain NE levels were determined and the injection sites examined histologically.

The DTB lesions did not alter consumption of the preferred ETOH solution nor the quinine detection level. Likewise, the DTB lesions did not affect open field activity and failed to affect the sedative effect of ETOH. The DTB lesions reduced hippocampal and cortical NE levels by 79 and 72%, respectively.

DTB NE lesions do not affect the oral intake of a preferred ETOH solution not the sedative property of ETOH. That the failure of such lesions to affect these measures may depend on the animals having been exposed to ETOH preoperatively is currently under investigation. The present results, nevertheless, do not support the view that ascending DTB NE fibers play a significant role in mediating the behavioral effects of ethanol.

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1164 SUBMAXILLARY SALIVARECTOMY ELEVATES PLASMA CATECHOLAMINES. S. Ritter, R.C. Ritter and C.R. Christianson*. College of Veterinary Medicine, Wash. State Univ., Pullman, WA 99164.

Persistence of significant amounts of plasma and tissue nerve growth factor (NGF) in adult mice suggest a possible role for NGF beyond its tropic and trophic developmental functions. The source of most NGF in the adult rodent is the submaxillary salivary glands. In order to determine whether removal of the main source of NGF affects sympathoadrenal function, we removed the submaxillary salivary glands from 18 adult male rats and subjected an additional 18 adult male littermates to sham salivarectomy. Fourteen days after surgery, these animals were deeply anesthetized with sodium pentobarbital, and the adrenal glands, vas deferens, spleen, heart and brain were removed for catecholamine (CA) assay. A blood sample was also taken by cardiac puncture after removal of the adrenal glands.

Our most striking finding was that plasma norepinephrine (NE) concentrations were 2.1 to 6.3 times higher in salivarectomized than in sham operated rats. Plasma epinephrine (E) concentrations were more variable but in some instances were 5.2 times greater than average concentrations in shams. Adrenal content of both NE and E was reduced to 50% of sham values. CA concentrations from other tissues did not reveal any significant differences between salivarectomized and sham operated rats. The above data suggest that salivarectomy caused increased basal CA release. We are also investigating the effects of salivarectomy upon the sympathoadrenal response to footshock stress. Preliminary data reveals significant differences in plasma and adrenal CA between salivarectomized and sham operated rats exposed to footshock.

If removal of the submaxillary glands was by itself a severe stress, then one might expect markedly elevated plasma CA in salivarectomized rats. However, the fact that salivarectomized rats gained weight at the same rate as shams, displayed no measurable changes in body temperature and did not drink prandially, suggests that severe stress was probably not a factor in generating the increased CA concentrations. Therefore, we suspect that elevated plasma CA in salivarectomized rats resulted from the absence of a sympathetic modulating hormone such as NGF. Experiments to determine the precise mechanism by which salivarectomy-induced CA alterations are brought about are in progress.

1165 LATERALIZATION OF BEHAVIORAL AND CATECHOLAMINERGIC RESPONSE TO EITHER INFARCTION OR 6-HYDROXYDOPAMINE LESIONS OF THE CEREBRAL CORTEX IN RATS. Robert G. Robinson. Dept. of Psychiat. Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

Right middle cerebral artery ligation in the rat leads to a 2 to 3 week period of spontaneous hyperactivity as compared with sham operated controls or preoperative baseline (Nature 255:322, 1975). Significant decreases in norepinephrine (NE) and dopamine (DA) concentrations in the cortex and brainstem occur concomitantly with this hyperactivity (Nature 255, 332, 1975). In marked contrast, left middle cerebral artery ligation, although producing a comparable lesion, does not lead to hyperactivity and these animals are behaviorally indistinguishable from controls. (Neurosci. Abst # 229, 1978). In addition, there are no significant postoperative changes in NE or DA concentrations in either the cortex or brain stem (Life Sci. 24: 943, 1979).

In an effort to compare the effects of focal ischemic lesions with the effects of focal lesions of catecholaminergic terminals, microinjections of the neurotoxin, 6-hydroxydopamine (6-OHDA) in a concentration of 2 ug/ul plus 1 mg/ml ascorbic acid were injected at a rate of 0.5 ul/min 1 mm below the surface of the cortex in approximately the same area where infarcts occur. Following injections into the right hemisphere, a dose response curve could be established with 2ug (N=6) producing a period of increased activity that was indistinguishable from post-ischemic hyperactivity. A 6ug dose (N=7) produced a period of increased activity which lasted 1 to 2 weeks longer than the 2ug dose, although the degree of hyperactivity was about the same for all doses (ie maximum activity 150-160% of control). In contrast to these findings, injections of either 2 ug or 4 ug of 6-OHDA into the symmetrical area of the left cerebral hemisphere did not lead to hyperactivity. These results cannot be attributed to a non-specific suppression of activity following left hemispheric injection because the left hemispheric injected animals were just as active as the controls and their food and water intake were not significantly different. These results clearly implicate the catecholaminergic pathways in the development of postinfarction hyperactivity and suggest that the underlying cerebral asymmetry in the rat may either be in the catecholaminergic pathways or in another postsynaptic neuron which is also involved in the development of hyperactivity.

1166 NOREPINEPHRINE ACTIVATES LATERAL GENICULATE NEURONS AND FACILITATES RETINAL INPUTS VIA AN α-ADRENOCEPTOR. Michael A. Rogawski and George K. Aghajanian. Depts. of Pharmacology and Psychiatry, Yale University School of Medicine, New Haven, Connecticut 06510.

The dorsal lateral geniculate nucleus (LGN) receives a dense noradrenergic innervation from the locus coeruleus (LC). It has previously been demonstrated that electrical stimulation of the LC enhances the responsiveness of principal LGN neurons possibly by depressing inhibitory interneurons (Y. Nakai and S. Takahori, Brain Res., 71:47-60, 1974). In the present study, the action of NE on LGN neurons was examined directly using microiontophoresis in the chloral hydrate anesthetized or unanesthetized cerveau isolé rat. Low iontophoretic currents of NE (1-15 nA) produced a delayed increase in the firing rate of most spontaneously active LGN neurons (latency 20-50 sec). This effect was mimicked by various sympathomimetic amines. The relative potency series of agonists was typical of postsynaptic α-adrenergic receptors: epinephrine > NE > phenylephrine > α-methylnorepinephrine > dopamine > isoproterenol. Prolonged (2-5 min) iontophoretic application of either d- or ℓ-amphetamine, which release NE from noradrenergic nerve terminals, also activated LGN neurons. The α-antagonists phentolamine, piperoxane and WB-4101 at low iontophoretic currents (<10 nA) produced a selective, dose-dependent and reversible blockade of the response to NE but not to glutamate. The β-antagonist sotalol had weak and variable effects at equivalent iontophoretic currents.

In order to examine the effects of NE on the response to afferent inputs, LGN neurons were orthodromically activated by electrical stimulation of the optic chiasm or with light flashes delivered above the eyes. The postsynaptic (r) component of the field response to optic chiasm stimulation, which represents the mass activity of principal LGN neurons, increased in amplitude during NE iontophoresis. With subthreshold shocks, iontophoretic NE markedly enhanced the probability of spike generation during both the early (∿2 msec) and late (100-300 msec) components of the evoked response. NE also enhanced the responsiveness of LGN units to light flash. The facilitation of the evoked activity was greater than the increase in spontaneous rate.

It is concluded that NE activates LGN neurons via a postsynaptic or α_1-type adrenergic receptor. Furthermore, these results suggest that NE released from axon terminals of coeruleo-geniculate neurons may serve to specifically enhance the transmission of visual information through the LGN.

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1167 ACTIVITY OF STRIATAL NEURONS IN THE BEHAVING MONKEY: IMPLICATIONS FOR THE NEURAL BASIS OF SCHIZOPHRENIA AND PARKINSONISM

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In the tail of the rhesus monkey caudate nucleus, which receives a projection from temporal visual cortex, a population of single neurons has been found which respond with latencies of 90-140 ms to visual stimuli. The visual responses were selective, often on the basis of physical characteristics of the stimuli such as orientation, colour or size. However, unlike the responses of neurons in the inferotemporal visual cortex, the responses of many of these caudate neurons to a particular visual stimulus diminished rapidly over the first 1-5 trials. Eye movement recordings showed that this occurred even though the animal was still fixating the visual stimulus. The responses to a particular stimulus were in some cases partially restored after a delay, or by intervening visual stimuli, or by rotation of the stimulus, or by changing its color. Thus the habituation was often to specific features of the initially effective stimuli. The major responses of these neurons were to the first few presentations of completely novel stimuli which possessed the identified subset of physically effective features. Arousal could not account for the responses of these neurons, in that the neurons responded only to a subset of arousing and non-arousing visual stimuli, and were relatively uninfluenced when arousal (as shown by the GSR) was induced by touch or by auditory stimulation. In the head and body of the caudate nucleus, and in the nucleus accumbens, different populations of neurons were found which responded either unconditionally to sensory stimuli, or conditionally to environmental events which were cues to the animal to prepare for the initiation of behavior, or throughout performance of a task, or in relation to particular movements (Rolls et al., 1979). Together, these different populations of striatal neurons could be involved in orienting to significant environmental stimuli and then enabling performance. Two possibilities follow. First, reduced function of these neuronal systems produced by dopamine depletion leads to Parkinson's disease. Second, increased responsiveness or effectiveness of these neurons associated with over-effectiveness of the dopamine systems leads to such symptoms as distractability, failure to maintain attention, and orientation to the slightest or even non-existent environmental stimuli, found in schizophrenia, which can thus be alleviated by neuroleptic drugs which block dopamine receptors.

Rolls, E.T. et al (1979) In "The Neostriatum", eds. I. Divac and R.G.E. Oberg. Oxford: Pergamon.

1168 PIMOZIDE'S EFFECTS ON ICSS DEPEND ON THE INTERACTION OF REWARD AND EFFORT. William H. Rosenblatt*, Kim Hutchins*, and H. M. Sinnamon (SPON: D. Adams). Lab. of Neuropsychology, Wesleyan University, Middletown, CT 06457.

The maintenance of instrumental behavior is determined by the amount of work required as well as by the magnitude of reward. However the work factor has generally not been systematically varied in studies concerned with effects of neuroleptics on reward. This omission appears partly responsible for the question frequently raised about the drugs' behavioral specificity. This problem prompted the study of pimozide on performance of a progressive fixed ratio (FR) schedule in a situation where required work was varied by increasing the FR and reward value was varied by increasing the pulse frequency of 1-sec trains of stimulation to the ventral midbrain. Pimozide increased the latency to reinitiate responding after each reward but had minimal effects on the rate of barpressing after an FR sequence began. In general, the increase in latency occured only on FR sequences that were near the maximal supported by any one value of reward. Thus latencies increase at low FR's for low rewards and at high FR's for high rewards. Were simple motor debilitation produced by pimozide, the depression in performance would have appeared at all FR's beyond a certain value and be independent of reward value. At any given reward value, lower FR's are completed sooner than higher FR's and therefore are associated with higher stimulation densities and greater behavioral facilitatory afteraffects. The possibility that lower FR's were more resistant to pimozide because of density-related factors was eliminated by imposing a delay between the reward and the availability of the manipulandum. This delay was held constant for all FR's. Similar patterns of affects were found on progressive FR performance for varying amounts of water reward. The effects of pimozide suggest that dopaminergic systems are involved in the process by which memory of reward and anticipated effort interact to control the initiation of instrumental behavior.

1169 LOCUS COERULEUS LESIONS: EFFECTS ON CEREBRAL OXIDATIVE METABOLISM IN VIVO. I - CYTOCHROME OXIDASE IN THE STEADY STATE. Myron Rosenthal, Sami I. Harik, Joseph C. LaManna and Andrew I. Light,* Dept. Neurol., U. Miami Med. Sch., Miami, FL 33101.

The role of norepinephrine (NE) in oxidative energy metabolism of the cerebral cortex remains unknown. Since most NE supply of the neocortex emanates from the nucleus locus coeruleus (LC) discrete lesions produced by local stereotaxic microinjection of 6-OH-dopamine produce ipsilateral depletion, providing a means of assessing NE actions. Such lesions were made unilaterally in Wistar rats (250-300 gm). Two weeks later, these rats were anesthetized, tracheotomized, artificially ventilated and holes were drilled in the skull, bilaterally exposing the frontoparietal regions. Oxidative metabolic functioning was assessed by evaluation of changes in reduction/oxidation ratios of cytochrome a,a_3 measured through intact dura by dual wavelength reflection spectrophotometry. As in controls, hypoxia induced by changing the inspired gas mixture from normal (30% O_2/70% N_2) to 100% N_2 was accompanied by increased cytochrome reduction. Transition from 30% O_2 to 100% O_2 or 95% O_2/5% CO_2 produced increased oxidation, indicating that the cytochrome oxidase redox state in LC lesioned rats is also partially reduced as in cats, rabbits and human neocortex. When ratios of oxidation to reduction produced by hyperoxic and hypoxic transitions were calculated, no differences were apparent between hemispheres ipsilateral to LC lesion (NE depleted hemispheres) and contralateral hemispheres. This demonstrates that if NE does have an effect on cortical metabolism, this effect is not apparent under "resting" conditions of low energy utilization. Differences did become apparent, however, when these brains were stimulated by electrical pulses delivered directly to the cortical surface. Both sides responded to such stimulation with transient oxidation followed by re-reduction of cytochrome a,a_3 back to baseline. However, in NE depleted hemispheres, the rate of re-reduction of cytochrome a,a_3 was markedly slowed. These results indicate that NE plays a role in cortical energy metabolism under conditions of increased energy demand rather than under steady state conditions. (Supported by NS 14319, NS 14325 and the Rita Cohen Memorial Fund)

1170 CATECHOLAMINE INVOLVEMENT IN ABNORMAL CORTICAL EEG BURSTING IN DBA MICE. Lawrence J. Ryan* and Seth K. Sharpless. Dept. Psych., Univ. Colorado, Boulder, CO 80309.

Brief spindle episodes (BSE) have previously been observed in the cortical electroencephalogram of DBA/2 and rarely in C3H mice, but not in C57BL/6 or BALB/c mice. BSEs are characterized by 1-5 sec bursts, with rapid onset and completion, of 6-7Hz monomorphic waves which may attain amplitudes of 1200 μv or more. These episodes occur spontaneously and may be provoked by pentylenetetrazol.

Three lines of evidence suggest that the locus coeruleus norepinephrine (NE) system may be involved in modulating BSE activity.

1. Continuous recordings from 4 chronically implanted, freely moving DBA mice for a total of 168 hrs revealed that the average rate of BSE occurrence was 1.42 BSEs/hr. They primarily occur when the animal is awake and active, occasionally when drowsy and rarely during sleep. Catecholamine depletion by either reserpine (2.5 mg/kg, i.p.) or α-methyl-p-tyrosine (250 mg/kg, i.p.) produced a 6-50X increase in the rate of occurrence, beginning approximately 4 hrs after drug administration.

2. Eight chronically implanted DBA mice received 1.0 mg/kg mecamylamine, 2.0 mg/kg haloperidol and 10 mg/kg propranolol, presented in random order with 3 days between each drug presentation. During the 30 min recording after injection neither haloperidol nor mecamylamine affected BSE occurrence. After propranolol, BSEs occurred at a mean rate of 127/hr (13.2 SEM). In C57 mice, 10 mg/kg propranolol produced a few irregular 1 sec or less bursts of spike and polyspike activity which may or may not be homologous to BSEs in DBA mice.

3. Acute transection of the brain stem of DBA mice caudal to the locus coeruleus (LC) had no effect on the occurrence of BSEs, and they could still be elicited by 10 mg/kg propranolol. Transection immediately rostral to LC released BSEs. In 5 mice, 116.4 (21.6 SEM) BSEs were recorded in the period from 1-1.5 hrs after transection. Physostigmine (0.25 mg/kg), given 1.5 hrs after transection of 3 of these mice, had no effect on BSE occurrence in the next 30 min, although this dosage was sufficient to produce cortical activation.

Thus, a beta blocking agent, propranolol, greatly exacerbates the occurrence of an EEG abnormality characteristic of DBA mice, whereas a dopaminergic blocking agent, haloperidol, does not. Transection of the ascending LC adrenergic tract also exacerbates the abnormality. It is interesting that DBA mice show a deficiency in cortical NE during development. This suggests that a genetic defect in the LC noradrenergic system of DBA mice is responsible for the appearance of BSEs.

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1171 POSSIBLE NORADRENERGIC MEDIATION OF THE GLYCOGENOLYTIC RESPONSE TO APOMORPHINE AND INSULIN IN RAT BRAIN. C.F. Saller* (SPON: R.J. Ertel). Psychobiology Program, Univ. of Pittsburgh, Pittsburgh, PA 15260

The effects of apomorphine (APO), a dopamine (DA) receptor agonist, on tissue glycogen levels were examined in the striatum, a brain region with a rich dopaminergic innervation, and in the hippocampus, a region with very low DA levels and few, if any, DA receptors. APO (5mg/kg, s.c., injected 20 min. before sacrifice) decreased glycogen levels in the striatum and in the hippocampus (see table). Fluphenazine (FLU), a DA receptor blocker prevented these decreases, suggesting a role for DA in promoting glycogenolysis. Pretreatment of rats with propranolol (PROP), a β-noradrenergic receptor antagonist, also prevented APO-induced glycogenolysis and, by itself, increased tissue glycogen levels. This result suggested a similar role for norepinephrine (NE). To determine if APO was acting by directly stimulating NE receptors or by indirectly activating NE-containing neurons, rats were depleted of NE and DA by administering reserpine (R) and α-methyltyrosine (AMT). This treatment elevated tissue glycogen levels and blocked APO-stimulated glycogenolysis. In contrast to the ability of all three pretreatments to block APO-induced glycogenolysis, only FLU blocked APO-induced stereotypy. Thus, APO may affect behavior by directly activating DA receptors, while its glycogenolytic effects may require the activation of NE-containing neurons.

The glycogenolytic response to insulin was examined in rats pretreated with FLU (0.5 mg/kg, s.c.) or PROP (5 mg/kg, s.c.) 30 min. prior to receiving insulin (4-24 units, s.c.). Rats were sacrificed 30 min. after the insulin injection. Insulin depleted striatal glycogen by 44%. This was unaffected by FLU, but completely blocked by PROP. Thus, the glycogenolytic response to insulin and to APO may be mediated by noradrenergic neurons.

PRETREATMENT	STRIATUM		HIPPOCAMPUS	
	Saline	APO	Saline	APO
Saline vehicle	-	-24*	-	-22*
FLU (0.5mg/kg, s.c., 30 min.[1])	-2	13*	4	8*
PROP (1mg/kg, s.c., 30 min.)	9*	19*	14*	18*
R (10 mg/kg, i.p., 24h) and AMT (250mg/kg, i.p., 4h)	20*	3	14*	14*

Data is expressed as the percent difference in glycogen levels between saline treated controls (striatum 1.93 ± 0.05 μmoles/g and hippocampus 2.51 ± 0.09 μmoles/g) and drug treated animals. (1) Times before saline or APO. *Significantly different from controls ($P < 0.05$).

1172 ALTERATIONS IN THE PERMEABILITY OF THE BLOOD-BRAIN BARRIER INDUCED BY AMPHETAMINE IN NORMOTENSIVE VS SPONTANEOUSLY HYPERTENSIVE RATS PRETREATED WITH HALOPERIDOL. Raman Sankar*, Floyd Domer, David Wellmeyer* and Perry Scallon*. Dept. of Pharmacology, Tulane University and College of Pharmacy, Xavier University, New Orleans, La. 70112.

Quantitative evaluation of the change in the permeability of the blood-brain barrier (BBB) to radioiodinated serum albumin (RISA) induced by amphetamine sulfate has been carried out in normotensive (WKY) and spontaneously hypertensive (SHR) rats. Approximately 50 μCi of RISA was administered intravenously 15 minutes after 2 mg/kg haloperidol to rats anesthetized with sodium pentobarbital. The mean femoral arterial blood pressure response to intravenous administration of saline or doses of amphetamine sulfate (2.5 and 5 mg/kg) i.v. given with the RISA was recorded. Fifteen minutes thereafter, the thoracic cavity was opened to permit the obtaining of a cardiac blood sample and the subsequent perfusion through the heart with 30 ml of saline to clear the cerebral vessels of radioactivity. The brain was dissected out and the blood and brain samples were weighed and counted for their content of radioactivity. The ratio of radioactivity in the brain to that in blood was then calculated as a measure of the permeability of the BBB. Administration of haloperidol caused a decrease in extrusion of RISA in both WKY and SHR rats. When given prior to amphetamine that had previously been found to increase the permeability of the BBB, there was a marked, significant decrease in permeability. This reversal of change in permeability of the BBB could be due to unmasking of action by amphetamine at a group of receptors not blocked by haloperidol. (Supported in part by USPHS Grant No. 5D18MB 160003-06A2 and 5T32 HL07299-02).

1173 EFFERENT CONNECTIONS OF THE SUBCOERULEUS REGION IN THE RAT. C.B. Saper and A.D. Loewy. Dept. of Anat./Neurobiol., Washington Univ. Sch. Med., St. Louis, MO 63110 and Dept. of Neurology, Cornell Univ. Med. Ctr., New York, NY 10021

The subcoeruleus region (SC) in the rat is in ill-defined portion of the dorsolateral reticular formation of the pons and caudal midbrain, so named for its ventral relationship to the locus coeruleus. In the rat, the SC contains a subpopulation of catecholaminergic neurons. The connections of these neurons, which have been studied using fluorescence histochemistry, remain the only part of the SC to have been so studied (Olson & Fuxe, Brain Res., 43: 289, '72). We have traced the efferent connections of the SC in the rat using 20-30 nl of a 20-30 μCi/μl solution containing equal proportions of ^{3}H-proline, ^{3}H-leucine and ^{3}H-lysine into the SC, the *ascending* labeled projection, in contrast to earlier reports, was found to be rather sparse. Labeled fibers traveled rostrally primarily through the midbrain reticular formation and then into the medial forebrain bundle. Most fibers distributed to the midbrain reticular formation, the lateral hypothalamic and preoptic areas and the posterior hypothalamic area. A similar though more sparse projection was seen on the contralateral side of the brain. The major *descending* projection labeled after SC injections was a paramedian pathway. A large contingent of labeled fibers ran medially from the injection site into the dorsal paramedian reticular formation. These formed densely labeled fascicles which turned ventrally as they descended through the medulla, sweeping laterally over the dorsal surface of the inferior olivary complex, then into the spinal cord, where they formed a dense band of heavily labeled fascicles in the superficial part of the ventral and ventrolateral funiculi. The intensity of the labeling progressively diminished caudally as labeled fibers apparently turned into the gray matter where they ended in a light terminal field over the entire ventral horn and intermediate gray matter, with a few fibers innervating the dorsal horn. A few fibers were followed through the contralateral reticular formation where they collected at the ventral tip of the spinal trigeminal nucleus and were traced caudally into the medial part of the lateral funiculus of the spinal cord. These fibers contributed to the sparse labeling of the contralateral spinal gray matter in a distribution which mirrored the ipsilateral side. The SC has a massive input to the ventral horn of the spinal cord at all levels, which may be an anatomic substrate for the locomotor properties which certain physiological experiments have imputed to the region. Supported by USPHS grant NS12751 and American Heart Assocation Grant 77 797.

1174 DOPAMINE AGONIST EFFECTS ON CYCLIC NUCLEOTIDES IN RAT BRAIN. Michael J. Schmidt, Eli Lilly and Company, Indianapolis, IN 46206, and David J. Jones and William B. Stavinoha, Univ. Texas Health Sci. Ctr., San Antonio, TX 78284.

Conflicting data have been reported concerning the ability of dopaminergic stimulation to elevate cyclic AMP (cAMP) in the striatum. Failure to confirm early studies has been attributed to differing doses of agonists, variable treatment times or use of inadequate techniques for arresting brain metabolism at the time of sacrifice. The present studies were undertaken to repeat exactly some experiments in the literature with regard to dosage of dopamine agonists, animal strains and injection-sacrifice schedules. In addition, a newly developed microwave unit optimized for rats was used to arrest brain metabolism instantaneously and thereby prevent artifacts associated with sacrifice procedures. The temperature of the rat brain reaches 90°C within 0.5 sec with this unit (915 mHz, 17 kwatts).

Administration of L-DOPA (100 mg/kg), which increases dopamine in the brain, did not increase cAMP in the striatum or cerebellum. Cyclic GMP was increased 62% in the cerebellum, but no change was detected in the striatum. Amphetamine (2.5 mg/kg), which releases endogenous dopamine, did not consistently elevate cAMP in the striatum or cerebellum. Amphetamine elicited a rise in cGMP in the cerebellum but not in the striatum. Apomorphine (10 mg/kg), a direct dopamine receptor stimulant, did not change cAMP in the striatum or cerebellum, but enhanced cGMP accumulation in the cerebellum. Lergotrile (5 mg/kg), another direct-acting dopamine agonist, did not change cAMP levels in the striatum or cerebellum, but increased cGMP in the cerebellum. The lergotrile-induced elevation in cGMP was also detected in 7 other brain regions. The changes in cGMP produced by all dopamine agonists were prevented by pretreatment with haloperidol, indicating dopamine receptors were involved.

We conclude that measuring cAMP concentrations in the striatum is not a suitable means of assessing dopaminergic effects in vivo. Measuring cAMP synthesis in homogenates of striatum in vitro is also unsuitable, since apomorphine is only weakly active in this system and lergotrile and bromocryptine are inactive, despite the ability of these agents to compete for dopamine binding to receptors in vitro and to cause functional changes indicative of dopamine receptor stimulation in vivo.

1175 HUMAN BRAIN CORTEX MONOAMINE OXIDASE (MAO): ONE MOLECULAR ENTITY, TWO ACTIVE SITES. A. Schurr, B. T. Ho and B. M. Rigor* Department of Anesthesiology, University of Texas Medical School, Houston, TX 77030 and Department of Neurochemistry and Neuropharmacology, Texas Research Institute Mental Sciences, Houston, TX 77030.

Although it is generally accepted that monoamine oxidase (MAO) exist in two different forms, this conception was recently questioned by various investigators. Our findings with human brain cortex MAO indicating that this enzyme probably exists as a single molecular entity containing two different activities. This interpretation is based on the following findings:

A) The enzyme has similar sensitivities to the irreversible inhibitors clorgyline and deprenyl with type B substrates like 2-phenethylamine (PEA) and benzylamine (BA).
B) Deprenyl competes with these substrates for MAO while clorgyline behaves as a noncompetitive inhibitor.
C) Two Km values found for both PEA and BA which appear as two maxima on the Michaelis-Menten curves. The second maximum (high Km Value) was obolished upon applying low concentrations of clorgyline but not by deprenyl. 5-Hydroxytryptamine (5-HT) was able to mimic the effect of clorgyline.
D) 5-HT found to be a noncompetitive inhibitor of BA deamination when low concentrations of BA were applied and a competitive one with high concentrations of BA.
E) The MAO type A activity measured with 5-HT as a substrate, shows positive cooperativity, as indicated by the sigmoidity of the Michaelis-Menten Curve. The Hill coefficient caculated to be 2.25, pointing at the possibility that four (4) interacting binding sites for 5-HT are exist in human brain cortex MAO.
F) As for PEA and BA, the activity of 5-HT deamination has two Km Values. This activity is very sensitive to clorgyline but only partially sensitive to deprenyl. However, both inhibitors compete with the substrate for the enzyme.
G) The enzyme is affected differently by certain phospholipids and fatty acids depending on the substrate used, indicating the possible involvement of such molecules in the regulation of its activity.

1176 BEHAVIORALLY-DERIVED ESTIMATES OF CONDUCTION VELOCITY AND REFRACTORY PERIOD IN A REWARD-RELATED PATHWAY DIFFER FROM THE CHARACTERISTICS OF MONOAMINERGIC NEURONS. Peter Shizgal and Catherine Bielajew*, Dept. Psychol., Concordia U., Montreal, Que., H3G 1M8 and John Yeomans, Dept. Psychol., U. of Toronto, Toronto, Ont., H5S 1A1.

When an axon bundle is stimulated concurrently at two sites, the orthodromic action potential (a.p.) from the upstream electrode collides with the antidromic a.p. from the downstream electrode and only a single a.p. reaches the synaptic terminals. If the time between stimulations at the two sites is sufficiently long, then collision will be avoided and both orthodromic a.p.'s will reach the terminals. The critical interval at which collision is just avoided will depend on conduction velocity (c.v.) and refractory period (r.p.).

Such collision effects can be inferred from behavior if the vigor of stimulation-elicited performance is related to the frequency of firing in the stimulated pathway. (Collision reduces the frequency at which orthodromic a.p.'s arrive at the terminals.) Our study of the pathways mediating the rewarding and aversive effects of lateral hypothalamic (LH) and ventral tegmental (VTA) stimulation in the rat was based on this rationale. A frequency threshold scaling method (Yeomans, Physiol. Behav., 15: 593-602, 1975) was used.

The principal findings and implications were as follows: 1) The LH and VTA are directly linked by reward-related axons. 2) The estimated c.v. in these fibers ranged from 2.6 - 7.7 m/sec, values substantially higher than the reported c.v.'s of monoaminergic fibers. 3) Behaviorally-derived estimates of the r.p.'s of reward-related fibers were shorter than the reported r.p.'s of monoaminergic fibers. Findings 2) and 3) suggest that the directly-stimulated, reward-related fibers were non-monoaminergic. The c.v. data predict that these fibers were myelinated and from .47 - 1.4 μM in diameter, a range that includes the majority of myelinated LH axons. 4) In two subjects, we observed collision-like effects in a self-stimulation task but not in a stimulation-escape task. The same electrodes and current intensities were used for both behaviors. This result supports the idea that the rewarding and aversive effects of medial forebrain bundle stimulation are mediated by different fibers with different trajectories.

1177 MULTIPLE PITUITARY DOPAMINE RECEPTORS: EFFECTS OF GUANINE NUCLEOTIDES. David R. Sibley* and Ian Creese. Dept. Neurosciences, Sch. Med., UCSD, La Jolla, CA 92093.

A number of recent studies have suggested the existence of multiple dopamine receptors. One categorization is that "D1" receptors are linked to adenylate cyclase while "D2" receptors are not. The anterior pituitary mammotrophs have been suggested to possess prototype D2 receptors since the dopamine inhibition of prolactin release does not appear dependent on cAMP.

Guanine nucleotides have been shown to play a regulatory role in a variety of receptor-adenylate cyclase systems. They appear to facilitate coupling between receptors and adenylate cyclase and to selectively decrease receptor affinity for agonists but not antagonists. We now show that guanine nucleotides decrease dopamine agonist affinity for ^{3}H-spiroperidol binding sites in bovine anterior pituitary membranes suggesting the presence of D1 receptors.

At 0.1 mM the nucleotides GTP, GDP, GMP, ATP, ADP and AMP had no effect on total or nonspecific ^{3}H-spiroperidol binding. However, the ability of agonists to displace ^{3}H-spiroperidol binding was markedly influenced by GTP and GDP (0.1 mM) which increased the IC_{50}'s of dopamine, apomorphine, and ADTN by approximately 6-11 fold. GTP and GDP were maximally effective at 0.1 mM with EC_{50}'s of 10 μM. Other nucleotides were ineffective in reducing the potency of dopamine in displacing ^{3}H-spiroperidol binding. GTP did not influence the ability of antagonists haloperidol or chlorpromazine to displace ^{3}H-spiroperidol binding. Pituitary dopamine receptors have also been labeled by a new radioligand, the potent agonist, ^{3}H-N-propylnorapomorphine. These binding sites exhibit all the characteristics expected of dopamine receptors. Specific ^{3}H-N-propylnorapomorphine binding is more than 50% reduced by 0.1 mM GTP.

The finding of GTP regulation of agonist affinity for ^{3}H-spiroperidol binding sites in pituitary suggests that these binding sites are associated with adenylate cyclase. These dopamine receptors would appear not to be associated with the control of prolactin release as their agonist affinities are much lower than the concentrations required to inhibit prolactin release in vitro. Furthermore GTP does not regulate the affinity of bromocriptine for ^{3}H-spiroperidol binding sites in the pituitary although it is more potent than dopamine in inhibiting prolactin release. Bromocriptine is, however, an antagonist of the striatal dopamine-sensitive adenylate cyclase where its affinity for ^{3}H-spiroperidol binding sites is also insensitive to GTP.

These data suggest that bovine anterior pituitary contains two distinct dopamine receptors, one not associated with cyclase which controls prolactin release, the other adenylate cyclase linked and regulated by GTP with unknown physiological function.

1178 STIMULUS-DEPENDENT ANALGESIC ACTION OF MONOAMINE REUPTAKE INHIBITORS: RELATIONSHIP TO 5-HT AND NE FUNCTION IN CNS. K.J. Simansky* Dept. of Psychiatry, Cornell Univ. Med. College, White Plains, N.Y. and J.A. Harvey, Dept. of Psychol., Univ. Iowa, Iowa City, Iowa 52242 (SPON.: William T. Lhamon).

Neurotoxin-induced depletion of central nervous system serotonin (5-HT), but not norepinephrine (NE), lowers jump thresholds to shock in the flinch-jump test whereas NE, but not 5-HT, depletion decreases paw-lick latencies in the hot-plate test (Neuroscience Abstract 890, 1978). This stimulus-dependent hyperalgesia suggested that central 5-HT and NE systems modulate different aspects of nociception in the rat. The present study therefore examined the ability of four monoamine reuptake inhibitors, with different relative selectivity for the 5-HT and NE reuptake mechanisms, to produce analgesia to shock or heat in neurologically-intact rats. Animals were injected with 10 mg/kg (i.p.) of the hydrochloride salts of fluoxetine (FLU), a highly selective 5-HT reuptake inhibitor previously shown to increase jump thresholds (Messing et al., Psychopharm. Comm., 1975, 1, 511-521); nisoxetine (NIS) or desipramine (DMI), selective NE uptake inhibitors; or chlorimipramine (CMI), a mixed-acting uptake blocker. Vehicle -injected controls (VC) received saline i.p.

All four reuptake inhibitors produced analgesia, but the efficacy of each drug in altering pain sensitivity varied with the assessment technique employed. FLU (+47%), CMI (+38%), and DMI (+24%) significantly increased jump thresholds compared to VC while NIS (+14%) did not. In contrast, DMI (+130%), NIS (+53%) and CMI (+45%) increased mean overall paw-lick latencies during testing at 30, 60, 120, and 180 min after injection while FLU (+13%) and VC (+2%) failed to alter reaction times compared to preinjection baseline. It was also found that FLU failed to produce analgesia on the hot-plate during a single determination (without preinjection testing) made 4 hr after injection--when 5-HT reuptake inhibition is maximal. DMI increased latencies by 100% when tested only at 90 min after injection.

In a test of the neurochemical mechanism of action of DMI in increasing paw-lick latencies, rats were depleted of either NE and dopamine (DA) by the intraventricular injection of 6-OHDA, or of DA alone, by the injection of 6-OHDA after DMI pretreatment. NE + DA depletion, but not DA depletion alone, blocked the analgesic action of DMI in the hot-plate test 30 days after surgery.

The above data are consistent with the hypothesis that inhibition of NE, but not 5-HT, reuptake produces analgesia to noxious heat whereas 5-HT reuptake blockade is correlated with analgesia to shock. These studies therefore provide further evidence that both 5-HT and NE normally suppress the response to painful stimuli but that their functions in nociception are dissociable.
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1179 NORADRENERGIC NEURONS IN THE LOCUS COERULEUS AND CARDIOVASCULAR FUNCTION IN NORMAL AND SPONTANEOUSLY HYPERTENSIVE RATS. Torgny H. Svensson, Göran Engberg* and Peter Thorén*. Depts of Pharmacology and Physiology, University of Göteborg, S-400 33 Göteborg, Sweden.

The pontine noradrenergic nucleus locus coeruleus (LC) has been claimed to participate in maintenance of apprehensiveness and arousal as well as in regulation of autonomic, e.g. cardiovascular function, including various reflexes. Thus, electrical stimulation of the LC produces a pressor response and, recently, afferent stimulation of the vagus nerve was found to cause inhibition of LC neuronal activity. We have used single cell recording techniques to study the response of LC neurons in the chloral hydrate anesthetized rat to a physiologically relevant stimulus for cardiovascular reflexes, namely volume load. Intravenous injection of e.g. 1-4 ml blood caused a volume-dependent reduction in firing rate of LC neurons. Subsequent bleeding of the same amount of blood induced a prompt return to base line activity. Also bilateral vagotomy in the neck caused this reversal of the NA-cell inhibition. Thus, LC neurons respond reciprocally to moderate changes in blood volume, an effect probably mediated via vagal afferents. In spontaneously hypertensive rats (SHR) the average firing rate of randomly encountered NA neurons in the LC was progressively reduced with increased blood pressure of the animals when compared with normotensive, agematched Wistar Kyoto rats (WKR). Bilateral vagotomy did not increase the reduced LC neuronal firing rate in SHR. The α_2-receptor antagonist yohimbine produced greater activation of LC neurons in SHR than in WKR suggesting possibly altered autoreceptor function at LC neurons. The LC NA neurons appeared biochemically equally active in awake SHR and WKR in contrast to e.g. nigroneostriatal dopamine neurons, which seemed hyperactive in SHR. The LC NA cell activity may thus like heart rate and cardiac output in SHR be depressed below corresponding parameters of WKR controls after elimination of environmental stimuli by anesthesia.

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1180 SEROTONIN AND GLUCOCORTICOID DEPLETION INDUCES RECEPTIVE FIELDS FOR GROOMING REFLEXES IN THE CAT. Richard M. Swenson & Walter Randall*. Dept. Anat., Sch. Med., & Dept. Psych., Univ. of Iowa, Iowa City, Iowa 52242.

Receptive fields for grooming reflexes (lick, bite, or scratch reflexes) are rarely seen in intact, adult cats or other species even though these reflexes can be elicited from newborns of some species (e.g., rats, dogs). Previous work has shown that lesions of the pons-midbrain tegmentum or of the frontal neocortex produce receptive fields for grooming reflexes in adult cats, and these lesions also cause a significant reduction in the activity of tryptophan hydroxylase, the "rate limiting" enzyme in the production of serotonin (5-HT), in the superior colliculus Randall, Pharm. Biochem. Beh., 1974, 2, 355-360). Also, inhibition of tryptophan hydroxylase activity by p-chlorophenylalanine (p-CPA) administration in conjunction with adrenalectomy (ADX) produces these grooming reflexes in adult cats although p-CPA or ADX alone is ineffective (Randall, Elbin, & Swenson, JCPP, 1974, 86, 747-750). P-CPA, however, is nonspecific in that it also affects catecholamine metabolism, and when administered systemically, depletes serotonin peripherally. The present study shows that when central 5-HT levels are reduced by lesions of the superior central and dorsal raphe nuclei or by injection of 5,7-dihydroxytryptamine (5,7-DHT) into the superior colliculus, in conjunction with ADX, receptive fields for grooming reflexes appear that are the same as those seen in cats after p-CPA and ADX or after CNS lesions. These receptive fields occur only after cortisol replacement is discontinued after ADX. Single, systemic injections of L-5-hydroxytryptophan (25mg/kg) or cortisol (100mg) significantly reduced receptive field size, whereas systemic administration of L-dihydroxyphenylalanine (50 mg/kg) was without effect. In cats with the single treatment of either raphe lesion or 5,7-DHT alone, small but significant receptive fields for grooming reflexes appeared only in the cats given 5,7-DHT. Raphe lesions led to significant reductions of 5-HT in hypothalamus(-71%), caudate(-68%), thalamus(-56%), and superior colliculus(-38%), whereas 5,7-DHT injection led to a significant reduction of 5-HT only in the superior colliculus (-70%). These data, in conjunction with our lesion and pharmacological work, indicate that serotonin in the superior colliculus and systemic glucocorticoids are involved in inducing grooming reflexes in cats. (Supported by NIH grant #5 RO1 MH15402-07).

1181 EFFECT OF ANTIDEPRESSANTS ON NORADRENERGIC AND SEROTONERGIC RECEPTORS. S.W. Tang* and P. Seeman. (SPON: F. Brawley), Pharmacology Department, University of Toronto, Toronto, CANADA.

In order to examine whether antidepressant drugs might act by inhibiting neurotransmitter receptors, the effects of these drugs were tested on noradrenergic and serotonergic receptors in vitro. The IC_{50} values or the concentrations of the antidepressants which 50% inhibited the binding of various ^{3}H-ligands to homogenates of calf brain regions are listed in the Table.

IC_{50} VALUES (nmoles/Liter) ± 10%						
^{3}H-ligand:	WB-4101	Clonidine	Dihydroalprenolol		Serotonin	LSD
nM ligand:	0.22	0.2	0.5	0.5	0.5	2
Region:	Frontal cortex	Frontal cortex	Frontal cortex	Cerebellum	Frontal cortex	Frontal cortex
Receptor:	alpha1	alpha2	beta1	beta2	serotonin	
Mianserin	56	12	11200	37000	90	100
Doxepin	24	2000	20500	7600	240	190
Amitriptyline	46	850	7000	4000	240	140
Clomipramine	130	7430	9300	21000	590	410
Imipramine	160	4930	13300	13500	1080	555
Trimipramine	67	1700	5300	13000	320	300
Nortriptyline	130	2980	5400	3600	380	350
Protriptyline	420	10360	8700	10000	1150	900
Desipramine	440	9400	14200	3000	3070	2000
Maprotiline	250	16770	7900	5400	220	1000
Nomifensin	980	2480	23300	66000	1370	2700
Iprindole	6150	16000	17000	9600	6000	5800

Since the therapeutic concentrations of these drugs in the patient's plasma water is known to be of the order of 50-150 nM we conclude that:

1. Mianserin and the tertiary amine tricyclic antidepressants may block alpha-adrenergic transmission by blocking the postsynaptic alpha1-receptors (data using ^{3}H-WB-4101).

2. Beta-adrenergic transmission is not inhibited by the drugs and thus may be enhanced by all antidepressants by either inhibition of noradrenaline re-uptake or by an increase in noradrenaline release (for the special case of mianserin which inhibited the pre-synaptic alpha2 receptor presumably labelled by ^{3}H-clonidine).

3. The weak but significant inhibition of serotonin receptors by some of the antidepressants may also contribute to the clinical antidepressant action.

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1182 NEUROCHEMICAL RESPONSES TO 5-HYDROXYTRYPTOPHAN IN RATS TREATED DURING EARLY DEVELOPMENT WITH LITHIUM CHLORIDE. Hillel Taub* and David A.V. Peters* (SPON: L. Maler). Dept. of Pharmacol., Sch. Med., Univ. Ottawa, Ottawa, Ont., Canada.

We have recently reported that several psychoactive drugs as haloperidol and chlorpromazine affect the development of central 5-hydroxytryptamine-containing neurons in the rat when administered during early postnatal periods (Gen. Pharmac. 9: 97, 1978; Fed. Proc. 37: 858, 1978). We now present data on the effects of neonatal treatment of rats with lithium chloride. Newborn Sprague-Dawley rats were given once daily subcutaneous injections of lithium chloride (LiCl, 1 μEq/g) on day 1 through 6 after birth. Control animals received injections of saline (1 μl/g) on the same schedule. No other treatment was given until either 25 or 60 days of age when the rats were injected with L-5-hydroxytryptophan (5-HTP, 30 mg/kg, i.p.) or its vehicle and killed 60 min later. The rat brains were dissected into 10-12 regions and assayed for 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA).

Neonatal lithium treatment alone produced small (15-30%) but statistically significant alterations in brain 5-hydroxyindole levels. At 25 days of age the 5-HT level was significantly elevated in the hippocampus, midbrain and thalamus and the 5-HIAA level significantly reduced in cerebral cortex, olfactory tubercle and thalamus. The increased 5-HT levels were no longer present at 60 days of age whereas the 5-HIAA levels were found to be significantly reduced in the cerebellum and corpus striatum in addition to the cerebral cortex, olfactory tubercle and thalamus.

As expected, 5-HTP injections at 25 and 60 days of age produced marked elevations in 5-HT (200-400%) and 5-HIAA levels (200-700%) in all brain regions studied. However, the magnitudes of the 5-hydroxyindole increases were significantly affected by the neonatal drug treatment. At 25 days of age the 5-HTP-induced elevations in both 5-HT and 5-HIAA were significantly greater (200-500%) in rats treated with LiCl during the neonatal period than in saline-treated controls in all brain regions except the hypothalamus. In contrast, the enhanced response to 5-HTP-injections was no longer apparent at 60 days of age and in several brain regions (cerebellum, motor and temporal cortices, hippocampus, olfactory tubercle, pons-medulla) and spinal cord the increases were significantly less in the LiCl group. These data suggest that exposure to lithium salts during the neonatal period may alter the development of 5-HT-containing neurons in the central nervous system. (Supported in part by OMHF).

1183 CHRONIC ADMINISTRATION OF AMPHETAMINE TO CATS: BEHAVIORAL AND NEUROCHEMICAL EVIDENCE OF DECREASED BRAIN SEROTONIN FUNCTION. Michael E. Trulson and Barry L. Jacobs. Program in Neurosci., Dept. Psychol., Princeton Univ., Princeton, NJ 08544.

Amphetamine is thought to exert its psychobiological effect primarily through an action on brain catecholamines. In fact, the behavioral effects of chronic amphetamine administered in high doses to animals and humans (amphetamine psychosis) provides one of the important underpinnings for the catecholamine theory of schizophrenia. A recent report by Ellinwood (In: Animal Models in Psychiatry and Neurology, I. Hanin & E.Usdin (eds.) 1977, pp. 61-74), however, led us to examine the effects of chronic amphetamine administration on brain serotonin metabolism in cats. He reported that such treatments led to the emergence of several behaviors, such as limb flicking and abortive grooming, that we had previously shown to be elicited exclusively by hallucinogenic drugs and to be dependent on decreased brain serotonin function (Brain Res. 132, 301, 1977). In the present series of studies, groups of cats were administered amphetamine sulfate in a dose of 7.5 mg/kg i.p. twice daily for up to 10 consecutive days. Approximately 3-4 days following the onset of the injections, the cats began to display limb flicking and abortive grooming. The occurence of these behaviors reached their peak frequency (e.g., 15 limb flicks/hr) on days 7-10, and persisted at a level significantly above baseline for approximately 6 days following drug withdrawal. Paralleling these behavioral effects, brain serotonin and 5HIAA levels (measured in six different areas) were decreased by approximately 30-40% after 3-4 days of amphetamine treatment, reached a maximum decrease of 50-60% at 10 days, and then returned to within 30-40% of normal levels 5 days following drug withdrawal. By contrast, brain catecholamines and metabolites (NE, DA, HVA and DOPAC) showed a much more rapid (1-2 days) and dramatic (60-90%) decrease, which persisted for a much longer period of time (greater than 14 days following drug withdrawal). These data indicate that high dose levels of amphetamine to cats can produce significant reductions in brain serotonin and 5HIAA, and furthermore, that these changes are of behavioral significance. Since the onset of "amphetamine psychosis" in humans shows a closer temporal correlation with the changes in brain serotonin than with the changes in brain catecholamines, we suggest that a decrease in brain serotonin function may be of significance in the production of this form of psychosis.

1184 SELECTIVE MODULATION OF RAT CORTEX β_1-RECEPTORS AND RAT CEREBELLUM β_2-RECEPTORS AFTER CENTRAL NORADRENERGIC DENERVATION. David C. U'Prichard, Terry D. Reisine, Stephen T. Mason, Hans C. Fibiger and Henry I. Yamamura. Dept. Pharmacol., Northwestern Univ. Sch. Med., Chicago, IL 60611.

The dorsal bundle (DB) containing ascending norepinephrine (NE) fibers was lesioned in rats by bilateral stereotaxic injection of 6-hydroxydopamine (6-OHDA). DB lesion decreased NE levels by 97% in the frontal cortex after one month. β-Adrenergic receptor number, measured by ^{3}H-dihydroalprenolol (DHA) binding, increased 62% from 84.5 to 136.9 fmol/mg protein. The contribution of β_1-receptors and β_2-receptors to the total β-receptor population in the frontal cortex was assessed by examining inhibition of DHA binding by the selective β_1-antagonist practolol and the selective β_2-agonistsalbutamol. In control cortex, practolol and salbutamol inhibition curves were shallow (n_H = 0.8). Eadie-Hofstee plots were biphasic for both drugs, and true kinetic constants for each component of practolol and salbutamol binding were determined by iterative computer analysis using a NONLIN program (Upjohn Co.). Salbutamol bound with high affinity (K_D = 0.2 μM) to 20% of the DHA frontal cortex sites, which represented β_2-binding, and bound with low affinity (K_D = 4.0 μM) to the other 80% of sites, which represented β_1-receptor binding. Conversely, practolol bound with high affinity (K_D = 0.4 μM) to the majority (β_1) population, and with low affinity (K_D = 10 μM) to the minority (β_2) population of DHA cortex sites. Analysis of inhibition of DHA binding by either practolol or salbutamol showed that in DB lesioned frontal cortex the number of β_1-sites approximately doubled, whereas the number of β_2-sites was unaffected by the lesion. Rats were also given 6-OHDA i.c.v., which depleted NE levels by 96% in the cerebellum, and increased overall cerebellar DHA β-receptor binding by 39%. Eadie-Hofstee plots of practolol and salbutamol inhibition of cerebellar DHA binding were biphasic. However, contrary to the frontal cortex, the β_1-component of DHA binding accounted for only 20% of total DHA binding, and the β_2-component for 80%. Analysis of both practolol and salbutamol inhibition curves showed that the increase in cerebellar DHA binding was due to selective augmentation of the β_2-population, while β_1-receptor number was unchanged. Destruction of brain NE terminals thus causes a selective supersensitivity of β_1-receptors in the frontal cortex, and of β_2-receptors in the cerebellum. This suggests that functional neuronal postsynaptic β-receptors are of the β_1-type in the cortex and of the β_2-type in the cerebellum. The location and significance of cortical β_2-receptors and cerebellar β_1-receptors is as yet unclear.

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1185 EFFECTS OF IONOPHORES ON TYROSINE 3-MONOOXYGENASE ACTIVITY IN PHEOCHROMOCYTOMA CELLS. Karen K. Vaccaro*, Bruce T. Liang* and Robert L. Perlman* (SPON: Thomas O. Fox). Dept. of Physiology, Harvard Medical School, Boston, MA 02115.

Catecholamine secretion from the adrenal medulla and from sympathetic neurons is accompanied by an acute increase in the rate of catecholamine biosynthesis in these tissues. This stimulation of catecholamine synthesis results from an increase in the activity of tyrosine 3-monooxygenase (TH). We have been studying the regulation of catecholamine synthesis and secretion in cell suspensions prepared from a transplantable rat pheochromocytoma. The carboxylic ionophores monensin, lasalocid and ionomycin (1-10 μM) all stimulate secretion of norepinephrine from these cells (Perlman, Fed. Proc. 38: 525, 1979). We have now studied the effects of these ionophores on TH activity in intact pheochromocytoma cells. TH activity was assayed by measuring the formation of ^3H_2O by cells which were incubated for 90 minutes at 37°C in the presence of L-(3,5-^{3}H)tyrosine.

TH activity in cells incubated in a control medium (123 mM Na^+) was 52.6±2.9 pmol/min/mg protein. Monensin, an ionophore for monovalent cations, caused a 40-75% reduction in TH activity in the cells. Lasalocid, an ionophore for both monovalent and divalent cations, stimulated TH activity by up to 100%. Incubation of the cells in a Na^+-free medium (choline chloride substituted for NaCl) increased TH activity by up to 85%. Monensin did not inhibit the TH activity of cells incubated in Na^+-free medium. Lasalocid stimulation of TH activity was the same in Na^+-free as in control medium. Ionomycin, a divalent cation ionophore, had no effect on TH activity in either medium. Thus, these carboxylic ionophores are useful for studying the coupling of catecholamine synthesis and secretion.

1186 Contrasting roles of dopamine and β-phenylethylamine in Type I behavior-related neocortical low voltage fast activity. C.H. Vanderwolf, T.E. Robinson and B.A. Pappas. Dept. Psychol. Univ. Western Ontario, London, Ont. Can., N6A 5C2; Neurosci. Lab., Univ. Mich., Ann Arbor 48109; Dept. Psychol., Carleton Univ., Ottawa, Can. K1S 5B6.

Following large doses of atropine SO_4 rats display large amplitude slow waves in the neocortex during immobility, tremor, tooth-chattering, and face-washing, (Type II behavior) but display atropine resistant low voltage fast activity (ARLVFA) during walking, struggling, postural changes, and head movement (Type I behavior). ARLVFA is abolished by prior treatment with reserpine but not by chlorpromazine, trifluoperazine, haloperidol, pimozide, α-methyl-p-tyrosine, p-chlorophenylalanine, methysergide, LSD or promethazine. However, since many of these drugs reduce the emission of Type I behavior, they produce a correlated reduction in the occurrence of ARLVFA. The effect of reserpine on ARLVFA is blocked by prior treatment with nialamide, suggesting the involvement of a monoamine. Replacement experiments showed that following reserpine, ARLVFA was not restored by l-dopa, apomorphine, clonidine or 5-hydroxytryptophan. However, β-phenylethylamine (PEA) appeared to restore normal ARLVFA, together with some active behavior. Restoration of ARLVFA was not affected by the addition of α-methyl-p-tyrosine, trifluoperazine or chlorpromazine, although these drugs eliminated most PEA-produced behavior. PEA may play a direct role in ARLVFA independent of catecholamines.

Although l-dopa and apomorphine were ineffective in restoring ARLVFA in reserpinized rats, they did produce: a) a sharp increase in Type I and other behavior, and b) an increase in atropine sensitive LVFA. Dopamine may play an indirect role in ARLVFA by stimulating the emission of Type I behavior and may also play some role in the atropine sensitive LVFA which is normally present during waking immobility.

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1187 DORSAL RAPHE NUCLEUS PROJECTING TO THE SUPERIOR COLLICULUS AND THE LATERAL GENICULATE NUCLEUS IN THE RAT. Marcelo J. Villar*, Marcela Huerta* and Daniel A. Pasquier. (SPON: C. Avendaño) Instituto de Neurobiología, Serrano 665, 1414 Buenos Aires, Argentina.

The presence of aminergic fibers in both the superior colliculus and the lateral geniculate nucleus have been shown in the rat by using histofluorescence methods (Fuxe, Acta physiol. scand. 64 (suppl. 247): 37-84, 1965). Since then much effort has been done to demonstrate them with other techniques (Conrad et al., J.Comp. Neur. 156: 179-206, 1974; Moore et al. J.Comp. Neur. 180: 417-438, 1978), being the results controversial.

In this study the axonal transport of the horseradish peroxidase (HRP) was used to trace the afferents to the superior colliculus and the lateral geniculate nucleus. Injections up to 0.05 microliters of 30-50 % HRP saline solution were made through micropipettes. In addition iontophoretic deposit of HRP was also performed to avoid diffusion of the marker to neighbor regions. After 18-24 h of survival the animals were killed by perfusion through the heart with Karnowsky fixative; then the brains were removed and processed according to a routine method (Pasquier & Reinoso-Suárez, Brain Res. 120: 540-548, 1977).

Large HRP injections in the lateral geniculate nucleus labeled neurons in the rostral dorsal raphe nucleus. Most of these neurons were placed close to the midline, others, however, were in the lateral wings of the dorsal raphe nucleus. Very small iontophoretic deposit of HRP in the lateral geniculate nucleus also labeled dorsal raphe neurons primarily in its lateral wings. A few of these latter neurons appeared even after iontophoretic HRP deposit in the dorsal part of the lateral geniculate nucleus.

Large HRP injections in the superior colliculus labeled numerous neurons in the dorsal raphe nucleus, which were placed almost exclusively in its lateral wings. HRP deposit by iontophoresis labeled consistently some neurons in the lateral wings of the dorsal raphe nucleus.

These results suggest, 1) that an aminergic projection from the dorsal raphe nucleus reaches the superior colliculus and the lateral geniculate nucleus in the rat, and 2) that this projection is originated mainly from a lateral caudal extension of the dorsal raphe, which has remained largely ignored.

1188 TYROSINE HYDROXYLASE ACTIVATION AND INACTIVATION UNDER PROTEIN PHOSPHORYLATION CONDITIONS. Kent E. Vrana*, Carin L. Allhiser* and Robert Roskoski, Jr. Dept. Biochem., LSU Medical Center, New Orleans, Louisiana 70112.

Tyrosine hydroxylase, the rate-limiting enzyme in catecholamine biosynthesis, catalyzes the conversion of tyrosine to DOPA. Apparent enzyme activity is changed by altering its affinity for the reducing agent 6-MPH_4 (6-methyl-5,6,7,8-tetrahydrobiopterin) or the end-product feedback inhibitor dopamine. In agreement with other laboratories, when cAMP and Mg·ATP are included during activity measurements using rat corpus striatum homogenate, there is a decrease in 6-MPH_4 Km, an increase in the Ki for dopamine and no change in the apparent Vmax of the enzyme. At low 6-MPH_4 concentrations, therefore, the apparent enzyme activity is increased. Omission of ATP or Mg^{2+} abolishes the response; the response is markedly decreased by omitting cAMP. Enzyme activity was measured during a 15 minute incubation following pre-incubation of the homogenate under phosphorylating or control (no cAMP or Mg·ATP) conditions. After a 3 minute pre-incubation under phosphorylating conditions, there is a 2-fold increase in enzyme activity (11.0 vs 5.7 nmoles/mg·hr). A 30 minute pre-incubation, on the other hand, is associated with inactivation (3.1 experimental vs 5.1 nmoles/mg·hr control). Both phosphorylation activation and inactivation are enhanced by the addition of electrophoretically homogeneous bovine brain protein kinase catalytic subunit. The 6-MPH_4 Km's for control, activated and inactivated enzyme are 0.86, 0.32 and 0.38 mM, respectively. Inactivation, therefore, is not due to decreased affinity of the enzyme for cofactor. We also find that the Ki for dopamine is increased in both the phosphorylated activated and inactivated enzyme: control, 6.1 μM; activated, 37 μM; inactivated, 59 μM. Phosphorylation inactivation, therefore, is not due to an increased sensitivity of the enzyme to an end-product feedback inhibitor. Phosphorylation inactivation is associated with a decrease in the apparent Vmax. Additional experiments are required to determine whether this mode of inactivation has a counterpart in the physiological situation.

Concurrent with this work, we developed a new DOPA decarboxylase coupled assay, in which the labeled dopamine is resolved from the tyrosine substrate using low voltage paper electrophoresis.

This work was supported by USPHS Grant NS-11310.

1189 CHANGES IN THE SENSITIVITY OF β-ADRENERGIC RESPONSES IN THE RAT CEREBRAL CORTEX DURING THE ESTROUS CYCLE AND AFTER OVARIECTOMY. H. Ryan Wagner* and James N. Davis. (Spon: A. D. Roses), VA Medical Center and Duke University Medical Center, Durham, N. C.

We have previously reported a decrease in β-adrenergic receptor-mediated cyclic 3',5'-adenosine monophosphate (cAMP) response in the cerebral cortices of ovariectomized rats chronically exposed to 17α-ethynyl estradiol (Neurosci. Abstr. 4: 524 1978). Continuing with these studies, we now report differences between β-adrenergic receptor-mediated responses in the cerebral cortices of adult female rats during the four day ovarian cycle as well as between cycling females and adult male and ovariectomized female rats of the same age. Accumulation of cAMP (pmols/mg protein) elicited by the β-adrenergic agonist (-) isoproterenol (ISO) in slice preparations of cerebral cortex was lowest at proestrous (Vmax = 34), increased at estrous (Vmax = 38) and diestrous day-1 (Vmax = 38) and at a peak on diestrous day-2 (Vmax = 46). In contrast with values in cycling females, β-adrenergic cAMP accumulations were elevated in both male rats (Vmax = 70) and in ovariectomized female rats (Vmax = 53). The differences in β-adrenergic sensitivity to ISO appeared to be mediated in part by differences in the density of β-adrenergic receptor membrane binding sites. As measured with the β-adrenergic radioligand [^{3}H]-dihydroalprenolol (^{3}H-DHA), β-adrenergic receptor membrane binding (fmols ^{3}H-DHA bound/mg protein) was lowest at proestrous (Bmax = 77), slightly increased at estrous (Bmax = 91) and on diestrous day-1 (Bmax = 99) with a peak on diestrous day-2 (Bmax = 110). As with β-adrenergic cAMP responses, ^{3}H-DHA binding was elevated in both age-matched male rats (Bmax = 126) and ovariectomized females (Bmax = 150) relative to cycling females. No significant differences were seen in the EC_{50}'s for ISO stimulation of cAMP or in the apparent dissociation constants (K_d) for ^{3}H-DHA binding between any condition. Results suggest that estrogens may cause 1) a cyclical decrease of β-adrenergic responses during the ovarian cycle with the lowest values occuring at proestrous and 2) a general decrease in β-adrenergic sensitivity in adult female rats in comparison with both age-matched male and ovariectomized female rats. Receptor binding studies suggest at least part of the differences may be mediated by differences in the density of β-adrenergic membrane receptors.

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1190 CHRONIC TREATMENT OF RATS WITH TRICYCLIC ANTIDEPRESSANTS INCREASES RESPONSIVENESS OF AMYGDALOID NEURONS TO SEROTONIN AND NOREPINEPHRINE. R.Y. Wang & G.K. Aghajanian, Depts. Psychiat. & Pharmacol., Yale Univ. Sch. Med., New Haven, CT 06508.

Recently deMontigny and Aghajanian reported (Science 202: 1303, 1978) that after 1-2 wks treatment of rats with tricyclic antidepressant drugs, there was a selective increase in the depressant response of hippocampal and lateral geniculate neurons to microiontophoretically applied serotonin (ionto-5-HT). The aim of the present study was to determine whether this finding holds for amygdaloid (AMYG) cells. In addition, the effect of chronic tricyclic antidepressants on the inhibition of AMYG cells by stimulation of the ascending 5-HT pathway was tested.

Drugs or saline were given ip for 2 wks. Chloral hydrate anesthetized rats were used for single-unit recording and microiontophoresis. Glutamate (Glu) was used to activate cells so that both spontaneously active and quiescent neurons could be studied. To standardize the activation level of cells, the maximum firing rate (prior to depolarization block) induced by ionto-Glu was determined for each cell. One half of the maximum firing rate was then maintained by adjusting the current of Glu. The sensitivity of AMYG neurons to 5-HT and other drugs was evaluated by the charge (the product of the current and the time) required to obtain a 50% decrease in the baseline firing rate.

The response of AMYG cells to 5-HT and lysergic acid diethylamide (LSD, a partial 5-HT agonist) was enhanced (1.5-3 fold) 24 hrs after 2 wks treatment with tricyclic antidepressant drugs (imipramine, desipramine or iprindole). However, the increased responsiveness of AMYG neurons was not specific for 5-HT; the response of cells to norepinephrine (NE) was also enhanced. AMYG neurons were more sensitive to endogenous 5-HT released by stimulation of the 5-HT pathway near the dorsal raphe nucleus (DRN). Compared to controls, the threshold for DRN-induced depressant effects on AMYG cells was significantly decreased. Chronic treatment with chloropromazine, a tricyclic antipsychotic drug, failed to alter the responsiveness of AMYG neurons to 5-HT or NE. Two or more days after discontinuation of chronic treatment with tricyclic antidepressants, the enhanced receptor sensitivity began to disappear; in some cases, subsensitivity was observed.

The results of the present study show that chronic treatment of rats with tricyclic antidepressant drugs increase responsiveness of AMYG neurons to 5-HT and NE. It is suggested that modulation of the sensitivity of postsynaptic 5-HT and NE receptors may be related to the delayed therapeutic actions of tricyclic antidepressant drugs (Supported by USPHS Grants MH-17871 and MH-14459).

1191 ALPHA, BETA PHARMACOLOGICAL CHARACTERIZATION OF NORADRENERGIC MODULATORY ACTIONS IN RAT SOMATOSENSORY CORTEX. Barry D. Waterhouse, Hylan C. Moises and Donald J. Woodward. Dept. Cell Biology, Univ. Tx. Health Sci. Ctr., Dallas, Tx. 75235

We previously reported that excitatory and inhibitory responses of rat somatosensory cortical neurons, produced either by natural stimulation of afferent synaptic pathways or microiontophoretic application of putative transmitters, are enhanced during norepinephrine (NE) iontophoresis. The issue considered in the present study was whether facilitatory effects of NE on cortical neuronal responses to putative transmitter substances could be defined in terms of classical alpha or beta adrenergic receptors. Extracellular activity of somatosensory cortical neurons was recorded from halothane-anesthetized albino rats using multibarrel micropipettes. Responses to iontophoretic pulses (10 sec duration at 45 sec intervals) of acetylcholine (ACH) and gamma-aminobutyric acid (GABA), putative cerebrocortical neurotransmitters, were examined before, during and after iontophoresis of NE, phenylephrine (PE) or isoproterenol (ISO) (4-30 nanoamps). Drug response histograms were used to quantitate effects of the adrenergic agonists on spontaneous and drug-induced activity.

In 15 of 24 (63%) cells, the alpha agonist PE exerted differential effects on spontaneous discharge and ACH-induced excitation which mimicked those exerted by NE. ACH responses of 7 cells were potentiated above control levels with doses of PE which produced no change or moderate depression of background discharge. In 8 neurons, ACH excitation was preserved relative to PE suppression of spontaneous firing rate such that signal to noise ratios were enhanced. In contrast, the beta agonist ISO had no effect or antagonized ACH-induced excitation in 17 of 19 (89%) cells tested. In 3 other cells, iontophoretically applied phentolamine, a specific alpha antagonist, reversibly blocked NE-induced enhancement of ACH excitatory responses. Unlike NE which consistently augments GABA-induced inhibition, PE and ISO at doses which suppressed spontaneous discharge produced no effect or antagonized the GABA response in 15 of 20 neurons.

In summary, these findings suggest that NE modulation of ACH responses in rat somatosensory cortex results from activation of an alpha type receptor, whereas NE augmentation of GABA inhibition occurs via a receptor interaction not easily categorized as an alpha or beta type. Furthermore, the pharmacological specificity demonstrated by NE, PE and ISO with respect to enhancement of GABA and ACH actions indicate that noradrenergic modulatory effects occur independent of a common depressant effect on spontaneous discharge. (Supported by grants from NSF BNS77-00174, NIDA DA-02338 and the Biological Humanics Foundation to DJW and NIH 5 F32 NS05699-02 to BDW)

1192 EFFECTS OF INTRACISTERNAL 6-HYDROXYDOPAMINE ADMINISTRATION ON THE ACQUISITION AND PERFORMANCE OF A SPECIFIC AND NOVEL LOCOMOTOR TASK IN RATS. Watson, M.* and McElligott, J.G. (SPON: S. McElligott). Department of Pharmacology, Temple University School of Medicine, Philadelphia, PA 19140.

Numerous investigators have proposed that catecholamines, notably norepinephrine (NE), are involved in learning processes. A method for evaluating the effect of central NE depletion on the acquisition and performance of a specific task requiring precise paw placement has been developed. Water-deprived rats were trained to traverse horizontally placed rods spaced at regular intervals (standard rod arrangement) for a water reward. Daily performance was assessed by determining average running time for approximately 25 trials using a photodetection system. In addition, foot placement was videotaped by focusing a T.V. camera on a full-length mirror angled beneath the runway. With 4 days of successive exposure to the task, rats ran 40% faster than on day one. The effect of central NE depletion on post acquisitional performance of this specific locomotor task was determined after rats were infused with 6-hydroxydopamine (in 0.1% ascorbate/saline vehicle). Three doses of 6-OHDA (25 µg in 25 µl) were administered intracisternally over a seven day period to enhance the preferential reduction of NE. Subsequent testing, which began 10-14 days following completion of treatment, showed no effect on post-acquisitional performance when compared to vehicle controls. A second group of rats was given 6-OHDA after 4 days on the standard rod arrangement and subsequently exposed to a similar but more difficult locomotor task. They were compelled to traverse an altered arrangement which consisted of irregularly spaced rods. Vehicle controls ran 44% faster by day four. 6-OHDA treated rats ran only 19% faster, showing impaired acquisition of this new task. Determination of intertrial intervals (latency between trials) showed no significant difference. In addition, no differences in open field behavior were noted between the two groups. Biochemical determinations were made using High Pressure Liquid Chromatography (HPLC) with electrochemical detection. Cerebellar NE was reduced by 73%, while limbic forebrain showed a reduction of 41% in NE and 29% in Dopamine (DA). While these data are consistent with a general neuromodulatory role for NE, the observed change in locomotor behavior may be the result of noradrenergic deafferentation of a specific brain region such as the cerebellum. Depletion of NE at cerebellar Purkinje cells may result in impaired acquisition of certain coordinated motor behaviors.

1193 IDENTIFICATION OF BULBOSPINAL SEROTONERGIC NEURONS ON THE BASIS OF CONDUCTION VELOCITY. M.W. Wessendorf*, E.G. Anderson, H.K. Proudfit. Depart. Pharmacol. U. of Illinois Col. of Med., Chicago Illinois 60612

The Nucleus Raphe Magnus (NRM) contains cell bodies of bulbospinal serotonergic neurons. The nucleus, however, contains cells of varied morphology, many of which may not be serotonergic. We have sought to establish criteria for identifying serotonergic cells on the basis of their conduction velocities.

Recordings from NRM cells were made using 4-barrel glass microelectrodes. Serial electrode drives along the rostral-caudal axis of the nucleus sampled bulbospinal units, which were identified by antidromic activation via ball electrodes placed on the dorsal lateral surfaces of the spinal cord between T_8 and T_{10}. Antidromic activation was assumed when observing invariance of latency in response to 3 Hz. stimulation, the ability of a cell to follow 60 Hz. stimulation, or collision between spikes simultaneously initiating at the soma and the spinal cord. Spikes were assumed to be of somatic origin if microiontophoretic application of d,l-homocysteic acid produced excitation.

In 160 bulbospinal cells recorded in the NRM, the conduction velocities were bimodally distributed. The conduction velocities of the majority of cells occurred in the range between 3.1 and 20 M/sec; however a large number of cells were also observed with conduction velocities between 0.7 and 1.0 M/sec. Very few cells had conduction velocities between 1.1 and 3.0 M/sec., or greater than 30 M/sec.

To determine the degree of representation of serotonergic cells in these ranges, conduction velocities were measured in rats injected intraventricularly with 150 mcg 5,7-dihydroxytryptamine (5,7-DHT), a serotonin neurotoxin. The treated animals were mixed with vehicle-injected controls in a blind experimental paradigm, and at 12 to 16 days a search was made for antidromically activated cells in the NRM. Data were normalized by expressing them as numbers of cells per electrode drive.

Treament with 5,7-DHT moderately reduced the number of cells encountered per drive through the NRM. On the basis of conduction velocity, cells in the 0.7-1.0 M/sec range were the most dramatically reduced, declining from 1.05 (± S.E. 0.32) cells per drive in controls to 0.16 (± S.E. 0.065) in treated rats. We therefore conclude that these cells are predominantly serotonergic. The units in the 3 to 6 M/sec range also declined significantly and may contain serotonergic cells. From the moderate decreases in other conduction velocity groups we cannot exclude the possibility that some faster conducting serotonin cells may exist.

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1194 DIFFERENTIAL DESCENDING PROJECTIONS FROM THE LOCUS COERULEUS AND THE SUBCOERULEUS/PARABRACHIAL NUCLEI IN MONKEY. K.N. Westlund and J.D. Coulter. Marine Biomedical Institute, Departments of Psychiatry & Behavioral Sciences and Physiology & Biophysics, University of Texas Medical Branch, Galveston, Texas 77550.

Descending projections from the locus coeruleus and the subcoeruleus/parabrachial region were examined using the anterograde autoradiographic tracing technique in monkey (*Macaca fascicularis*). Single injections of 0.1-0.3 µl of an equal parts mixture of [^{3}H] leucine and proline (50 µCi/µl) were made either into the region of the locus coeruleus or into the subcoeruleus/medial parabrachial nuclei. Labeled fibers descend ipsilaterally both medially, with the medial longitudinal fasciculus, and laterally beneath the trigeminal complex. Some fibers seen crossing the midline terminate in the contralateral locus coeruleus and subcoeruleus/parabrachial nuclei respectively. Common terminations in the brainstem include the raphe complex, the intermediate cell group of the facial nucleus, the nucleus prepositus hypoglossus, the spinal nucleus of the trigeminal and the medial reticular formation. Specific terminations of descending locus coeruleus fibers include the ventral portion of both the dorsal motor nucleus of the vagus and the nucleus of the solitary tract, as well as the subjacent reticular formation. Subcoeruleus/parabrachial specific terminations include the nucleus hypoglossus and the nucleus retroambiuus. Heavy fiber labeling from both regions continues into the spinal cord ipsilaterally in the ventrolateral white matter through cervical, thoracic, lumbar, and sacral cord levels. Fibers are seen crossing the midline at all levels of the medulla and spinal cord. At all cord levels both locus coeruleus and subcoeruleus/parabrachial terminations include heavy label ipsilaterally over the ventral horn in the region equivalent to Rexed's laminae VII-IX, particularly over the motor neurons. Laminae I and X contain fairly heavy terminations ipsilaterally while dorsal horn laminae III-VI are lightly labeled. Lighter label is also seen in the same general pattern contralaterally. The major descending termination of the subcoeruleus/parabrachial region is bilaterally at levels T_2-T_4 in association with the sympathetic intermediolateral cell column. The locus coeruleus, in contrast, projects heavily upon the parasympathetic lateral gray, bilaterally at levels S_2-S_4. Since localization of the terminal zones seen in this study corresponds closely with the pattern demonstrated histochemically for noradrenergic terminals, the locus coeruleus and the subjacent subcoeruleus/parabrachial regions appear to provide differential descending noradrenergic innervation of cranial and spinal somatic sensory, motor and visceromotor cell groups. (Supported by NS12481).

1195 HISTOCHEMICAL AND HISTOFLUORESCENT LOCALIZATION OF BIOGENIC AMINE DEPOSITS IN ALTERNATE SECTIONS. Joe Wood and Robert E. McClung. Department of Neurobiology & Anatomy, University of Texas Medical School, Houston, TX 77025.

Biogenic amines can be readily detected in the CNS by histofluorescent methods. These techniques are highly sensitive and yield striking photographs; however, the reaction products are sensitive to light and tend to fade. Moreover, histofluorescent techniques utilize dark field microscopy which reveals little of the non-fluorescent cytoarchitectural components. Electron microscopic localization of biogenic amines using glutaraldehyde-dichromate has been used successfully in the central nervous system, and there have been light microscopic observations made from small plastic embedded sections. Difficulties of correlation of histofluorescence with light and electron microscopy are now more apparent than real. Fresh brain material can be frozen on dry ice and sections made on a cryostat at approximately 10-20 microns. Tissue was obtained from rats, monkeys and cats, frozen, and sectioned on a cryostat. Sequential sections were alternated, with section A being used for histofluorescence, section B for a modified glutaraldehyde dichromate staining procedure, and section C as an untreated control. Glyoxylic acid histofluorescence shows norepinephrine and serotonin-containing cell bodies and processes, especially in the midbrain region. The next section was treated with a mixture of glutaraldehyde, sodium chromate and potassium dichromate at pH 4.1. Following incubation the tissue was stained with conventional histologic stains, i.e. cresyl violet and/or toluidine blue. Neurons correlative with histofluorescent areas were seen to contain catecholamine and/or indolamine chromium deposits in cell bodies and in some processes. Visualization of these deposits range anywhere from low-power light microscopy to oil immersion, and with care, pigments can be differentiated from amine deposits. In the unstained, untreated control sections, only pigment deposits are visible. This technique should lead to a better understanding of the cytoarchitecture of the central nervous system and the relation of amine-containing cells to other cell structures, i.e. glial cells and other neurons. The technique will be useful in localizing amine-positive areas for electron microscopy, and no doubt will serve as the bridge from mapping to electron microscopy. Supported by USPHS grant NS-10326.

1196 DEVELOPMENTAL CHANGES IN THE CEREBELLUM INVOLVING GRANULE CELLS, PURKINJE CELLS AND THE SEROTONIN INNERVATION INDUCED BY RAPHE TRANSPLANTS. Miyuki Yamamoto-Yoshida* and Victoria Chan-Palay (SPON: S.L. Palay). Depts. of Anatomy and Neurobiology, Harvard Medical School, Boston, MA 02115.

Serotonin neurons in several raphe nuclei contribute to the innervation of the cerebellar cortex and nuclei through mossy fibers, parallel fiber-like axons, and diffusely branching free endings. Whereas serotonin neurons and their processes develop early in prenatal ontogeny, the cerebellum develops late, postnatally. The aim of these investigations was to examine the effect of raphe transplants upon the subsequent development of the cerebellum in early postnatal rats. A series of experiments was conducted to determine the optimal ages of donor and host animals. Donor rats of ages 6 days to adult and host animals of 4 to 14 days postnatal age were used. The most consistent results were obtained with both donor and host animals of 6 postnatal days. The midline raphe structures of the medulla were carefully dissected from brains of donor animals. Host animals were anesthetized with ether and the IVth ventricle exposed to receive the transplant without mechanical traumatizing the overlying cerebellum. Post-transplantation survival was 4 weeks and longer.

The cerebellum and structures bounding the IVth ventricle were examined in one group of experimental animals following perfusion fixation with aldehydes and staining of the sections with thionin. Cerebellar structures close to the transplant consistently showed developmental anomalies. The cerebeller cortex displayed (1) folial malformation particularly in the vermis, (2) foci of arrested granule cells due to nondescent of the external granular layer, (3) foci of disruptions in the Purkinje cell layer. These foci of changes were directly related to the position of the transplant.

In a second group of transplanted animals, the serotonin innervation of the cerebellum was examined after three hour intracisternal or intraventricular infusions of ^{3}H-serotonin (50 µl, 10^{-6} M in saline, specific activity 10.8 Ci/mmol) followed by perfusion fixation with aldehydes. Light microscope autoradiograms revealed an unusual profusion of labeled serotonin axons in the foci with arrested granule cells and disrupted Purkinje cells, compared to normal and to control animals. Control experiments were performed using transplants of liver tissue or gelfoam sponges soaked in serotonin. These cases showed folial malformation but not the foci of neuronal changes, indicating that changes in folial pattern alone may be produced by transplants of various tissues. These results indicate that transplants of raphe can cause developmental changes in the cerebellum of the host. These anomalies may be due in part to a detectable increase in the serotonin innervation of the cerebellum, most probably originating from the transplant.

1197 COMPARISON OF NORADRENERGIC MODULATORY ACTIONS ON PURKINJE CELL RESPONSES TO IONTOPHORESIS OF γ-AMINOBUTYRIC ACID, β-ALANINE AND TAURINE. Hermes H. Yeh, Hylan C. Moises, Barry D. Waterhouse and Donald J. Woodward. Dept. Cell Biology, U. Tx. Health Science Center, Dallas, Tx. 75235

We have previously shown that both iontophoretically applied norepinephrine (NE) and stimulation of the locus coeruleus can potentiate γ-aminobutyric acid (GABA)-mediated inhibition of cerebellar Purkinje cells (PC). Glycine-induced inhibition was not enhanced and dopamine did not mimic the facilitatory effect of NE. The specificity of the observed NE modulation was studied further here in a series of iontophoretic experiments comparing depressant amino acids structurally related to GABA.

Multibarrel micropipettes were used to apply drugs and record extracellular PC unit responses in halothane-anesthetized rats. Inhibitory responses to iontophoretic pulses (10 sec duration at 30-35 sec intervals) of β-alanine, taurine and GABA were examined before, during and after NE iontophoresis (5-20 na). Drug response histograms were computed to quantitate NE effects on spontaneous and drug-induced neuronal activity.

Consistent with previous findings, NE at doses which had little or no effect on PC spontaneous activity produced marked augmentation in all 13 cells tested. In 3 cells, GABA applied continuously at low currents showed no ability to potentiate PC responses to superimposed GABA pulses. This suggests that the observed modulation with NE cannot be explained by summation of NE depressant effects with hyperpolarizations produced by GABA. NE interaction with β-alanine-induced inhibition, tested in 19 PC's, was clearly different from GABA, with a predominance of 10 cells showing no effect, 7 cells augmented and 2 cells antagonized. NE effects on taurine-induced inhibition was also dissimilar to GABA. In 14 cells tested, 6 showed no significant changes, 4 cells were potentiated and 4 cells were antagonized. NE facilitatory effects, when seen with β-alanine and taurine, were moderate in comparison to that of GABA. Picrotoxin reversibly blocked GABA (3 cells) and β-alanine (3 cells) inhibitions whereas taurine blockade (3 cells) appeared to be of distinctly longer duration. This preliminary result, in addition to the apparent specificity in NE modulation, would argue for a specificity residing within different amino acid receptors or, perhaps, reuptake processes.

In summary, these data confirm the previously reported NE modulation of GABA-mediated inhibition. Evidence is provided here that NE preferentially enhances the inhibition of PC's produced by iontophoretically applied GABA in contrast to that produced by other amino acid depressant agents. (Supported by grants from NSF BNS77-01174, NIDA DA-02338 and the Biological Humanics Foundation to DJW).

1198 ELECTROPHYSIOLOGICAL EVIDENCE OF INHIBITORY INPUTS TO THE VENTRAL TEGMENTAL AREA FROM THE PREOPTIC AREA AND THE NUCLEUS ACCUMBENS. C. Y. Yim*, H. Maeda* and G. J. Mogenson. Dept. of Physiology, Univ. of Western Ontario, London, Canada N6A 5C1.

Previous anatomical studies have shown that limbic forebrain structures project to ventral tegmental area (VTA) of the midbrain (Nauta, Brain 1958, 81, 319). The present study investigated with electrophysiological recording techniques possible inputs to the VTA from the anterior hypothalamic-medial preoptic area (AHPO) as well as from the nucleus accumbens (NA).

Action potentials were recorded extracellularly from neurons in the VTA of urethane anesthetized rats using glass micropipettes filled with 4M NaCl. The effects of single pulse stimuli delivered to the NA and AHPO on the neuronal activity of the ipsilateral VTA were investigated. In some experiments, 7 barrel micropipettes were used for recording and iontophoretic application of picrotoxin (a GABA antagonist) and nipecotic acid (a GABA uptake inhibitor) to study the effect of these drugs on the response of VTA neurons to NA stimulation.

The electrophysiological characteristics of the population of units recorded suggested two types of VTA neurons: one type had long spike durations and slow rates of discharge and a second type shorter spike durations and fast rates of discharge. These observations confirm previous findings in which the two types were considered to be dopaminergic mesolimbic and non-dopaminergic neurons (Yim & Mogenson, Brain Research 1979, in press). Approximately 20% of VTA neurons were antidromically activated from the nucleus accumbens.

Both types of VTA neurons received convergent inputs from the AHPO and NA. Electrical stimulation of the AHPO inhibited the majority of the sample of VTA neurons tested with a relatively short latency(<10 ms). Stimulation of NA inhibited both types of VTA neurons tested but in addition, activated the first type of VTA neurons as well. Activations usually had longer latencies of > 10 ms. Inhibition of the VTA neurons by stimulation of NA was blocked by iontophoretically applied picrotoxin and prolonged by nipecotic acid with no change in latency.

These observations provided evidence of an inhibitory input from AHPO and NA of the limbic forebrain to dopaminergic and non-dopaminergic neurons in the VTA. The effects of the iontophoresis of picrotoxin and nipecotic acid suggest that the descending inhibitory pathway from the NA to the VTA is GABAergic. Nature of the transmitters that mediated the excitatory response from NA and the inhibitory response from AHPO stimulations remains unknown. The descending GABAergic projection from the NA to the VTA appeared to be similar to the feedback GABAergic pathway present in the nigral striatal dopaminergic system.
(Supported by MRC of Canada)

1199 PRENATAL PHENOBARBITAL EXPOSURE ALTERS MOUSE BRAIN NEUROTRANSMITTER UPTAKE. John W. Zemp, Thomas N. Thomas and Lawrence D. Middaugh, Department of Biochemistry and of Psychiatry and Behavioral Sciences, Medical University of South Carolina, Charleston, S.C. 29403.

We have previously reported that prenatal exposure to phenobarbital during the last third of pregnancy produces alterations in the brains of C57 BL/6J mice. These alterations include delays in some correlates of brain growth and development[1] changes in the circulating levels of corticosterone on the day of birth and at 21 days of age[2] and long lasting changes in behavior which may reflect a change in the arousal level of the exposed animals[3] . In this report we demonstrate that prenatal exposure to the barbiturate produces changes in the ability of crude synaptosomal preparations to accumulate several neurotransmitters. High affinity uptake of catecholamines, serotonin and γ amino butyrate(GABA) were estimated using crude synaptosomal preparations obtained from the brains of mice exposed to phenobarbital (20 or 40 mg/kg maternal body weight) (P_{20} and P_{40}) or to saline injections during the last week of gestation. The radioactive neurotransmitters was added to the crude synaptosomal preparation and incubated for five minutes at 37° C. Following filtration and washing, the filters were soaked overnight in sodium dodecyl sulfate and counted. Results were expressed as pmoles uptake/mg protein/5 minutes. Results indicated that for the P_{40} group there was a significant increase in the uptake of both dopamine and norepinephrine at low concentration (0.01μM) and a significant decrease in the uptake of GABA at low concentrations (0.01μM). No effect on the uptake of these neurotransmitters was observed in the P_{20}groups. By contrast the uptake of serotonin was significantly and substantially increased at all concentrations of serotonin studied with both the P_{20} and P_{40} groups. These results are consistent with a change in synaptic membrane function as a result of prenatal phenobarbital administration. (Supported by Grant # DA 06124 from ADAMHA)

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MOTOR SYSTEMS

1200 FREQUENCY CHARACTERISTICS OF THE HUMAN ELECTRO-OCULOGRAPHIC RESPONSE Larry A. Abel*, A. Terry Bahill and B. Todd Troost Dept. Neurology, Univ. Pittsburgh School of Medicine and Biomedical Engineering Program, Carnegie-Mellon University, Pittsburgh, PA. 15261.

Human tissue is commonly modeled as a resistor in series with a parallel resistor-capacitor combination. This circuit should produce an inherent low-pass filtering of electro-oculographic (EOG) data, if the orbital structures involved are, in fact, adequately described by this model.

Using standard EOG electrodes and typical AC amplifiers we first found the EOG current to be 20 pA. We could not make our impedance measurements at currents this low, so we used 10 nA of current, which produced a current density of 3×10^{-4} mA/cm^2.

In an effort to find the cutoff frequency of the obligatory low-pass filtering we then measured the impedance of the head in a fixating subject and of the EOG electrodes as a function of frequency. The impedance of the head held constant at about 4 kohms over the entire frequency range. The impedance of the electrodes, however, showed a surprising increase in magnitude and phase angle at frequencies above 10 kHz. Electrode phase angle went from a 7 deg lag at lowest frequencies to zero at 100 Hz, then becoming an increasingly large lead at higher frequencies.

The lack of a pronounced attenuation at 25 Hz as predicted by a simple R-C tissue model indicates that the high frequency components of saccadic eye movements are not inherently eliminated by EOG recording. The presence, however, of concurrent EMG activity originating in the facial muscles still masks the fine structure of saccades, as can be seen by spectral analysis. Thus, other recording techniques are preferable when eye movements are to be studied in detail.

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1201 THE NATURE AND DISTRIBUTION OF NECK MUSCLE AFFERENTS PROJECTING TO THE MEDULLA. V.C. Abrahams and T. Yokota, Department of Physiology, Queen's University, Kingston, Ont. Canada. K7L 3N6.

Electrical stimulation of muscle afferent nerves provides a convenient technique for studying central projection pathways. Afferent nerves serving the large dorsal neck muscles, unlike the more commonly studied hindleg nerves have a unimodal skewed distribution with a single peak at 3-4μ. Recordings have been made of conduction velocity and stimulus threshold from 238 neck muscle afferent fibres. Fibres activated by threshold (T) to 1.2T had conduction velocities of 20 m/sec and above. Increasing stimulus strength from 1.2T to 2T led to the appearance of a few (4 of 80) fibres conducting at velocities below 10 m/sec. All myelinated fibres were activated by stimulation at 10T. Spindle and Golgi tendon organs (GTO) afferents in neck muscle nerves conduct at velocities of 20 m/sec or more. Thus stimulation of the large dorsal neck muscle afferent nerves at strengths of T to 2T will lead mainly to excitation of spindles and GTO's. It is only when stimulus strengths from 2 to 10T are used that GPIII afferent fibres are activated. Stimuli of 10T and greater (and usually requiring longer pulse widths) are necessary for unmyelinated afferents to be excited.

These stimulation criteria were used to re-examine the nature of muscle afferent projections to the medulla just posterior to the obex. Fast green dye-filled glass micro-electrodes were used so that recording sites could be accurately noted. The largest group of units activated by neck muscle afferents at this level of the medulla were high threshold afferents and few spindle or GTO projections were found, and then in widely scattered regions. The largest population of units excited (43 of 63 examined) had muscle afferent thresholds mostly between 6 and 8T and never below 4T. The same units responded to mechanical stimulation of the ipsilateral cornea (usually light touch of cotton, but in 3 units needing a firm stroke), pinch of both pinnae with toothed forceps (but not light touch) and a sharp tap to the nose. These units were located deep to the trigeminal subnucleus caudalis in lamina V and VI. A few units with high thresholds (2.5 to 21T) and with large nociceptive cutaneous fields were found ventromedial to pars magnocellularis of trigeminal subnucleus caudalis. Low threshold units were found as previously described in the most lateral region of the cuneate nucleus and between the cuneate nucleus and trigeminal subnucleus caudalis.

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1202 EFFECT OF VIBRATION ON THE ANKLE STRETCH REFLEX IN HUMAN. Gyan C. Agarwal and Gerald L. Gottlieb. Dept. of Physiology, Rush Presbyterian-St. Luke's Medical Center, Chicago, Illinois 60612 and College of Engineering, University of Illinois at Chicago Circle, Chicago, Illinois 60680.

Vibration applied to the tendon of the extensor muscles of the ankle joint produces the tonic vibration reflex (TVR) which increases when the vibration frequency is increased. The TVR produces changes in the mechanical properties about the joint as well as in the spinal reflexes associated with the vibrated muscle.

Torques were applied to dorsiflex the ankle of seated human subjects evoking a myotatic reflex and post-myotatic responses. Vibration inhibits the myotatic component of the stretch reflex in soleus. The degree of inhibition is proportional to the vibration frequency. This is in contrast to the facilitation of the myotatic reflex produced by tonic voluntary contraction.

Vibration does not influence the post-myotatic component of the stretch evoked response. This component is also not significantly influenced by the tonic voluntary contraction. These results indicate that the post-myotatic responses to limb perturbation are not only different from the myotatic reflex in their latency but also in their functional dependence to peripheral and central influences.

The mechanical effects of vibration were measured by applying sinusoidal or random torque inputs. Although the myotatic response to discrete stretches is suppressed by the TVR, the repeated stretches of sinusoidal oscillation produce an average EMG response which is not different in magnitude from the no vibration case with tonic voluntary contraction. The TVR affects a joint's response as measured in terms of mechanical parameters (mainly joint stiffness) in the same facilatory manner that is seen with tonic volunatry contraction.

(This work was supported by NSF grant ENG-7608754 and NIH grants NS-00196 and NS-12877)

1203 MULTIPLE UNIT ACTIVITY ASSOCIATED WITH VOLUNTARY HAND MOVEMENTS AND SOMATOSENSORY STIMULATION IN THE SENSORIMOTOR CORTEX OF THE MONKEY. Joseph Arezzo* and Herbert G. Vaughan, Jr. Dept. of Neuroscience, Albert Einstein Coll. of Med., Bronx, N.Y. 10461.

The firing patterns of cortical neurons associated with conditioned hand movements have been extensively studied in the monkey using single unit techniques. These studies are usually limited to recordings from a restricted region and are inherently biased toward sampling of larger neurons. In this investigation, we have examined digitally rectified and averaged multiple unit activity (MUA), recorded simultaneously from portions of the frontal and parietal cortex of monkeys trained to perform self-initiated hand movements. The timing and intracortical distribution of this activity has been compared with the MUA elicited by peripheral stimulation. Our recording methods provide a weighted sum of the neural firing within a sphere of tissue roughly 350 u in diameter, and thus, provide an index of net changes in neural population activity within a circumscribed cortical locus. These data are particularly useful in interpreting field potential recordings, which provide the sole available method for studying human sensorimotor processes.

Antecedent increases in MUA begin 90-100 msec before EMG monitored contractions and are recorded throughout the hand area of the precentral gyrus, including the anterior bank of the central sulcus. The antecedent activity is maximum in amplitude within the fifth lamina but can also be recorded in the fourth and fifth lamina. In the more superficial laminae of area 4, the initial increase in MUA is coincident with movement onset and continues for up to 200 msec following the end of EMG activity. Peripheral stimulation of the median nerve which is sufficient to produce a thumb twitch results in activation limited to the posterior portion of area 4. Within area 3a, a phasic burst of MUA begins 10-20 msec after movement and is followed by a second discharge which peaks 100 msec after movement onset. Area 3a receives the earliest somatosensory input with MUA beginning 6.0 msec following stimulation. Movement related MUA in areas 1 and 2 follows the onset of contraction and also peaks at 100 msec. Additional MUA which begins 150 msec after movement onset is recorded from area 5. No changes in MUA are found in area 7 associated with self-paced movements.

This work was supported by Grant MH 06723 from the USPHS.

1204 GRADED EFFECTS OF STATIC FUSIMOTOR STIMULATION ON THE FREQUENCY RESPONSE OF MUSCLE SPINDLE PRIMARY ENDING. R.A. AURIEMMA*, G.P. MOORE, J.R.ROSENBERG‡ M. DUTIA* Dept.Biomed.Engr.,USC,Los Angeles Ca. 90007 and Institute of Physiology, Univ. Glasgow, Scotland.

The effect of gamma fusimotor innervation on the transducing properties of the spindle muscle receptor were studied in the tenuissimus muscle of the cat. Functionally single primary afferent axons and static gamma motor axons were isolated from dorsal and ventral rootlets at the L7-S1 level. Length perturbations having a Gaussian amplitude distribution of 10-100μ RMS level and a flat frequency spectrum from DC to 120 Hz were applied to the proximal half of the muscle and the Ia discharge recorded, with and without concurrent, independent Poisson pulse trains of 40-80 pulses/sec mean rate being delivered to one or more gamma axons. Crosscorrelation of the Ia spike train with the input length waveform allowed the calculation of the average prespike length epoch and an estimate of the impulse response function of the muscle receptor. The Fourier transform of the impulse response function provided an estimate of the receptor's frequency response.

In the absence of gamma stimulation the gain of the frequency response curve increased at 20db/decade, suggesting a system responding to the velocity of the applied perturbation When individual gamma axons were stimulated the relation was shifted by varying amounts for each axon, towards 40db/decade, a characteristic of an acceleration-sensitive system. When pairs of gamma axons were independently stimulated, the shift was generally greater than that seen from either axon alone, but was never greater than 40db/decade even for cases where one of the axons produced such a shift when stimulated alone. Increase in mean spike rate and an absolute decrease in the magnitude of the gain function at each frequency were observed to accompany the gamma effect on the slope of the gain curve (Supported in part by NIH grants NS 11298 & GM 23732.

1205 EFFECTS OF ALTERED GRAVITY ON A HUMAN OTOLITH-SPINAL REFLEX. S.B. Backman* and D.G.D. Watt. Dept. of Anaesthesia Res. and Aviation Med. Res. Unit, McGill Univ., Montreal, Canada H3G 1Y6.

The sensitivity of the vestibular otolith organs is lowered whenever the head is tipped out of its normal orientation. Recently, Melvill Jones & Young (1978) have demonstrated that "the threshold of sensitivity to saccular stimulation is significantly lower when in its normal orientation relative to gravity than when tilted 90° away from that orientation". This experiment extends this finding by replacing subjective assessment of linear movement with an objective test of otolith sensitivity.

Ten subjects were exposed to sudden, unexpected falls of 15cm (200 to 450 msec, depending on Δg) while surface emg activity was recorded from their left calf muscles. Step changes of .33, .67 and 1.0 g were employed, using counterweights to reduce acceleration below 1.0 g in the vertical direction. Subjects were also suspended horizontally, substituting for gravity with bungee cords running from the waist to the wall, and similar .33, .67 and 1.0 g step changes were used.

Emg activity consisted of 2 components, an early burst related to the onset of fall, and later activity concerned with landing. On average, emg activity began 74 msec after release for vertical Δgs of .67 and 1.0, and a horizontal Δg of 1.0. The early activity was reduced and sometimes absent for a vertical Δg of .33 and horizontal Δgs of .33 and .67, extending the average latency to as much as 116 msec in these cases. Later activity concerned with landing built up before contact and continued afterwards, its pattern being less consistent and of lesser amplitude when the subject was landing from a horizontal fall. Emg activity was rectified, all falls were averaged for each Δg and orientation, and the area under the resulting curve was measured from 50-150 msec after release. (This activity is considered to be predominantly otolith-spinal in origin, being of short and relatively invariant latency, time-locked to the acceleration stimulus, and too early for a voluntary response (Melvill Jones & Watt, 1971). It can also be selectively abolished by labyrinthectomy in cats (Watt, 1976) and is absent in labyrinth-defective human subjects (Greenwood & Hopkins, 1976)). The area of the early burst varied with the size of the acceleration stimulus when the subject was tested in either orientation. Furthermore, the gain of this otolith-spinal reflex was significantly reduced when the subject was horizontal. This confirms the findings of Melvill Jones & Young, and provides a means of quantitizing changes in otolith function in man during prolonged changes of gravity, as during space flight.

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1206 THE SIZE PRINCIPLE: EFFECT OF INHIBITORY INPUTS TO MOTIONEURONES IN HUMAN SUBJECTS. P. Bawa, Dept. of Kinesiology, S.F.U., Burnaby, B.C., V5A 1S6.

In the decerebrate cat, Henneman and coworkers (1965, 1974) demonstrated that the effect of an inhibitory input to a motoneurone pool with an existing excitatory input is to reduce the effect of excitation, thus keeping the fixed order of recruitment of motoneurones. The following experiments were done to test the effects of inhibition in normal human subjects. The subject sat comfortably in a chair with his foot strapped to a plate carrying strain gauges so that the contractions of T A and triceps surae (G-S) muscles were isometric.

(i) Disynaptic spinal inhibition: The subject recruited a pair of distinct single motor units (SMUs) from T A and maintained their firing rate. Stimulus to the tibial nerve to elicit H-reflex in soleus resulted in inhibition of T A motor units. The high threshold (HT) SMU dropped out before the low threshold (LT) SMU for an interval up to 300 msec. No reversal in derecruitment observed.

(ii) Vibration of Achilles tendon: The subject recruited two distinct SMUs from T A and maintained their firing rate. Vibration of Achilles tendon at 60Hz, resulted in TVR in soleus and inhibition of T A-SMUs. The HT-SMU derecruited before LT-SMU without exception.

(iii) Descending inhibition: Achilles tendon was vibrated at 60Hz to recruit two distinct SMUs from soleus. The subject was asked to derecruit these units voluntarily while the vibration was still on. Out of the 50 pairs studied, 34 derecruited with HT-SMU before LT-SMU. In 12 pairs, LT unit dropped before HT unit while 5 pairs were "confused", the order of recruitment & derecruitment changed on rechecking.

The net excitability of a motoneurone is determined by the temporal and spatial relationships of excitatory and inhibitory synaptic inputs. In the first two cases, if there is only one inhibitory input which has a fixed spatial relationship to the excitatory input, a uniform reduction in the excitatory conductance change would result in a regular pattern of derecruitment. In the last case, in addition to the descending inhibition, inhibition from G.T.O.s (60Hz recruits G.T.O.s), Renshaw cells and PAD could interact in a more complicated way to result in the observed pattern of derecruitment. This implies that conductance changes are not the only determinants of decrecruitment, the interaction of IPSPs versus EPSPs have to be taken into account.

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1207 DORSAL COLUMN NUCLEI LESION ELIMINATES DISUSE OF DORSAL RHIZOTOMIZED FORELIMB. A.J. Berman, D.E. Teodoru,* T.A. Tran,* and A. Blau.* Dept. of Neurosurgery, V.A. Hospital, Bronx, N.Y. 10468

After unilateral dorsal rhizotomy in the monkey, the forelimb is not used purposively unless the intact limb is restrained or incapacitated. This movement deficit has variously been attributed to learned non-use, cross-spinal inhibition, sensory inattention, or, most parsimoniously, preference for use of the fully innervated limb. Results of the present study indicate a further possibility, that dorsal column and dorsal column nuclei are implicated in the processes underlying loss of function after dorsal rhizotomy.

Eight monkeys underwent dorsal rhizotomy C2-T3 (DR) and/or dorsal column (DC) section and/or dorsal column nuclei (DCN) lesion. The time between procedures was four to six months. The lesions were: in three animals, bilateral DCN lesions and DC section followed by unilateral DR; in two, bilateral DCN followed by unilateral DR lesion; in two, unilateral DCN lesion followed by bilateral DR; and, in one, unilateral DC section followed by bilateral DR. Animals were maintained in a 14' x 9' x 8' cage with wire mesh walls and narrow beams affording opportunities for ambulating and climbing.

In every case in which DR was combined with DCN lesion or DC section, use of the DR limb was seen from the first postoperative day. This was true when DCN lesion or DC section preceded DR and was also the case when DCN lesion followed DR. The limbs with the combined lesions were used for support, climbing, ambulating, reaching for food placed on the floor or hanging from the ceiling, and for bimanual tasks. The animals with bilateral DR used combined-lesion limbs to the exclusion of contralateral DR-only limbs. The limb with both DR and DCN lesion was also often preferred over the limb with DCN lesion-only for performance of tasks requiring fine digital coordination. This presumably was due to the poor coordination of fingers in the hand ipsilateral to DCN lesion. This lack of coordination was significantly alleviated by superimposed dorsal rhizotomy.

It would appear that, after DR, the dorsal column, dorsal column nuclei system exerts inhibitory effects on movement. Lesions of these pathways remove this inhibition, allowing return of motor function.

1208 CONTROL OF NECK MUSCULATURE BY TECTAL EFFERENT PATHWAYS. G. Bilotto, K. Fukushima, J. H. Fuller and B. W. Peterson. The Rockefeller University, New York, N.Y. 10021.

Mechanisms underlying head movements elicited by activation of the superior colliculus have been investigated by recording activity of neck muscles and reticulospinal neurons in precollicular decerebrate cats during microstimulation of the intermediate to deep tectal layers. Trains (duration 30-400 msec, rate 300-400 Hz) of 100 μsec pulses with amplitudes of 5-100 μA produced saccadic eye movements and excitation of contralateral dorsal and ventral neck muscles plus inhibition of corresponding ipsilateral muscles. Excitatory EMG responses of neck muscles typically consisted of an initial burst at a latency of 5-13 msec (median 8 msec) followed by rebound depression and then by tonic excitation which lasted throughout the train. The strongest responses were produced by stimulation at posterior points. Muscles with upward pulling directions (rectus major, biventer, complexus) tended to be more strongly activated from medial tectal points which also produced oblique upward eye movements while longus capitis, which has a downward pulling direction, was best activated from lateral points which also produced oblique downward eye movements. Responses of muscles with primarily horizontal pulling directions (splenius, obliquus capitis inferior) were less sensitive to the medio-lateral position of the stimulus point. The pattern of tectally evoked neck muscle activity is therefore consistent with the retinotopic organization of the colliculus.

Extracellular recording revealed that 21/75 medial (MRST) and 8/34 lateral (LRST) reticulospinal neurons had reciprocal responses to stimulation of the ipsi- and contralateral tectum which resembled those of neck muscles. Most of the remaining neurons were silent and failed to respond to stimulation. Latencies of contralaterally evoked discharge ranged from 2.5 to 10 msec with a median of 5 msec. Some neurons were activated for the entire duration of the stimulus train, others responded only at train onset or exhibited a combination of the two patterns. Discharge of these neurons is therefore appropriate to contribute to the responses of neck muscles to tectal stimulation. Such neurons were most prevalent among MRST neurons located in the periabducens region which is consistent with the idea that this region is an important center for organization of both eye and head movement components of horizontal gaze shifts. The same MRST neurons may participate in vestibulo-neck reflexes since a number of tectally activated neurons also responded to stimulation of contralateral semicircular canal nerves.

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1209 COMPRESSION EFFECTS ON THE COMPOUND ACTION POTENTIAL OF IN SITU SEVERED AND INTACT NERVES. L. Jack Bloom*, L. Donald Gilmore*, Carlo J. De Luca, (SPON: W. Bradley). Liberty Mutual Research Center, Hopkinton, MA, Children's Hospital Medical Center, Harvard Medical School, Boston, MA.

For the past six years our laboratory has been developing an implantable nerve electrode interface capable of recording neuroelectric signals from the surface of severed peripheral nerves. An investigation was undertaken to determine the extent of nerve injury resulting from compressive forces introduced in the surgical procedure and post-surgical trauma occurring when such an electrode is implanted.

Severed and intact peroneal nerves of New Zealand white rabbits were supermaximally stimulated and the compound action potential (CAP) was measured. The application of direct compressive force on a 3 mm length of nerve produced a corresponding decrease in CAP for both intact and severed nerves. The onset of these changes depended on the rate of force application. Experiments in which the force was rapidly applied exhibited a threshold value of 25 to 40 grams for both severed and intact nerve, above which further increases in force caused a rapid decrease in the amplitude of the CAP. The signal decreased to 50% initial value after application of 50 to 70 grams for the severed nerve and 90 to 100 grams for the intact nerve. Experiments in which a 20 gram sustained force was applied to severed and intact nerves produced similar decreases in the CAP during the initial 60 seconds of application. After that time a more pronounced decrease in CAP was observed in the severed nerve, with complete loss of the CAP observed at 25 minutes. A comparison of the 10 and 20 gram sustained force application showed a 50% decrease in CAP occurring at 2 minutes for the 20 gram weight and 60 minutes for the 10 gram weight. The initial positive phase of the CAP decreased in amplitude as a function of compressive force, indicating a decrease in the population of larger diameter fibers. The other phases of the CAP remain relatively unchanged throughout the course of the experiments. Control experiments indicate that ischemia and nutrient blockage seem to play a minor role in the decrease in the CAP during the observable period. The compressive force appears primarily to affect the structural integrity of the larger fibers. The rate and extent of the damage is dependent on the magnitude and duration of the compressive forces. The relatively low magnitude of the forces that cause injury requires that nerves be manipulated with care. These effects are more pronounced in a severed nerve.

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1210 DISTURBANCES OF BOTH CONTROLLED PREHENSION AND GRASP RELEASE FOLLOWING UNILATERAL ABLATION OF THE SUPPLEMENTARY MOTOR AREA. Daniel Bourbonnais*, Allan M. Smith and Gilles Blanchette*. Centre de Recherche en Sciences Neurologiques, Université de Montréal, Montréal, Québec.

The effects of unilateral supplementary motor area (SMA) lesions on the performance of a learned precision grip of the contralateral hand was study in fascicularis monkeys. Animals were first trained to exert an isometric force between the thumb and forefinger for a one-second duration to obtain a fruit juice reward. Finger pressures were measured by a hand-held strain gauge and a tone signal indicated to the monkey that the exerted pressures were within an upper and a lower force limit. On both rewarded and unrewarded trials the animals were required to release the grasp on the transducer in order to initiate the next trial. Pre-operative surface recordings from the forearm flexor and extensor muscles of the wrist and fingers indicated that the maintained prehension was accomplished by a co-contraction of these muscles. Immediately following ablation of the SMA a grasp reflex appeared in the contralateral hand which persisted from 2 to 3 weeks. Grasping was easily elicited by cutaneous stimulation of the volar surface of the hand and could be augmented by stretch of the finger muscles. The most striking disturbance of the conditioned precision grip was the animals' inability to release the force transducer from its grasp with the contralateral hand. In contrast, release from the ipsilateral hand remained normal. During conditioned prehension a significant increase in the rectified EMG activity of the forearm flexor muscles and a significant decrease in the forearm extensor muscles was found to evolve post-operatively. A corresponding increase in the rate at which prehensile force was applied to the transducer developed during the three week post-operative observation period. Moreover, the mean finger pressures exerted by the hand were greater than the mean pre-operative levels. Repeated elicitation could habituate the grasp reflex and allow the monkeys to release objects from the contralateral grip under voluntary control. Frequently, release of the manipulandum was preceeded by a sudden violent increase in prehensile force as if this strategy enabled voluntary opening of the hand either by habituation of the grasp reflex or by the facilitation of opposing reflexes. The extent of the cortical lesions in both monkeys has been histologically confirmed by serial section.

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1211 INTRAPPENDAGE EMG ANALYSIS OF SCORPION WALKING MOTOR PROGRAM. Robert Bowerman and Joel Butler*. Dept. of Zoology & Physiology, University of Wyoming, Laramie, WY 82071.

The neural control of arthropod locomotion has been extensively studied in insects and crustaceans, where considerable basic information about the structure and physiology of both nervous and muscular systems is available. A number of functional similarities between these arthropod walking control systems and those of vertebrates have been described. In contrast, little is known concerning arachnid systems. Even though the arachnid evolutionary line is distinct from that of the other arthropods, they face similar functional demands in terms of walking coordination, a similarity of functional solutions might be anticipated. With these thoughts in mind, the scorpion has been selected for study of the locomotory system, as a representative arachnid.

This report focuses on an EMG analysis of scorpion leg 4, from behaviorally important muscles during free-walking, forward motion. In addition to the EMG's the sensory discharge from two cuticular stress sensing elements, one in each tarsal claw, was recorded. These phasic receptors provide an accurate electrical record of timing of both swing phase initiation (tarsal claw burst cessation) and stance phase initiation (tarsal claw burst initiation). In this way, EMG's can be related to whole leg movements without the need for cinematographic analysis. The stepping relationship between legs,[1] and joint angle changes within legs[2] have been described previously.

The major EMG relationships between the elevator and depressor muscles of the trochanter-femur joint are described. These two muscles are primarily responsible for the movements of the swing and stance phases respectively. The following relationships will be reported. (1) EMG burst length vs. step cycle time, (2) integrated EMG burst vs. step cycle time, (3) phase relationships between elevator and depressor EMG bursts, (4) switching relationships between elevator and depressor EMG bursts, (5) timing between tarsal claw activity and elevator/depressor burst activity vs. step cycle time, and (6) tarsal claw burst length vs. step cycle time.

Also, during the stance phase the femur-patella and patella-tibia joints are extended by a single double extensor muscle but flexed by separate muscles.[3] The timing of motor outputs between these muscles will also be described.

[1]Bowerman (1975) J. Comp. Physiol. 100, 183-196.

[2]Root & Bowerman (1978) Comp. Biochem. Physiol. 59A, 57-63.

[3]Bowerman & Root (1978) Comp. Biochem. Physiol. 59A, 49-56.

1212 EFFECT OF HEMISECTION ON MOTOR DEVELOPMENT IN KITTEN HINDLIMB. Barbara S. Bregman and Michael E. Goldberger. The Medical College of Pennsylvania, Philadelphia, PA 19129.

At birth, immature proprioceptive placing is present in all directions, but is unreliable, high threshold and hypermetric. By 3 weeks, it is smooth and readily elicited. Tactile placing is seen at 22-23 days but is immature until 6 weeks when it is smooth, accurate and of small excursion. Monopedal hopping is present backward and lateral at birth, in all directions by 3 weeks. Vestibular response to drop is first seen at 3 days and is characterized by slight extension. By 24-26 days the mature response (extension, abduction and weight bearing) is present. Locomotor development reflects this reflex maturation. During week 1, the kitten pulls itself forward by the forelimbs. Weight bearing in the hindlimbs begins during week 2, when balance is poor; it improves by day 24. Locomotion is characterized by a wide based support with symmetrical abduction and external rotation at the hip. The foot's dorsum frequently drags along the surface at the beginning of swing until 21-23 days. Locomotion is wide based, ataxic, and over-deliberate until 6 to 7 weeks.

Kittens hemisected at T13/L1 on day 1 show initially (for 3-4 weeks) only a few, transient deficits. Ipsilateral proprioceptive placing and monopedal hopping are abolished or impaired for 1-3 days. These recover and then mature on schedule. Emergence of the immature vestibular reaction is delayed 3-6 days but its maturation is not delayed. During the first 3-4 weeks the hemisected kittens show a slight deficit in proximal fixation ipsilaterally. Then the kitten grows into some deficits, i.e. they don't appear until the 2nd month. They do not recover. Ipsilaterally, tactile placing is essentially abolished, although occasionally a gross placing response to tactile stimulus is elicited. Proprioceptive placing and hopping which had appeared to recover become abnormal (high threshold, hypermetric). As use of the hindlimb matures in the normal animal, locomotor deficits in the hemisected animal appear. Ipsilaterally, the dorsum of the foot always drags at the initiation of swing phase. The limb flexes hypermetrically and instability at the hip becomes even more severe. Reflex and locomotor development are integrated in both normal and hemisected kittens. Some reflexes are transiently impaired and then appear to recover. As the reflex normally becomes more dependent on higher control, recovery decompensates. Some reflexes and locomotor patterns are initially dependent on supraspinal control; they normally appear late in development. Hemisection arrests the development of these reflexes and locomotion reveals permanent immaturities.

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1213 EFFECT OF UPPER CERVICAL AFFERENTS ON VESTIBULOSPINAL TRACT NEURONS. Emily Brink*, Naoki Hirai*, and Victor J. Wilson. Rockefeller Univ., New York, N.Y. 10021.

To study a possible site of interaction between vestibular and neck afferents, we have recorded extracellularly from identified vestibulospinal tract (VST) neurons in Deiters' nucleus and in the descending nucleus of decerebrate cats while stimulating the C_2 ganglion and dorsal rami bilaterally. Like the vestibulooculaf (VOC) neurons studied by Hikosaka & Maeda (Exp. Brain Res. 18:512, 1973) many VST neurons, in both nuclei, were excited by stimulation of the contralateral C_2 ganglion; this excitation often had a latency < 6 msec (early excitation). In contrast to VOC neurons, VST neurons were typically not inhibited from the ipsilateral ganglion. Early excitation could be followed by late excitation, which was sometimes the only effect seen. In some neurons, mainly in the descending nucleus, only inhibition (latency 5-10 msec) was observed. Early excitation was due to proximal, perhaps joint area, afferents because stimulation of the dorsal (or ventral) rami almost invariably caused only late excitation. Both early and late excitation were seen in lateral and medial VST neurons that project only to neck segments, as well as in others that were excited antidromically from the cervical enlargement. In many cases the same neurons were excited at short latency by stimulation of the contralateral C_2 ganglion, and monosynaptically from the vestibular nerve (usually whole nerve, but sometimes canal nerve stimulation). The results show that interaction between reflexes evoked by neck and vestibular afferents and acting on neck and limbs may take place in the vestibular nuclei.

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1214 SUPRASPINAL AND SEGMENTAL INFLUENCE ON MEDIAL AND LATERAL LONGISSIMUS NERVE ACTIVITY IN RATS. Emily Brink* and Donald W. Pfaff. The Rockefeller University, New York, NY.

Medial longissimus (ML) is a proximal tail muscle, innervated by L_6-S_2 nerve branches. Lateral longissimus (LL) is a lumbar back muscle, innervated by dorsal rami along its length. Inputs to these muscles were studied in urethane-anesthetized female rats by recording from ML and LL nerves while stimulating ipsilateral lumbosacral dorsal roots (DR), medial medullary reticular formation (RF), vestibular nuclei complex (VN), midbrain central gray and areas lateral and ventral (CG area), and ventromedial hypothalamus (VMH). Stimulation of appropriate DR evoked short-latency (probably monosynaptic) compound potentials in ML (ave. latency 2.2 msec, n=40) or in LL (2.1 msec, n=14)nerves. Stable short-latency responses were more consistently obtained for ML (40/40 cases) than LL (10/17 cases). Generally, ML response required double shock (1.2-2 msec interval); LL response required 3-4 shocks. Supraspinal influence was studied using a condition-test paradigm. Subthreshold pulse trains of variable length (cathodal pulses, .1 msec duration,≤100 μA, monopolar) preceded subthreshold DR single shocks. For ML nerves, conditioning stimulation of RF (n=14), VN (n=15) or CG area (n=20) facilitated occurrence of short-latency segmental responses to single DR shocks. Conditioning by RF (requiring 1-5 shocks) appeared with condition-test intervals (Cti) of 0.5-2 msec. VN conditioning (5-12 shocks): optimum Cti 2-4 msec. Conditioning by CG (9-20 shocks): optimum Cti 5-20 msec. In the fewer LL nerves where stable segmental responses were obtained, similar facilitation by VN (n=4) and CG area (n=2) conditioning was seen. Suprathreshold RF, VN or CG area stimulation could provoke sizeable potentials in ML (n=3,1,2) and LL (n=3,1,4) nerves. Stimulus trains of up to 50 shocks, 200 μA applied to VMH never facilitated ipsilateral ML or LL nerve activity. Results indicate that excitatory paths from RF, VN, CG area to ML and LL exist and suggest some difference in spinal organization of ML and LL.

ML motoneurons localized by antidromic stimulation were found in L_6-S_1 spinal cord, posterior to the lumbar enlargement, ventrolaterally in the ventral horn. LL motoneurons were found medially in the ventral horn of the lumbar enlargement.

1215 MODIFICATION OF MOVEMENT-RELATED EMG ACTIVITY BY PERTURBATIONS APPLIED BEFORE ONSET OF VOLUNTARY HUMAN ARM MOVEMENTS. S. H. C. Brown* and J. D. Cooke, Dept. of Physiology, Univ. of Western Ontario, London, Canada.

A triphasic pattern of EMG activity has long been recognized in association with voluntary movements. This pattern consists of an initial burst of agonist activity followed sequentially by a burst of activity in the antagonist and a final period of agonist activity. The experiments described here were designed to investigate the interaction between this common pattern of EMG activity and imposed perturbations in limb position.

Experiments were performed on normal human subjects performing a visual step-tracking task using flexion/extension movements about the elbow. Torque pulse perturbations were applied randomly during trials and were timed to occur following the change in target position but before the onset of movement. Perturbations both opposing and assisting flexion were applied (flexion load and flexion unload forces).

The response to the force opposing movement appeared as a second peak of biceps activity occurring in the later phase of the first biceps burst. This peak was present in some subjects in the absence of perturbation. Changes in delay time of the perturbation relative to movement onset did not alter the latency of the response. Little change was seen in the other movement-related EMG activity except for an increase in the amplitude of the triceps burst. No EMG responses were seen corresponding to the M1 or M2 reflex responses. These reflex responses were, however, present when perturbations were applied following movement onset or when the subject was required to maintain a fixed position. Application of perturbations assisting movement (flexion unload) produced a decrease in this later component of the initial biceps burst.

The results suggest that for some period prior to movement onset, reflex systems and the systems generating the initial burst of agonist activity are inaccessible to peripheral modification.

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1216 EFFECTS OF VIBRATORY MUSCLE STIMULI APPLIED DURING VOLUNTARY HUMAN ARM MOVEMENT. C. Capaday* and J. D. Cooke, Dept. of Physiology, Univ. of Western Ontario, London, Canada.

Vibration of muscle tendons has been used by several investigators to study the "passive sense of position". In the present study the effect of such vibratory muscle stimulation on active arm movements has been investigated. Subjects performed a step-tracking task making alternate flexion/extension movements about the elbow. They were instructed to move 'briskly and accurately' between the target zones. All subjects were well practised at the task. No differences were seen in movement trajectories if the subjects performed the task with or without visual guidance.

A small DC eccentric motor enclosed in a plastic casing (weight approx 70 gm) was mounted on either the biceps or triceps tendon using adjustable Velcro straps. During continuous vibration of either tendon the amplitude and peak velocity of both flexion and extension movements were decreased. The changes were more marked in trials where the movements were performed without visual guidance (eyes closed). Similar changes in movement amplitude and velocity were seen when the vibration was applied just during the movements. Activity in reflex pathways was tested by application of torque pulse perturbations while the subject was required to maintain a fixed arm position. During vibration, of either tendon, short and long-latency reflex responses to the perturbation were markedly depressed in both biceps and triceps muscles.

The above results are consistent with activation of Ia muscle afferents by the vibration and with the results obtained from 'passive position sense' experiments. The results suggest that the effects of vibratory muscle stimulation on the parameters of active movements are produced by an action of muscle afferent input on supra-spinal structures.

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1217 SPINAL PROJECTIONS FROM THE MEDULLA OF THE ALBINO RAT. A.J. Castiglioni, Jr. and J.D. Coulter. Marine Biomedical Institute, Departments of Psychiatry & Behavioral Sciences and Physiology & Biophysics, University of Texas Medical Branch, Galveston, Texas 77550.

Horseradish peroxidase (0.5-1.0 µl, 40% solution) was injected into various levels of the spinal cord of adult albino rats. The brainstems were cut into 50µm sections and the neurons containing HRP were visualized by reaction with tetramethylbenzidine (Hardy-Heimer method). At the level of the pyramidal decussation retrogradely labeled neurons were found in a dense band arching from the region dorsal to the lateral reticular nucleus toward the central canal ipsilaterally, and contralaterally in small numbers in the ventral reticular formation (R.F.) following injections of the thoracic cord. Injections of the cervical cord produced many labeled cells predominantly medially and dorsally in the R.F., while lumbar injections yielded small numbers of labeled neurons in the ventral reticular formation. In the medullary R.F. at levels of the inferior olive, lumbar injections produced large numbers of labeled cells in the ventral R.F. overlying the inferior olive contralateral to injections. Cells labeled from the thoracic cord were found latero-dorsal to lumbar projecting cells, and cells labeled from cervical injections were found medially and dorsally in the R.F. contralateral to injections. Injections in all spinal levels labeled cells throughout the medial reticular formation ipsilaterally at these medullary levels. Small cells in the marginal layer of the spinal nucleus of V were labeled in caudal portions of the nucleus from injections of the upper cervical cord (C_1-C_3). Rostrally many large neurons in deeper regions of the nucleus were seen after injection of either upper cervical cord or cervical enlargement. Many small labeled neurons were found just ventral to the lateral tip of the inferior olive following lumbosacral injections, as well as occasional labeled cells lying among fibers of the pyramid. At caudal levels of the inferior olive many labeled cells were seen in the nuclei of the raphe after lumbar injections. At slightly rostral levels many cells are seen ventrally in the raphe, between the inferior olives, but relatively few were seen dorsally. A similar pattern is seen after both thoracic and cervical injections, however, the number of labeled cells is reduced. Many cells of the nucleus parvocellularis of the medullary R.F. were labeled after cervical injections, mainly ipsilaterally, and reduced numbers of cells in this region were labeled from injections into lumbar levels. These results indicate that spinal projections from medullary reticular structures are less diffusely organized than previously thought. (Supported by NS12481).

1218 AN ANALYSIS OF PRE AND POST SYNAPTIC MECHANISMS FUNCTIONING TO CONTROL TRIGEMINAL MOTONEURON ACTIVITY DURING SLEEP STATES. Scott H. Chandler*, Yoshio Nakamura* and Michael Chase (SPON: R.E. Hall). Departments of Physiology and Anatomy, Sch. Med., UCLA, Los Angeles, Calif. 90024.

In a previous communication (Chandler et al., Neuroscience abstracts, p292, 1978.) we have shown that the antidromic field potential recorded in the motor nucleus of the trigeminal nerve is depressed during active sleep as compared to quiet sleep. Additionally, we demonstrated that intracellularly recorded antidromic spikes elicited by masseter nerve stimulation were specifically blocked during the transition from quiet to active sleep. This was correlated with the onset of muscular atonia and membrane hyperpolarization. In this report we will present data indicating that these events are due to a process of postsynaptic inhibition of trigeminal jaw closer motoneurons during active sleep.

Intracellular recording from trigeminal jaw closer motoneurons with 2M K citrate micropipettes (8-15mΩ) during quiet and active sleep was performed in unanesthetized, fully conscious, head restrained cats. Antidromic spike potentials in masseter motoneurons were elicited by stimulation of the masseter nerve. The antidromic spike showed a 7% decrease in spike peak potential, a 40% decrease in after-hyperpolarization amplitude and a 20% increase in time to peak (TTP) as measured from the foot of the action potential to its peak during active sleep as compared to quiet sleep. It was also found that the monosynaptic EPSP induced in jaw closer motoneurons by mesencephalic V stimulation decreased 12% in peak amplitude, 13% in TTP, and 17% in half width during active sleep as compared to quiet sleep. Additionally it was found that only the peak amplitude of the EPSP decreased during rapid eye movement periods of active sleep (AS-REM) as compared to non-rapid eye movment periods of active sleep.

These results provide the most direct evidence to date that the spontaneous hyperpolarization that occurs during active sleep in trigeminal motoneurons is due, at least in part, to active postsynaptic inhibition of these motoneurons. Furthermore, the data suggest that the phasic inhibition of the masseteric reflex during AS-REM is due to presynaptic inhibition of group Ia muscle afferents from the masseter muscle.

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1219 SUBTHRESHOLD MEMBRANE ACTIVITY IN SPINAL CORD MOTONEURONS DURING ACTIVE SLEEP. Michael H. Chase and Francisco R. Morales*. Departments of Physiology and Anatomy and the Brain Research Institute, UCLA, Los Angeles, CA 90024.

During active sleep there is a decrease in the tone of the somatic musculature which occurs in conjunction with hyperpolarization of the membrane of α-motoneurons. Also, during this state, there are phasic increases and decreases in tone and reflex excitability. The present report describes lumbar α-motoneuron membrane potential changes which may provide the substrate for these phasic phenomena. A total of 35 antidromically identified motoneurons were examined throughout cycles of sleep and wakefulness, utilizing a technique for recording intracellularly in the chronic, undrugged, normally respiring cat (Morales and Chase, Intracellular Recording of Lumbar Motoneuron Membrane Potential during Sleep and Wakefulness, Exp. Neurol., 62:821-827, 1978). Records were obtained which satisfied the following criteria: (1) antidromic spike amplitudes greater than 60 mV (range 60-95 mV); (2) a stable membrane potential (greater than 50 mV) for more than 10 minutes; and (3) repetitive discharge following intracellular injection of depolarizing current. During active sleep the following three types of subthreshold membrane activity occurred which were predominant during bursts of rapid eye movements: (1) rapidly depolarizing potentials (3-5 mV; 10-30 msec); (2) slowly depolarizing potentials of large amplitude (4-8 mV, some 10 mV; 40-120 msec) occurring as rhythmic membrane potential oscillations; and (3) rapidly hyperpolarizing potentials (3-5 mV; 10-30 msec). Although rapidly and slowly depolarizing potentials were occasionally accompanied by bursts of spontaneous discharge, spike activity was rarely present. The fact that even large depolarizing potentials only occasionally promoted motoneuron spike discharge indicates the potency of the inhibitory influences acting on the final common pathway during active sleep. These data demonstrate the presence of considerable subthreshold membrane activity in spinal motoneurons during active sleep, especially in conjunction with rapid eye movement periods. We suggest that these subthreshold potentials may reflect the continuous descending excitatory and inhibitory influences that are responsible for the phasic motor events of active sleep.

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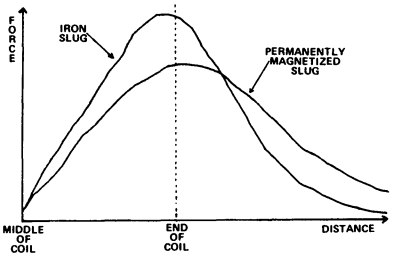
1220 ELECTROMAGNETIC MUSCLE STRETCH: TECHNICAL ASPECTS AND APPLICATIONS. T. R. Colburn*, J. R. Wolpaw, W. Vaughn*, and J. L. Christensen* (SPON: H. C. Lansdell). Res. Services Br., NIMH; Neurobiol. Dept., Armed Forces Radiobiol. Res. Inst.; Bethesda, MD 20014.

Use of an external solenoidal coil to apply force to a slug chronically implanted in the musculotendinous junction (Wolpaw & Colburn, Br. Res. 141:193, 1978) allows stretch of an individual muscle in an awake, intact animal without joint rotation or shortening of antagonist muscles. It is thus of value in studying the role of muscle stretch in performance (Wolpaw, Science 203:465, 1979). Coated iron slugs have been implanted for 3 mos in Rhesus monkey forearm muscles. The forearm passes through the center of the coil. The design goal is to maximize force on the slug and thus on the muscle and to minimize input power to the coil. Force exerted on a slug on the axis of a coil is dependent on slug length, cross-sectional area, material, and rate of change of the magnetic field. Rate of change of the magnetic field is determined by the location of the slug, by coil length, diameter, wire size, number of turns, and current. Force is always directed toward the middle of the coil. An iron slug gives a greater force peak, while a permanently magnetized slug gives a broader force peak. For the latter, slug orientation affects force amplitude. Slug location for peak force differs slightly, but for both slugs occurs near the end of the coil (Fig.). Coil inside diameter should be as small as practically possible, to maximize both force and frequency response. AC current can be used to deliver vibratory force, but should be rectified to reduce power consumption. An iron sleeve around the coil gives a 50% increase in force. Water cooling by means of copper tubing wrapped around the sleeve greatly reduces heating. Our coil measures 50 mm long, 42 mm i.d., 100 mm o.d., has 493 turns of #14 wire, an iron sleeve, water cooling (allowing a 5% duty cycle at maximum current for 1 hr), and a 40-amp, 25-volt power supply. It can exert a force of 420 gm (7-ms rise time) on a 2-gm, 20x5x3-mm iron slug which is easily implanted in any of several monkey forearm muscles.



Force vs distance from middle of coil for a soft iron (Armco) slug and a permanently magnetized (Alnico) slug.

1221 QUANTITATIVE SUBCORTICAL DISTRIBUTION OF EFFERENT PROJECTIONS FROM MOTOR CORTEX IN RAT. Robert C. Collins and Terry Der*. Dept. of Neurology, Wash. Univ. Med. Sch., St. Louis, Mo. 63110

Subcortical projection sites from motor cortex have been identified in many animals using electrophysiological or anatomical techniques. The relative distribution of efferent projections among all the different terminal fields has not been established. As part of an on-going study of the use of motor pathways during focal seizures it became important to know the site and relative density of first order projections from motor cortex.

^{14}C-proline alone, or mixed with ^{14}C-leucine (0.03 to 0.1 µl; 1µCi/µl) was injected through a 20 µm tip micropipette into the forelimb area of motor cortex of rat, 2-3 mm lateral and anterior to bregma. Animals were killed 24 hours later, perfused fixed with 3.3% paraformaldehyde buffered to pH 7.3 with 0.2 M cacodylate. Serial coronal or horizontal sections were cut at 20 µm at -15°C, picked up on cover slips and mounted on cardboard for exposure to Kodak SB-5 film. These were developed at 10-20 days to study major projections, then re-exposed for 1-3 months to enhance faint projections. Sections were subsequently stained with thionin to match autoradiographic densities with nuclear groups. A Leitz MPV microdensitometer was used to measure optical densities (OD). Cross sectional areas of terminal fields were measured planimetrically after enlargement and the volume of terminal fields computed. The quantity of projection to each subcortical nucleus was calculated as mean OD x mm^3.

The injection sites measured 1-3 mm in diameter, involved all cortical layers, and were confined to motor cortex. Over 50% of the total amount of subcortical projections ended in VA (26.5%) - VL (30.1%) complex. Ipsilateral (16.1%) and contralateral (2.9%) caudate/putamen terminal field densities occurred in a bi-laminar pattern in dorsolateral quadrants. The ventral medial n. (VM) received 11.5%, and the central lateral n. (CL) 8.5%. 1-2% of the total was found in reticularis, zona incerta, pretectal, and the posterior nuclear group each. Less than 0.5% appeared in central medial n. (CE), subthalamic n., the parafascicularis n. and the peri-rubral field each. In addition to caudate there were very faint projections across midline in CE, CL and VM.

1222 EYE MOVEMENT CONTROL: THE EFFECTS OF PAIRED SUPERIOR COLLICULUS AND FRONTAL EYE FIELD ABLATIONS. Janet Conway*, Peter H. Schiller*, and Sean True* (SPON: S. Corkin) Dept. Psyc., MIT Cambridge, MA 02139.

Ablation of either the superior colliculi (SC) or the frontal eye fields (FEF) produces relatively small deficits on eye movements. In both of these structures, however, single units discharge in association with saccadic eye movements and stimulation triggers saccades at low current levels and with short latencies. Recently it has also been shown that ablation of the SC does not interfere with stimulation elicited saccades from the FEF (Schiller, P.H. Brain Res 122: 154, 1977.). This finding suggests that these two structures form a parallel pathway to the brainstem for the control of saccadic eye movements.

To test this hypothesis, we removed, in successive stages, both the SC and the FEF in 10 rhesus monkeys, the majority of which had a search coil implanted for monitoring eye movements. The animals were trained to pick apple pieces out of randomly oriented slits in various locations in a plexiglass "apple board" which faced them. A minicomputer was used to measure saccadic and fixation parameters. Normal monkeys performed well on this task and the subsequent analysis disclosed a clustering of fixations at positions corresponding to the locations of the apple pieces on the board.

Removal of either the FEF or the SC alone produced only small deficits on the apple board task. FEF ablation yielded a temporary neglect of peripheral targets which completely disappeared 1-4 weeks after surgery. SC ablation alone reduced saccade frequency and target acquisition accuracy to some extent. By contrast, paired ablation of the FEF and the SC produced a dramatic deficit: monkeys made very few visually triggered saccades. The deficit was still profound more than a year after the operations. In spite of the fact that the animals were unable to direct their eyes appropriately, they attended to visual targets without looking, and cleared the apple board in the experimental situation.

The results suggest that the FEF and the SC form two relatively independent channels for the control of visually triggered saccades. Either pathway can subserve this function. A severe, non-recoverable deficit ensues only when both channels are disrupted. (Supported by NSF grant #BNS 76-82543 and NIH grants #5 R01 EY00676 and #1 T31 GM07484)

1223 ELECTROMYOGRAPHIC CONTRIBUTIONS TO REFLEX REGULATION OF MUSCLE PROPERTIES IN THE DECEREBRATE CAT. P. J. Cordo* and W. Z. Rymer (SPON: G. H. Collins). Dept. Physiol., SUNY Upstate, Syracuse, NY.

The stretch reflex response of the soleus (SOL) (n=4) and medial gastrocnemius (MG) (n=18) muscles was observed by recording tension and EMG from whole muscle and single motor units during imposed 5 mm/sec stretches. Whole muscle EMG was recorded intramuscularly, fully rectified, filtered and integrated; single unit EMG was also intramuscularly recorded, with fine-wire bipolar electrodes. During stretch responses, muscle force increased linearly while integrated EMG increased abruptly and then leveled off. We observed a rapid divergence of the force-EMG relations of isometric from that of lengthening muscle, indicating a transient reduction in the net capacity for force production shortly after stretch onset. This increase in activation is generated by reflexively induced motor unit recruitment and rate modulation.

Recruitment in order of increasing size and the sharp decline in numbers of newly recruited units with increasing force appear to be preserved in dynamically lengthening muscle. Force thresholds of motor units are increased in lengthening, compared to isometric muscle suggesting an overall broadening of the force range of the recruitment mechanism. In dynamically lengthening muscle, recruitment was observed throughout the force range obtained with MG stretch reflexes and at forces above 1 kg in SOL.

Rate modulation was observed to occur with three basic patterns during stretch reflex activation: 1) step increases in rate just after stretch onset, in motor units activated prior to stretch initiation, 2) doublets, in units activated both prior to and during stretch and 3) smooth increases in firing rate in units recruited during stretch. The mean slope of the force versus firing rate relation obtained for dynamically recruited units (smooth rate changes) in MG (n=102) was 2.0 pps/100 g increase in muscle force and in SOL (n=22) 0.2 pps/100 g. The mean±s.d. step increase in rate observed for previously active units was 3.7±1.8 pps in SOL (n=22) and 6.1±2.4 pps in MG (n=18). Motor units were observed to fire with doublets more frequently at higher initial forces, but the overall probability of doublet occurrence in all trials of all MG units (n=102) was 18%.

Although the lengthening SOL possesses a smaller dynamic range of recruitment and rate modulation than MG, it appears to be significantly greater than that previously reported (1). Additional EMG observed with lengthening, compared to isometric contractions is thought to represent reflex compensation for intrinsic nonlinear muscle properties. The most prominent compensatory mechanism is likely to be motor unit recruitment, a proposition supported by previously reported electrical stimulation studies (2).

(1) Grillner,S. and Udo,M. Acta Physiol. Scand. 81:571-573, 1971.
(2) Cordo,P.J. and Rymer,W.Z. Soc. Neurosci. Abstr. 4:294, 1978.

1224 THE DEPENDENCE OF MUSCLE TENSION ON STIMULUS INTERPULSE INTERVAL AND MUSCLE LENGTH. Patrick E. Crago. Case Western Reserve University, Cleveland, Ohio 44106.

Quantitative assessment of the contribution of temporal summation to total muscle force would be improved by the availability of an analytical model relating firing rate to force. Models that are linear in the frequency domain (Milner-Brown, Stein and Yemm, J. Physiol. 230, 371, 1973) are only applicable over restricted ranges of modulation because of the non-linear, sigmoidal relationship between force and firing rate. An analytical model has been developed that relates steady-state force to firing rate over a wide frequency range and that has an explicit dependence on muscle length.

The model was derived empirically from plots of steady-state force as a function of stimulus interpulse interval (IPI) at different muscle lengths. The data were from isometrically constrained cat soleus muscles activated by synchronous electrical stimulation of the whole muscle nerve or by distributed stimulation of ventral root filaments. Some data published previously by other investigators was also used.

The following analytical model describes the results:

$$F = FO\ (1+(a+bL)IPI)$$

where F is muscle force and L is length. FO, a and b are empirically derived constants.

At a fixed muscle length, force increases linearly with decreasing IPI, and the linearity is good from about 10% to 90% of the maximal force regardless of muscle length. The slope of the linear region (calculated as the linear regression of force on IPI) decreases approximately linearly with increasing muscle length. The intercept of the linear regression line on the zero IPI axis remains constant as length changes, expect near maximal physiological length. Maximal force, which is achieved at a small but non-zero value of IPI, varies only about 10% over a wide range of lengths (-20 to -4 mm with respect to maximum).

In practice, limits must be placed on the calculated value of F to insure that it does not exceed the maximal force (at short IPI's and long lengths) or go below zero (at long IPI's and short lengths). With these restrictions the model is reasonably accurate over a large portion of the physiological ranges of length and firing rate.

This model is currently being used in studies of force modulation under physiological conditions and in the design of control systems for orthoses employing electrically stimulated muscles. (Funded by NIH, NINCDS Contract Number NO1-NS-2-2314).

1225 DIFFERENCES IN THE REFLEX EFFECTS OF DIGITAL NERVE STIMULATION ON THE FIRING OF LOW AND HIGH THRESHOLD MOTOR UNITS IN HUMAN FIRST DORSAL INTEROSSEOUS MUSCLE. A.K. Datta* and J.A. Stephens* (SPON: A. Taylor) Sherrington School of Physiology, St. Thomas's Hospital Medical School, London, ENGLAND.

Previous observations in both cat and man indicate that the pattern of recruitment of motor units can be altered by cutaneous stimulation (1,2). During controlled ramp contractions of 1st dorsal interosseous muscle (1DI), for example, continuous stimulation of the digital nerves of the index finger raises the recruitment threshold of units normally recruited at contraction strengths <1.5N (6% maximum) and lowers the threshold of those normally recruited at strengths >1.5N. One explanation for this finding would be that cutaneous stimulation has an overall excitatory effect on high threshold units but an inhibitory effect on low threshold units. To investigate this hypothesis we have performed the following experiment.

Subjects were required to maintain a contraction of 1DI such that the motor unit under study fired steadily at 10pps. 80ms after every third unit action potential the digital nerves of the index finger were stimulated using single shocks at 3x threshold for perception. Histograms were then constructed of the interval between the two motor unit spikes following each stimulus. Given an afferent plus efferent conduction delay of 30ms the reflex effects of the stimulus begin some 10ms after the start of each measured interval. We can assume that if the overall reflex effect of the stimulus is excitatory then this will cause a shortening of the measured interspike interval. Conversely if the effect is inhibitory then we can expect the measured interval to be prolonged.

All units recruited at contraction strengths >1.5N had their measured interspike interval shortened by digital nerve stimulation. All these units had fast twitch contraction times (30-62ms). The excitatory drive to these motoneurones had been increased by the stimulus. The net reflex effect of cutaneous stimulation for these units was excitatory. In contrast all but two slow twitch (>75ms) low threshold units (<1.5N) had their intervals lengthened. The overall reflex effect of the cutaneous stimulus for these units was inhibitory. The behaviour of low threshold (<1.5N) fast twitch (<75ms) units was more mixed. 5 were excited and 8 were inhibited.

We conclude that the changes in the pattern of recruitment produced by cutaneous stimulation in man as in cat can be attributed to differences in the reflex connections of motoneurones which are related to motor unit mechanical properties (3).

1. Kanda, Burke & Walmsley. Exp Brain Res, 29, 57-54 1977
2. Garnett & Stephens. J Physiol, 282, 13-14P 1978
3. Burke, Jankowska & Ten Bruggencate. J Physiol, 207, 709-732

1226 [^{14}C]-2-DEOXYGLUCOSE UPTAKE IN MONKEYS WITH HYPOTONIC HEMIPLEGIA AFTER PRECENTRAL CORTICAL ABLATION. George Dauth, Sid Gilman, Kirk Frey*, John Penney*, Bernard Agranoff. Depts. of Neurol. and Biochem., Sch. Med., The Univ. of Mich., Ann Arbor, MI 48109.

Lesions of the precentral cortex in humans and infrahuman primates result in a contralateral hemiparesis, initially hypotonic and subsequently hypertonic. The anatomical substrates and mechanisms underlying these effects are unknown. We have investigated this problem in monkeys with ablations of the precentral cortex using the [^{14}C]-2-deoxyglucose (2-DG) autoradiographic technique of Sokoloff. The hypothesis is that uptake of 2-DG will reflect alterations in the metabolic activity in brain structures important in the genesis of hypotonia and hypertonia. M. fascicularis monkeys were rendered hemiplegic by ablation of areas 4 and 6. One week later, when the contralateral limbs were hypotonic and paretic, the animals were given 100 μCi/kg of 2-DG intravenously. Forty-five minutes later the animals were sacrificed; the brains were removed and sectioned for autoradiography and histology. The results show a marked depression in uptake of 2-DG in structures receiving projections from areas 4 and 6. This is evidenced in the autoradiographs by decreased grain density in the following nuclei ipsilateral to the lesions as compared to the same nuclei contralaterally: caudate nucleus, putamen, red nucleus, pontine nuclei, and thalamus. The findings implicate these nuclei in the pathogenesis of hypotonic hemiplegia after cerebral cortical ablation.

1227 ULTRASTRUCTURAL CHARACTERIZATION OF CEREBELLAR TERMINALS ON RUBROSPINAL NEURONS IN RAT. A COMBINED ELECTRONMICROSCOPIC STUDY USING ANTEROGRADE AND RETROGRADE AXOPLASMIC TRANSPORT, Jan J. Dekker, Section of Neuroanatomy, Yale University School of Medicine, New Haven, Ct. 06510.

Although the ultrastructural morphology of the rat red nucleus has been described (Reid et al., J. Comp. Neur. 162, 1975), no data is available on the site of termination and synaptoarchitecture of cerebellar input to this nucleus. In the present ultrastructural study, the synaptic terminals of the cerebellar nucleus interpositus and their postsynaptic targets in the red nucleus were identified by means of anterograde axoplasmic transport of ^{3}H-leucine and retrograde transport of horseradish peroxidase (HRP).

In four 6 week old albino rats ^{3}H-leucine was injected into the left nucleus interpositus and in the same animals HRP was injected into the left rubrospinal tract at a level posterior to the obex. After a one day survival the animals were perfused and the contralateral red nucleus was dissected out and cut in slabs 250 microns thick. The slabs were then incubated in a 3,3'-diaminobenzidine solution and subsequently osmicated, dehydrated and embedded in Araldite. One micron thick sections were cut from the polymerized blocks and processed for light microscopic (LM) autoradiography. The silver grain distribution and the position of the HRP-labeled neurons in these sections were used to guide the trimming of the blocks into pyramids. Subsequently, ultrathin sections were cut from these pyramids and processed for electron microscopic (EM) autoradiography.

In the sections prepared for LM autoradiography the majority of the silver grains were located above the caudal part of the red nucleus, which also contained many densely packed giant and large neurons with HRP granules in their cytoplasm. In the rostral, parvocellular part, a few HRP-labeled neurons, but almost no silver grains, were present. EM examination of the ultrathin autoradiographic sections cut through the magnocellular part of the red nucleus revealed that the bulk of the silver grains were located above myelinated axons and synaptic terminals, whereas only a few grains were present over other profiles such as glial and neuronal perikarya. The labeled terminals were almost exclusively large, elongated and electronlucent, and contained dispersed aggregations of round vesicles. They usually established multiple asymmetrical synaptic junctions with smooth parts of giant and large cell somas as well as with smooth parts of proximal dendrites. Furthermore, HRP granules were present in most of the giant and large neuronal perikarya and in many proximal dendrites which were in synaptic contact with labeled terminals. This indicates that axonal terminals from the cerebellar nucleus interpositus establish direct axo-somatic and axo-dendritic contact with both large and giant rubrospinal neurons. (Supported by NS 14841).

1228 EFFECTS OF SPINAL CORD STIMULATION ON SEGMENTAL REFLEXES IN MAN
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Neurophysiological follow-up studies are in progress in 15 patients with implanted systems for epidural spinal cord stimulation. These studies are based on evoked spinal cord potentials, somatosensory evoked potentials, tendon jerks, vibratory reflexes and polyelectromyographic recordings of spinal reflexes and their dependence on brain influence. The procedures, done initially in all patients, were repeated at approximately 3, 6 and 12 month intervals post-implant. Evaluation of the evoked spinal cord and somatosensory potentials, and the interaction of tendon jerks and vibration revealed no significant changes in the neurophysiological characteristics of the four patients re-evaluated by the time of this writing. However, there was a noticeable decrease in the electromyographic features of spasticity and evidence of improved suprasegmental control in these patients.

1229 FICTIVE LOCOMOTION IN THE STINGRAY, *DASYATIS SABINA*.
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Electromyograms (EMG's) show that stingrays locomote with a rostral to caudal wave of elevation and depression of the pectoral fins. At each segmental level the activity in dorsal and ventral musculature alternates. Increased swimming velocity, due to exteroceptive stimulation, produces decreased cycle times and increased EMG activity. Although the overall cycle changes, the duration of bursts retains similar phase relationships.

Animals restrained by chronically implanted vertebral clamps swim with the same cycle times and EMG patterns as freely swimming animals. Unlike dogfish sharks, stingrays decerebrated at the mesencephalic/diencephalic junction locomote spontaneously while animals with high spinal transections do not. The EMG pattern recorded from decerebrate stingrays is the same as that recorded from intact animals.

In this report, compound neuronal activity was investigated in the restrained, decerebrate preparation. A cuff electrode was attached to the peripheral nerve innervating the dorsal fin muscles. Simultaneous recordings were made from the cuff and from bipolar EMG electrodes in muscle of the same segmental level. Rhythmic bursts of neural activity were recorded corresponding to the EMG bursts during spontaneous locomotion. The cycle times measured from either neurograms or EMG's were approximately 0.9 s. This is within the range observed in different types of preparations. The animals were then immobilized with curare and artificially respired. The dose was sufficient to block both spontaneous EMG activity and EMG evoked by stimulation of the nerve in the cuff electrode. Following immobilization, spontaneous rhythmic discharges continued in the neurogram. If the discharges stopped, they could be elicited by exteroceptive stimulation or electrical stimulation of the rostral midbrain tegmentum. In all cases the rhythmic activity showed the same cycle times and durations as observed before paralysis. Stimulation of the midbrain with a continuous 50-60 Hz train was particularly reliable in eliciting rhythmic activity. Such stimulation also evokes normal locomotor movements in nonparalyzed, decerebrated animals. Rhythmic EMG or neurogram bursts often continued for several cycles after cessation of the stimulus.

The observation that normal locomotor rhythms can be recorded from the motor nerve after immobilization establishes fictive locomotion in this preparation. The decerebrated, immobilized stingray thus provides a good experimental model for further investigation of vertebrate locomotion.

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1230 REFLEX EMG ACTIVITY IN PARKINSONIAN PATIENTS.
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The myotatic reflexes of Parkinsonian patients were tested by applying pseudo-random torques about their elbow joints. Rectified, surface EMG activity was obtained from the biceps and triceps muscles. A description of the reflex motor output was provided by impulse response functions giving the average EMG response to a 20 ms pulse of torque.

Two populations of Parkinsonian subjects could be distinguished on the basis of these impulse response functions. The first group yielded responses similar to those for normal adult subjects. Subjects in this group were observed to have little rigidity. In general, their data could be fit by a simple linear model relating EMG activity to forearm position, velocity, and acceleration.

The EMG impulse response functions for patients in the second group were significantly different, in that they contained a large damped oscillation at a frequency of 10-12 Hz. This oscillation was not present in normal subjects or in the first group of Parkinsonian patients. Moreover, their frequency was much higher than that for typical Parkinsonian tremor (3-5 Hz). They were also observed to outlast any large transients in the impulse response functions for forearm velocity and acceleration. Patients in this group had a significant level of rigidity in their "off" state. However, the oscillations were actually greater during the "on" state, when rigidity had been effectively suppressed by L-Dopa.

These results confirm and extend previous work showing that patients with rigidity had exaggerated M2 responses. They suggest that: (1) While oscillations in the EMG impulse response functions could be correlated with the clinical observation of "off" state rigidity, rigidity itself was not directly responsible for them. (2) The oscillations appear to be triggered, rather than sustained, by kinematic feedback from peripheral receptors.

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1231 RECRUITMENT ORDER OF MOTOR UNITS IN THE FLEXION REFLEX OF THE ACUTE AND CHRONIC SPINAL CAT.
R.G. Durkovic and K.E. Misulis*. Dept. Physiology, Upstate Medical Center, Syracuse, NY 13210.

As part of an investigation of the physiology and behavior of the flexion reflex in spinal cat the present study was designed to test motor unit recruitment order in the tibialis anterior (TA) muscle. The experimental preparation was the unanesthetized decerebrate cat with a T-10 spinal transection made either two weeks before or immediately before decerebration. Single motor unit EMG was recorded by means of a fine needle electrode inserted into the belly of the TA muscle. Electrical stimulation of the cutaneous superficial peroneal or saphenous nerves was used to evoke reflex TA muscle activation. Cutaneous nerve stimulus intensities were below those required to activate cutaneous C fibers.

The reflex discharges of pairs of motor units were monitored simultaneously and recruitment order was determined either as a function of varying stimulus intensity or by determining the probability of firing at a constant stimulus intensity. In the flexion reflex of acute spinal cats recruitment order was generally in order of increasing EMG spike amplitude. Such a relationship was observed in over 75% of motor unit pairs with reversal in about 20% of the cases. These results are significantly different from chance. In contrast, recruitment in chronic spinal cats was very different with large EMG spikes often being recruited with a greater probability and at stimulus intensities lower than units with small EMG spikes (in over 55% of motor unit pairs). Recruitment orders for pairs of units determined using superficial peroneal nerve stimulation were, with few exceptions, the same as those determined using saphenous nerve stimulation for both acute and chronic spinal cats.

Studies from other laboratories indicate that a direct relationship exists between motoneuron size and EMG spike amplitude. If this relationship applies to the present experiments, the results suggest that the majority of TA alpha motoneurons are recruited in an orderly fashion (from small to large) in the flexion reflex of the acute but not the 2-week chronic spinal cat.

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1232 MUSCLE AND MOTOR UNIT PROPERTIES OF EXERCISED AND NON-EXERCISED CHRONIC SPINAL CATS. V.R. Edgerton, L.A. Smith*, and E.Eldred. Dept. Kinesiology, Anat. and Brain Res. Inst., UCLA, Los Angeles, CA. 90024.

The role played by motor unit activity in determining the biochemical and physiological properties of muscle is thought to be critical. Lower spinal transections have resulted in a transformation of both the histochemical profile of some fibers in soleus (SOL) muscle of guinea pigs (Karpati and Engel, Arch. Neurol. 17:542, 1967) and the contractile properties of the whole SOL in cats (Buller, et al, J. Physiol. 150:399, 1960). It has also been hypothesized that the shorter contraction time (CT) of the SOL, after cord transection, is due to a virtual elimination of motoneuron discharge (Gallego, et al, J. Physiol. 281:253, 1978).

To study the role of neuromuscular activity, eight 2-week old kittens and eight 12-week old cats were completely spinalized at approximately T_{12}. Four of the 2-week and five of the 12-week cats were exercised 20 min daily on a treadmill for a period of 3 mo. These cats were situated so that only the hind limbs touched the treadmill belt and locomoted at speeds ranging from 0.15 to 0.75 m/s. The transected cats differed greatly in the ability to bear the weight of their hindquarters, but all exhibited contraction of ankle muscles (Smith, et al, Neurosc. Abst., 1979).

The mean muscle to body weight ratio for the gastrocnemius of the exercised 12-week (12-EX) cats was 90% of normal cats, while it was 60% for the other groups. The SOL muscle to body weight ratio was 60% of normal in the 12-EX and 40% of normal in the other cats. Mean whole muscle CT of the SOL for the 2-week non-exercised (2-NE), 2-week exercised (2-EX), 12-NE and 12-EX was 60, 30, and 44 ms, respectively. For the medial gastrocnemius (MG), mean CT for the same groups was 31, 31, 52 and 43 ms. Mean CT for motor units of the SOL were 46 and 47 ms for the 12-NE and 12-EX cats (N=81). MG mean CTs were 33, 28 and 25 ms (N=88) for the 2-NE, 2-EX and 12-EX cats. Mean motor unit tetanic tensions were 21, 10 and 24g for the 2-NE, 2-EX and 12-EX cats, respectively. Some conversion of histochemically identified fiber types from a slow-to-fast twitch profile occurred in the SOL, but the percentage was not consistent and did not have the degree of conversion seen in the physiological properties. Fatigue properties of the SOL and MG motor units remained within normal ranges for all groups tested.

This data suggests that absence of activity cannot explain the shorter CT for the SOL after transection, since this occurs even when the motor units are involved in considerable amounts of activity during locomotion.

Supported by a grant from the Easter Seal Foundation.

1233 STRUCTURAL, HISTOCHEMICAL AND ELECTROMYOGRAPHIC ANALYSIS OF INDIVIDUAL COMPONENTS OF THE CALF MUSCLE COMPLEX IN CATS. Arthur Wm. English* (SPON: S. L. Wolf). Dept. Anat., Emory Univ., Atlanta, GA 30322.

The calf muscle complex of cats consists of five anatomically distinct components. Medial gastrocnemius (MG) and soleus (SOL) are both unipennate muscles which insert onto the medial and ventral parts of the tendocalcaneus, respectively. Lateral gastrocnemius consists of at least three unipennate components. Its lateral head (LG_l) is readily distinguished from an intermediate head (LG_i) by their fiber orientation and insertion. Its medial head (LG_m) shares a separate tendon of insertion with plantaris and is thus a single mechanical component supplied by two nerves. At the middle of the leg, myosin ATPase staining at pH 4.55 reveals that the components can also be differentiated histochemically. LG_l and MG consist primarily of fast twitch, glycolytic fibers (FG). Slow twitch oxidative (SO) and fast twitch oxidative-glycolytic (FOG) fibers are found less frequently. Soleus contains almost exclusively SO fibers and those fast twitch fibers found are FOG. LG_m and LG_i lie intermediate; both contain 30-50% SO fibers and 10-20% FOG fibers. To begin to determine whether these individual components may subserve different functions, electromyograms were recorded from each of the components during repeated overground stepping trials in each of six adult cats. The onset, duration and intensity of EMG activity was analyzed for each with respect to individual step cycle elements, as determined from synchronous high speed cinematography. All of the muscular components examined showed a similar pattern of temporal spacing of EMG activity. Activity begins during the first extension epoch (E_1), prior to foot placement, continues throughout the yield or E_2 epoch and most of the following E_3 epoch, and terminates just prior to the removal of the foot from the ground. Modulation of the temporal patterns of activity of each of the components with changes in stepping frequency was similar. EMG intensity during each epoch, in each muscular component, as determined from rectified and integrated signals, generally increased with increases in stepping frequency. Differential increases in these intensity measures were noted, but not of the same order as has been reported for peak EMG amplitudes (E. G. Smith et al, J. Neurophys. 40:503-513, 1977). It is concluded that the individual muscular components may make differentail contributions to the overall force at the tendocalcaneus which reflect a division of labor in the complex. Supported by Grant AM19916-02.

1234 AXON COLLATERALS OF CAT MEDIAL AND INFERIOR RECTUS MOTONEURONS. C. Evinger*, R. Baker and R.A. McCrea (SPON: J.A. Zadunaisky). Dept. Physiol. & Biophys., New York Univ. Med. Ctr., 550 First Avenue, New York, NY 10016

Unlike the findings in the mammalian spinal cord, there has been neither anatomical nor physiological evidence supporting the presence of axon collaterals from cranial motoneurons. In particular, extensive electrophysiological investigations of extraocular motoneurons have failed to detect recurrent synaptic effects. Nevertheless, intracellular injections of horseradish peroxidase into ten medial and six inferior rectus motoneurons has demonstrated the existence of axon collaterals in every case. In seven cats, both the dorsal and ventral, as well as rostral and caudal subgroups, of these motoneurons were examined. Identification was based on antidromic activation from the isolated medial and inferior rectus nerves and the neurons' response to vestibular nerve stimulation. Reconstruction of stained motoneurons showed that each cell had somadendritic trees in excess of 1 mm in diameter with inferior rectus being slightly larger. The dendrites of these cells always extended into the periaqueductal gray and often into the MLF, but seldom crossed the midline. One or more collaterals emanated from the parent axon of every injected motoneuron. Even though these collaterals exhibited extensive branching, they all terminated within the ipsilateral oculomotor nucleus less than 1 mm from the parent soma. The terminals of the axon collaterals were large, averaging 3.5 μ (± 1.5, n = 114 medial rectus terminals; major and minor terminal diameter/2). Despite their extensive branching and termination within the oculomotor nucleus, there was neither morphological nor electrophysiological evidence for recurrent synaptic contact with any extraocular motoneuron. These results suggest that the collaterals of medial and inferior rectus motoneurons terminate on oculomotor internuclear neruons. This suggestion is supported by the observation that non-motoneurons within the oculomotor nuclei exhibit recurrent synaptic activation following IIIrd nerve stimulation. Thus, the efferent signals to other brainstem nuclei from the oculomotor internuclear neurons could, in part, be similar to that arriving at the extraocular muscle. This organization contrasts with that of the abducens nucleus where motoneurons and internuclear neurons exhibit qualitatively identical discharge patterns, but in which neither the motoneurons nor internuclear neurons have recurrent axon collaterals. Supported by USPHS Grants EY-02007, NS-13742 and Fellowships NS-05857 and NS-06163.

1235 **SHORT TIME SCALE CORRELATIONS BETWEEN DISCHARGES OF MEDULLARY RESPIRATORY NEURONS.** J.L. Feldman, D. Sommer* and M.I. Cohen. Dept. of Physiology, Albert Einstein College of Medicine, Bronx, N.Y. 10461 and Depts. of Physiology and Anesthesia, Northwestern Univ.,Chicago, Ill. 60611

The interactions on a short time scale among inspiratory (I) neurons and among expiratory (E) neurons of the cat medulla have been studied by analyzing simultaneous recordings of spike activity from two or more neurons. Microelectrodes were used to record activity in three regions: a) the ventrolateral nucleus of the solitary tract (vlNTS), containing I neurons almost exclusively; b) the rostral portion of the ventral respiratory group (rVRG), in the ventrolateral medulla rostral to the obex containing mostly I neurons; and c) the caudal portion of the VRG (cVRG), containing mainly E neurons. Crosscorrelation histograms (CCHs) between activites of two I neurons or two E neurons were used to ascertain the existence of correlated discharges on a short time scale (ca. 1 msec), as indicated by a sharp peak near zero lag in the CCH. Adjacent neuron pairs (recorded with the same electrode) have a high incidence of such correlated discharge: they were present in 16/26 pairs of I neurons and 28/45 pairs of E neurons. In contrast, such correlations were extremely rare for pairs of distant neurons (recorded with two microelectrodes on opposite sides of the medulla): only 1/43 I neuron distant pairs had a clear cut peak in the CCH and none of the 97 (0/97) E neurons distant pairs had such a peak. However, 18/43 I neuron distant pairs had common high-frequency oscillations (HFOs), with period 9-17 msec. These results indicate that: a) within local clusters of I or E neurons there is a high incidence of local interactions and/or locally shared inputs; b) only a small fraction of distant neuron pairs are oligosynaptically connected. Since respiratory neurons of a particular discharge type are ultimately connected, as indicated by synchronization of firing on a time scale of seconds or tens of milliseconds (HFOs), the coordination of activity between distinct regions is probably produced by limited and specific connections between specialized subpopulations. (Supported by N.I.H. Grant HL-20800).

1236 NEURONS INVOLVED IN PEDAL WAVE GENERATION IN APLYSIA. Steven M. Fredman and Behrus Jahan-Parwar. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

In our previous work on pedal locomotion in Aplysia californica, we have found that the motor program is centrally generated and that the neuronal circuitry for the pedal waves which drive locomotion appears to reside in the pedal ganglia. In the present study, we have examined pedal ganglia neurons using a semi-intact preparation. Simultaneous intracellular and extracellular recordings have permitted identification of neurons with axons in the pedal nerves. Two different classes of presumptive motor neurons have thus far been identified: i) neurons which fired bursts of spikes during the rising phase of the pedal contraction wave; ii) neurons which were inhibited during the rising phase of the wave, but fired during the falling phase of the wave. Physiological stimuli which elicit locomotion such as seaweed extract applied to the anterior tentacles increased the frequency of the pedal waves. Correlated with this was a change in the timing of the motor program with neurons in both classes exhibiting an increase in burst frequency and increased spike frequencies within each burst. The timing of neurons within each class relative to each other remained essentially constant. For neurons with axons in the same nerve, no synaptic connections have been observed either among neurons of the same class or between neurons in the different classes. Some neurons which fired during opposite phases of the pedal wave had common synaptic input, but of opposite signs. These data suggest that the coordination of the motor neurons is maintained by interneurons rather than by interconnections among themselves.

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1237 VESTIBULAR AND NECK REFLEXES IN CONSCIOUS RABBITS. James H. Fuller. Biol. Sci. Grp., U-42, Univ. Connecticut, Storrs, CT 06268

Rabbits were chronically prepared with head holders and DC oculogram electrodes and rotated about the vertical axis. Three reflexes were examined. First, during sinusoidal rotation in the horizontal plane without vision and with the head fixed to the platform, a torque produced in that plane mimics the eyes nearly perfectly, with counterrotating and resetting occurring consistently and in coordination with the eyes. If the animal's head is freed to move in the same plane, the resulting head movements (vestibulo-collic reflex, VCR) partially null the platform rotation (gain of 0.2 to 0.9). Second, if the head is mechanically (passively) stabilized in space and the body rotated beneath it, the collico-ocular (neck-eye) reflex (COR) results: a rotation of the body to the animal's left (i.e., a rightward deviation of the animal's neck) is accompanied by rightward eye movements (gain of about 0.1); thus, a self-initiated rightward movement of a free head on a stationary trunk would be accompanied by a rightward eye movement—or, opposite the vestibulo-ocular reflex (VOR)—as noted by Gresty (Acta Otolaryngol. 81: 386, 1976). Third, during rotation with a free head, all three reflexes ought to interact: a leftward platform movement results in a rightward head movement (VCR), a rightward eye movement due to neck deviation (COR), and a vestibularly-induced rightward eye movement (VOR)—the amount of eye movement is determined by the gain of the VCR, with the COR proportional and the VOR inverse to the magnitude of the VCR. If the animal's head is suddenly fixed to the rotating platform by an electronic brake (eliminating neck deviation), in principle, the COR is also eliminated. The vestibular component of gaze is not altered; rather, it is shifted wholly to the VOR. Elimination of the COR ought to bias the gaze towards platform movement: no such change was seen. Furthermore, during continuous rotation, the VCR may vary in gain between 0.2 to 0.8 over 1/2 min; this variation in neck deviation should affect the gaze by varying the COR. It does not.

In conclusion: 1) measurement of the COR by passively fixing the head in space is an artificial paradigm since neck muscles actively shorten and lengthen to stabilize the head in space and can only rarely be accomplished by the animal; 2) fixing the head to a rotating platform may not measure the VOR alone since the animal is isometrically contracting its neck muscles (revealed by head torque measurements), which may co-vary with the COR; 3) whatever the origin of the COR, it cannot be viewed as an universal reflex that assists or resists the VOR—it can do either, contingent on whether the animal's head movement is independent of or dependent on body movement. Its importance in intact animals is moot.

1238 SYNAPTIC EVENTS IN THE MEDIAL RECTUS MOTONEURONS DURING VESTIBULAR NYSTAGMUS IN THE CAT. Nobuhiko Furuya*, Yoshikazu Shinoda* and Jun-Ichi Suzuki*. (SPON: M. Maeda). Dept. of Otolaryngology, Teikyo Univ. Sch. of Med., Tokyo Japan

Intracellular recordings from the medial rectus motoneurons were made during vestibular nystagmus in encéphale isolé cats under local anesthesia. Repetitive electrical stimulation of the ipsilateral vestibular nerve produced slowly increasing nerve discharges followed by abrupt cessation of activity in the medial rectus nerve. In accord with the rhythmic change of the medial rectus nerve discharges, the membrane potential of medial rectus motoneurons showed a slowly increasing depolarization followed by a fast and then a slow hyperpolarization. The onset of the hyperpolarization coincided with that of the abrupt cessation of the nerve activity. After electrophoretic injection of Cl^- ions into the cell, the earlier fast hyperpolarization was not affected, but the later slow hyperpolarization was reversed to a depolarization. Mean rising slopes of the earlier fast hyperpolarizations before and after Cl^- ion injection were not significantly different in the motoneurons whose resting membrane potentials and frequencies during nystagmus were stable. Application of depolarizing current through the recording electrode filled with 2M K-citrate revealed a reduction of amplitude in the fast hyperpolarization; application of hyperpolarizing current resulted in an increase in amplitude.

These results indicate that disfacilitation is primarily responsible for the quick cessation of the medial rectus nerve activity; contributions of IPSPs to the fast hyperpolarization must be very small, if present at all. This pattern of activity differs from the synaptic events in the abducens motoneurons during vestibular nystagmus in which not only disfacilitation but also IPSPs are involved in the fast hyperpolarization. Clear evidence of IPSPs during the later slow hyperpolarization of medial rectus motoneurons indicates that post-synaptic inhibition does contribute to this phase of activity, however.

1239 INPUT RESISTANCE OF SPINAL MOTONEURONS DURING SLEEP. L.L. Glenn and W. C. Dement. Dept. Psychiatry, Sch. Med., Stanford Univ., Stanford, CA. 94305

During wakefulness and non-rapid-eye-movement (NREM) sleep, a background of continuous muscle tone exists in postural muscles. During REM sleep, all postural tone and electromyographic activity ceases except for occasional myoclonic jerks. After an analysis of central root recurrent discharge in combination with spinal cord sections, Gassel, Marchiafava and Pompeiano (1965) proposed that motoneurons are inactivated because of an increase in postsynaptic inhibition. In the present communication, we report the first confirmation of this hypothesis based upon measurements of motoneuron input resistance during wakefulness and the two phases of sleep. Seven cats were implanted for the recording of cortical, geniculate, ocular, and dorsal neck muscle potentials. The vertebrae over the lumbosacral cord were fused and prepared for later ventral horn microelectrode penetrations. After recovery from surgery, sleep deprivation, in combination with gradual adaptation to combined cranial and lumbar restraint, enabled the intracellular recording of motoneurons in restrained, but naturally sleeping and waking cats. Input resistance was measured by the passage of constant current pulses (-25nA to +25nA) through one barrel of a double micropipette (1-2 micrometers, potassium acetate) while recording voltage with the other. The input resistance of 11 motoneurons during NREM sleep ranged from 0.42 to 8.7 megaohms. Using NREM sleep as the baseline state, motoneuron resistance decreased a mean 67% ($p<0.01$) upon entering REM sleep. Wakefulness resistance averaged 106% of NREM levels (N.S.). Hyperpolarizing currents were passed through the microelectrode during the transition between NREM and REM sleep. In most motoneurons, the membrane depolarized towards an inhibitory equilibrium potential as the cat entered REM sleep. The diminished input resistance and reversal of the REM sleep hyperpolarization directly confirms the hypothesis that motoneurons are inactivated as a result of increased postsynaptic inhibition. Furthermore, this suggests that the synapses responsible for the inhibition terminate upon the motoneuron soma.

Gassel, M.M., Marchiafava, P.L., and O. Pompeiano. Arch. Ital. Biol. 103:25-44, 1965.

1240 ORIGINS OF CORTICAL AND CEREBELLAR PROJECTIONS TO THE RED NUCLEUS IN THE CAT. R. Gold, D.J. Reed*, M. Rowinski and D.R. Humphrey, Lab. of Neurophysiol., Emory Univ. Sch. Med., Atlanta, GA 30322.

The projections to the feline red nucleus (RN) were defined with retrograde labeling techniques. Injections of horseradish peroxidase (Sigma VI, 30-50 % solution, 0.05-0.2 µL) were made bilaterally into the RN of 9 animals. After a survival time of 48 hr, the brains were perfused, removed and reacted either with DAB, preceded by cobalt chloride (N=8; cf. Adams, Neurosci., 2: 141), or with TMB (N=1).

Labeled corticorubral (CR) cells were found in areas 4 and 6, with a smaller but consistent population in area 5. Very few labeled neurons were found in areas 3a, 3b, 1, 2 and 7. Cells within the distal forelimb region of area 4 were labeled only by injections into the rostral one-third of the RN. In contrast, cells were labeled in the proximal forelimb region of area 4 and in area 6 by injections in all parts of the nucleus; these areas included regions on the medial wall of the hemisphere and in both banks of the presylvian sulcus, which are thought to correspond, respectively, to the supplementary motor area and the frontal eye field. Injections into the caudal one-third of the RN also labeled cells within the hindlimb region of area 4. All labeled cells were pyramidal in shape, with the modal transverse soma diameter being 15 µm in areas 4 and 6, and 17-20 µm in area 5.

Injections into the caudal two-thirds of the RN labeled cerebellar cells principally within the contralateral nuc. interpositus (IP). In contrast, rostral RN injections labeled cells principally in the contralateral dentate nuc., although they were much fewer in number than the labeled IP neurons seen following more caudal injections. Labeled neurons were also seen in the entopeduncular nuc., in or near the VL nuc., and in the dorsal column nuclei.

These results indicate that the input to the RN originates principally from cortical areas 4 and 6, and from the IP and dentate nuclei of the cerebellum. Previous studies in this laboratory have defined similar origins for cortical and cerebellar projections to the RN in the monkey. CR neurons in the monkey are, however, smaller and more superficially located than is the case in the cat. The receptive field properties of corticorubral neurons are currently under investigation. (Supported by NIH Grant NS-10183).

1241 SPECIFICATIONS FOR THE VESTIBULOOCULAR TRANSFER FUNCTION: PHASE DATA FROM IDENTIFIED OCULAR MOTONEURONS IN CAT. Jefim Goldberg* and Edward L. Keller. Dept. of Electrical Eng. and Computer Sci., Univ. of California, Berkeley, CA 94720.

The frequency response of the vestibuloocular reflex (VOR) pathways has been studied in the decerebrate or anesthetized cat and monkey, and more recently, in the alert, intact monkey. These pathways include the vestibular end organ, primary vestibular neurons, pools of central, brain stem neurons, ocular motoneurons, and the oculomotor plant (globe, eye muscles, and other orbital tissues). During head rotations these pathways must provide a total of 180° of phase lag with respect to head accelerations in order to produce compensatory eye movements. Part of this phase lag, particularly at higher rotational frequencies, is generated by the dynamics of the oculomotor plant.

In the decerebrate cat the phase lag of vestibular nucleus neurons during sinusoidal rotations of the head (at frequencies above 0.1 Hz) is similar to that observed in the monkey. On the other hand, the response of abducens motoneurons (ABN) in the decerebrate cat lags head acceleration by a significantly smaller amount than in the alert monkey. Thus at a representative frequency of 0.25 Hz, it has been reported that ABN in the cat lead compensatory eye movements by about 70°. In the alert monkey discharges of presumed ABN show a phase lead of about 18°. To explain the large difference one would have to postulate an oculomotor plant in cat that is an order of magnitude slower than that in the monkey. This does not seem reasonable. Another alternative is that the units previously recorded in the monkey abducens nucleus, and not antidromically identified, are not all motoneurons and the sample is biased toward shorter phase-lead neurons.

To answer this question, we made phase measurements of neural discharge in alert, chronically implanted cats. Discharges of antidromically identified ABN were recorded while the animals were being rotated at frequencies of 0.1-1.5 Hz. At 0.25 Hz, motoneurons lead eye position by 24° on the average. Abducens nucleus units that are not driven antidromically from the 6th nerve exhibit an average lead of about 33°. Both types of neurons increase these phase leads during states of lowered alertness.

The 24° average phase lead for the alert cat ABN approximates the reported 18° monkey phase lead. The 70° lead reported for decerebrate cat motoneurons is most likely due to the preparation and its state of alertness.

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1242 HIERARCHICAL CONTROL OF LOCOMOTOR RECOVERY FROM THE EFFECTS OF DORSAL ROOT LESIONS. Michael E. Goldberger. Department of Anatomy, The Medical College of Pennsylvania, Philadelphia, PA 19129.

Recovery of motor function may be due not to the mass action of all undamaged pathways but to a controlled process in which the contribution of each pathway is somehow ranked or weighted; i.e. the recovery is regulated hierarchically. If this explanation were correct, one should be able to demonstrate that one pathway is more important than another in recovery after a particular lesion. The role of descending systems in recovery was assessed (using hemisections) after ipsilateral lumbosacral (l-s) deafferentation in which: 1) all l-s roots were cut, or 2) the L6 root was spared (spared root preparation) and were also compared with the effects of hemisection alone. Cats performed several food-rewarded locomotor tasks involving timed traverse of 9 ft. long runways. These included an obstacle course, boards with randomly placed holes of various sizes, pairs of one-inch wide bars with variable distance between them, 2" wide runways and 12" wide boards which were used as controls for locomotor speed. The more difficult tasks lengthened the time of a traverse in normal and lesioned animals by increasing the number of 'mistakes' the animals made (i.e. by requiring greater accuracy). Therefore, measurements of speed were used to indicate degree of impairment and course of recovery from it. L-S rhizotomies sparing L6 produced a mild deficit on all runways except the 12" wide board on which no deficit was seen. Recovery began when tactile placing returned at 7 days and was "complete" by 6 weeks. In another group of cats, L1 hemisection produced a severe deficit which began to show recovery on the 12" board on d2 and on the other runways on d8. Recovery was "complete" by 6 weeks. When ipsilateral hemisection was added in the recovered spared-root animals, a severe deficit resulted which also showed recovery on the 12" board beginning d2 and on the other runways beginning on d8 when proprioceptive placing returned. Recovery was "complete" by 6 weeks. In contrast, hemisection made in completely deafferented cats abolishes all recovery permanently. The recovery in deafferented cats is therefore dependent on the ipsilateral descending systems, but recovery in spared root cats may be independent of these systems. Since sparing a root (Goldberger and Murray 1978) also: 1) blocks sprouting of descending systems and 2) prevents the development of hyperactivity of descending (e.g. vestibulospinal) reflexes both of which are seen in deafferented cats, the results suggest: a) an inequality in importance of the spared root and descending systems in mediation of recovery and that b) recovery and sprouting show similar responses to a particular combination of lesions.

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1243 ABSENCE OF CROSS-AXIS PLASTICITY IN THE VESTIBULO-OCULAR REFLEX. Lionel O. Greene, Jr.* and Laurence R. Young. MIT, Department of Aero/Astro, Center for Space Research, Cambridge, MA 02139.

The vestibulo-ocular reflex (VOR) plasticity shown following the wearing of optically reversing prisms represents a change in gain in afferents originating in semicircular canals lying in the same anatomical planes, but not a transfer of innervation to the extra-ocular musculature from canals situated in differing orientations. The vestibular response to visual inputs requiring compensatory ocular movements across the semicircular canal planes was monitored in the present experiment by fitting two rhesus monkeys with dove prism spectacles that caused vertical visual field movements for horizontal head motion and horizontal visual field motion with vertical head movements. VOR gains (eye velocity/head velocity) in the horizontal and sagittal planes were assessed during passive trapezoidal oscillations (accelerations to 30,45,60 and 90°/s, 25 s constant angular velocity) about yaw (G_z, earth vertical) and pitch (G_y, aural) axes. Tests were conducted in both the light and dark.

OPTOKINETIC NYSTAGMUS. Compensatory eye movements were generated during G_y and G_z accelerations to 30 and 45°/s following 10 days of testing. Visual suppression of vestibular cues was incomplete during accelerations to 60 and 90°/s, as the gain was always less than 1.0 (range: 0.0-0.9); nor was the direction of ocular motion always orthogonal to the rotational plane. There were no differences for G_y or G_z accelerations.

VESTIBULAR NYSTAGMUS. The inability of the oculomotor apparatus to evoke saccades across the planes of semicircular canal orientation has been demonstrated, thus delineating a limit of VOR plasticity. After 15 weeks of 90° rotated vision:

GAIN PER ACCELERATION			POST ACCELERATION	
Rotation	HORIZONTAL	VERTICAL	HORIZONTAL	VERTICAL
yaw	0.3 of normal	none	0.5 of normal	none
pitch	none	0.4 of normal	none	0.6 of normal

To show that these results were valid and not due to an abnormal subject population, reversing prisms were fitted to the test animals and the horizontal and vertical VOR's monitored. Trapezoidal accelerations were used as test stimuli in addition to sinusoidal accelerations at 0.1 Hz, ± 140°.

GAIN/PHASE PER ACCELERATION			POST ACCELERATION	
	HORIZONTAL	VERTICAL	HORIZONTAL	VERTICAL
yaw	non-reversed; 0.4 of normal 25-45° phase lag	-	reversed;	-
pitch	-	non-reversed; 0.7 of normal 20-40° phase lag	-	non-reversed; 0.7 of normal

These results imply asymmetries in central nervous responses to accelerations about the horizontal and sagittal planes, the bias possibly originating in the otolith end-organ/macular structures.

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1244 VIBRATORY DECREMENT IN THE STRETCH REFLEX. Z. I. San and W. Z. Rymer. Upstate Med. Cent.,Syracuse,NY 13210 and Nortwestern Univ. Sch. Med.,Chicago,Ill. 60611.

The stretch reflex is known to be weaker in the presence of vibration superimposed upon the stretch than in its absence. The vibratory decrement in the stretch reflex is attributed to the occlusion of the stretch response of the primary endings in the presence of vibration. We have examined the question of whether the vibratory occlusion of the primary ending can explain the dynamics of the decrement in the stretch reflex. Ten experiments were carried out on decerebrate cats; the force, the EMG and the discharge of a primary ending pertaining to either the soleus or the medial gastrocnemius were monitored. Applying the same ramp stretch repetitively, a control series of averaged responses (force and EMG) was recorded for different values of pre-stretch force. (The latter was varied via the crossed extensor reflex.) Low amplitude vibration selected in order to synchronize the discharge of the primary ending was applied either (a)before and during the stretch, or (b)only for a period ending prior to the stretch. The force and EMG responses were compared with those interpolated from the control series according to pre-stretch force. We found that the responses in the presence and also in the aftermath of vibration were smaller compared to the appropriate controls. The decrement could not be explained simply by the occlusion of the primary endings' discharge. For instance, in paradigm (b) the decrement could be pronounced even though there was no occlusion of the primary ending's response. The time course of the development (or cessation) of the decrement in the stretch reflex was of the order of hundreds of milliseconds after initiation (or termination) of vibration. We found no comparable effect of preceding vibration upon the monosynaptic reflex evoked by tendon jerks. This is evidence against the involvement of presynaptic inhibition. The results point toward the possibility of polysynaptic circuits at the spinal level that respond with considerable lag to the Ia afferent signals.

1245 Skeletal Muscle Electrolyte, Water, and Lipid Concentrations in an Inherited Canine Neuromuscular Disorder. G.A.Hegreberg and M. J. Hamilton*, Dept. Veterinary Microbiology and Pathology, Washington State University, Pullman, WA 99164.

An inherited canine neuromuscular disorder is characterized by general muscular weakness, muscular atrophy, and an intolerance to cold and exercise stress. The disorder is inherited as an autosomal recessive trait and is clinically apparent at 3-4 months of age. The clinical course is slow and progressive in the maturing dogs but stabilizes after maturity. Urinary creatine excretion is elevated and urinary creatinine/creatine ratios are depressed in affected dogs. Serum electrolytes and serum creatine kinase activity are within normal limits in the affected dogs.

Pathologic changes in the skeletal muscle observed in the affected dogs are consistent, generalized, and severe and involve the distribution and size of the muscle fiber subtypes I and II. The affected dogs have a marked deficiency of type II fibers and an increase in the diameter size variation of both type I and II fibers. Other pathologic changes include centralization of nuclei, nuclear rowing, and an increase in interstitial connective tissue.

We have demonstrated that plasma aldosterone levels are significantly elevated in the affected dogs. Plasma renin activity is not significantly increased and plasma cortisol levels are comparable to levels found in nonaffected dogs.

Because of the alteration in electrolyte control mechanisms, intracellular electrolytes, C^{14} inulin space, and total lipid and water concentrations were measured in skeletal muscle from 4 adult affected and 4 adult nonaffected dogs. Electrolytes were measured using atomic absorption spectrophotometric methods and data expressed as meq/kg fat-free wet weight. Results indicate an increase in intracellular sodium and chloride concentrations and a decrease in intracellular potassium concentration in the affected dog skeletal muscle (Na- affected 71.6 ± 11.0, nonaffected 50.3 ± 12.5; K- affected 69.5 ± 5.6, nonaffected 93.4 ± 10.0). There was a significant increase in the lipid content of the affected dog muscle and a decrease in the water content. No significant change was observed in the C^{14} inulin space.

This disorder will provide a useful model to study electrolyte control mechanisms, muscle electrolyte shifts in disease, and to compare with similar changes which occur in aged muscle. (Supported by NIH grants RR00515, FR5465, GM07125, and the Muscular Dystrophy Associations of America, Inc.)

1246 SEGREGATED ORIGINS FOR SUPERIOR COLLICULAR PROJECTIONS BASED ON THEIR INTENDED TARGET STRUCTURES. [1] C. K. Henkel, [2] S. B. Edwards and [3] K. S. Kersey*, [1] Dept. Anat., Bowman Gray Sch. Med., Winston-Salem, NC 27103 and [2], [3] Dept. Anat., Univ. of Va. Sch. Med., Charlottesville, VA 22901.

In search for a general principle governing the anatomical organization of the deeper or motor layers of the superior colliculus, we analyzed the distribution of collicular cells which give rise to several functionally distinct projections and compared their laminar and spatial arrangements. The histochemical method of de Olmos ('77) for labeling the retrogradely transported HRP enzyme was used, and the position of labeled cells was recorded with the aid of an x-y plotter. In five cats iontophoretic HRP deposits were made in the cervical spinal gray to study the origin of the direct tectospinal projection controlling head movements. The data indicated that about 80% of the cervically-projecting collicular cells was found in stratum griseum intermedium (SGI). Most of them were found at an intermediate depth within SGI, and many were giant cells 40-70μ in diameter. Both the SGI and deeper cells projecting to the cervical cord were found predominantly in the caudal and lateral portions of the colliculus. In several additional animals HRP deposits in cervically-projecting areas of the reticular formation labeled collicular cells in a similar distribution, indicating that the indirect tecto-spinal pathway has a collicular origin similar in many respects to that of the direct pathway. In six cats HRP deposits were made in a paralemniscal zone of the ipsilateral, caudal midbrain reticular formation in order to compare the origin of an indirect tecto-facial pathway related to control of pinna movements (Henkel and Edwards, '78) to the above data. In striking contrast to the distribution of head movement-related collicular cells, 75% of the pinna movement-related collicular cells was found in stratum griseum profundum (SGP). Over 60% of these was present in the rostral half of SGP. Those cells labeled in SGI were located in its deepest portion (88%) and were seldom larger than 40μ in diameter. This information suggests that the laminar organization of cells in the motor layers of the superior colliculus reflects a functional segregation based on the peripheral target structure of collicular projections.

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1247 COMPARATIVE MORPHOLOGY OF CAT ABDUCENS INTERNUCLEAR NEURONS AND MOTONEURONS. S.M. Highstein, A. Karabelis*, R. Baker and R.A. McCrea (SPON: Morris B. Bender). Dept. Neurosci., Kennedy Ctr. for Res., Albert Einstein Coll. of Med., Bronx, N.Y. 10461; Dept. Physiol., New York Univ. Med. Ctr., New York, N.Y. 10016

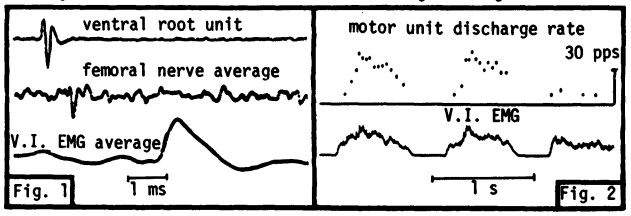
In anesthetized cats the abducens (Abd) nucleus was explored with microelectrodes containing 4% HRP. The purpose was to antidromically identify and stain Abd motoneurons (Mns) and internuclear (Int) neurons (Ns) in order to characterize their soma-dendritic trees and axonal trajectories. All neurons received disynaptic IPSPs and EPSPs following ipsi- and contralateral vestibular nerve stimulation. Abd Mns generally were larger and tended to lie more caudal in the nucleus than Int Ns, but both exhibited soma shapes extending from small fusiform to large multipolar. The axon hillocks of Abd Mns were directed toward all parts of the nucleus except laterally, yet all axons eventually turned ventrally to form the VIth nerve without any sign of collateralization either in the Abd nucleus or in the brainstem (n=24 Mns). Axon hillocks of Int Ns (n=22) were nearly always directed dorsally and axons left the Abd nucleus in a coronal trajectory spanning the entire dorsal-ventral extent of its medial border. Every Int axon headed toward the contralateral MLF before curving rostrally towards the IIIrd nucleus; but 30% exhibited a caudal collateral that coursed in the contralateral MLF towards the prepositus. The dendritic trees of all Abd Mns branched more extensively than those of Int Ns, especially at secondary and tertiary levels. Dendrites of Abd Mns often spanned the entire Abd nucleus. Abd Mns exhibited 6 to 8 primary dendrites as compared to 4-6 for Int Ns. Characteristically Abd Mns and Int Ns had long ventral dendrites which often extended into the reticular formation below the nucleus. In addition, dendrites of both populations curved around the facial genu, especially laterally toward, but not entering, the medial vestibular nucleus. The dendrites of Abd Mns were always extended more dorsomedially toward the MLF. We suggest that the morphological differences in soma-dendritic profiles (i.e. size) are responsible, in part, for the quantitatively different physiological responses in Abd Mns and Int Ns. We conclude that the absence of any recurrent axon collaterals within the Abd nucleus demonstrates that horizontal conjugate gaze signals are determined individually by all neurons within the nucleus and are not the result of intrinsic neural circuits. In fact, it is likely that the massive dendritic tree of Abd Mns might result from the necessity for receiving all information concerning vergence and conjugate gaze signals, much of which is shared by the comparatively smaller dendritic trees of Int Ns and medial rectus Mns. Supported by Grants EY-02007 and EY-01670.

1248 A METHOD FOR RECORDING AND IDENTIFYING SINGLE MOTOR UNITS IN INTACT CATS DURING WALKING. J.A. Hoffer, M.J. O'Donovan* & G.E. Loeb. Lab. of Neural Control, NINCDS, NIH, Bethesda, MD 20205.

A combination of three recently developed techniques has enabled us to obtain stable records from single motor neurons in normal cats during walking, and to characterize motor units by fiber type. Fine flexible wires (Loeb & Duysens, J. Neurophysiol. 42:420, 1979) are introduced through the dorsal root ganglion into the L5 ventral root. A recording cuff (Davis, Gordon, Hoffer, Jhamandas & Stein, J. Physiol. 285:543, 1978) is placed around the femoral nerve, and bipolar EMG probes sample each of the five muscles supplied by it (rectus, sartorius, and the vasti: medialis, lateralis and intermedius). Active ventral root electrodes generally sample one or more discriminable units. By spike-triggered averaging from the ventral root into the nerve and muscle electrodes, the orthodromic neural and EMG spikes can be extracted to obtain the conduction velocity, muscle of destination, and EMG signature of each unit (Fig. 1).

On occasion a recorded motor axon can be microstimulated in isolation through the ventral root electrode, and the mechanical properties of its muscle unit obtained under anesthesia. A heavy suture fixed to the patellar tendon, emerging through the skin, is attached to a myograph for measurement of twitch parameters, sag, and fatigue index. Further procedures could in principle include glycogen depletion and histochemical identification from muscle biopsy material (Garnett, O'Donovan, Stephens & Taylor, J. Physiol. 287:33, 1979). Criteria used to verify that the recorded and stimulated units are identical include replication of conduction velocity and EMG signature, and occlusion of the muscle response to spike-triggered microstimulation if the stimulus is delivered within the refractory period of the muscle, but outside that of the nerve.

Fig. 2 shows the normal firing pattern of the vastus intermedius (V.I.) unit from Fig. 1 (slow twitch contraction time; 90 ms). The motor neuron was recruited as the rectified/smoothed muscle EMG crossed a reproducible level, and its firing rate closely followed the whole muscle EMG during walking.



1249 A BASIS FOR PATHOLOGIC FIXATION EYE MOVEMENTS EXISTS IN NORMAL SUBJECTS. John R. Hotson, Dept. Neurol., Stanford Univ. Sch. Med., Stanford, CA. 94305.

Some patients with impaired supranuclear oculomotor control have pathologic large amplitude eye movements when fixating on a target. Small amplitude saccades and slow drifts are widely known to occur in normal subjects in similar test situations. The fixation eye movements in 12 control subjects were recorded using a purkinje image eyetracker and in addition to saccades and slow drift, small amplitude square waves, polyphasic square waves, flutter movements, and vertical nystagmus occurred.

Square waves consisted of an initial disruptive saccade which, after an intersaccade interval less than 350 msec., was followed by a second corrective saccade returning the eye toward the intended position. The mean amplitude of square waves (19.7', SD 10.4) and polyphasic square waves (40.0', SD 16.7) was significantly greater (p=0.001) than horizontal fixation saccades (9.2', SD 3.3).

Intersaccade intervals of square wave varied from 50 msec. to 350 msec. Square waves with an interval less than 200 msec. were greater in amplitude (p=0.004) than match paired square waves with 200-350 msec. intervals, suggesting that the corrective saccade occurred sooner when the initial saccade produced a large gaze deviation. The mean amplitude of the first saccade of polyphasic square waves (25.8', SD 9.9) was similar to the mean square wave amplitude, however the mean amplitude of the second saccade (48.6', SD 18.4) was significantly greater (p=0.016). The mean duration of the first intersaccade interval of polyphasics was also shorter than square waves (p=0.016), while the last polyphasic interval was the same. Polyphasics may be produced by a coupling mechanism similar to square waves, however the second corrective saccade occurs earlier and overshoots the position of intended gaze, requiring additional corrective saccades.

Four patients with diffuse cerebellar disorders had fixation eye movements qualitatively similar to control subjects, however the amplitude and/or frequency of saccades, slow drift, square waves and polyphasic square waves were increased. It is possible that defective supranuclear control of small fixation eye movements may lead to the development of large amplitude fixation instability. (Supported by NASA grant NGR 05-020-634 and Inst. Med. Res., San Jose, Ca.)

1250 A COMPARISON OF THE DISCHARGE PATTERNS OF MOTOR CORTEX AND SPINAL MOTORNEURONS IN THE MONKEY DURING THE 'VOLUNTARY' CONTROL OF WRIST POSITION. D. R. Humphrey and D. J. Reed*, Lab. of Neurophysiology, Emory Univ. Sch. of Med., Atlanta, GA 30322.

When monkeys are trained to grasp a small handle and to maintain a constant wrist position in the presence of perturbing forces, they employ two modes of operation, depending upon perturbation frequency. At low frequencies (0.1-0.5 Hz) and moderate force levels (100-200 gm), the position of the wrist is controlled principally by a smooth modulation in the firing rates of small motor units in the wrist flexor and extensor muscles. Spike-triggered averaging reveals that these units generate low twitch tensions (0.1-0.5 gm), and their discharge rates correlate highly with the time course of the force perturbation. At higher perturbation frequencies (0.6-2.0 Hz), these small units discharge at near constant rates, which appear to be near maximal levels (40-50 imp./sec). This occurs in both flexors and extensors, so that the wrist is partially 'clamped' by co-contraction, a manuever that is effective because of the increased mechanical impedance of tonically active muscle. Over this same frequency range, however, the control of wrist position is further aided by a progressive 'recruitment' into activity of larger motor units, which have larger twitch tensions; their firing rates are increasingly modulated as the perturbation frequency increases, despite a constant peak-to-peak force level.

Recordings from cells within the forearm area of the contralateral motor cortex reveal a similar pattern of behavior. Small (slowly conducting) pyramidal tract (PT) and corticorubral cells also exhibit a modulated firing rate during the tracking of low frequency perturbations, and tend to fire at a more constant rate during rapid perturbations. In contrast, rapidly conducting PT cells, as well as unidentified neurons in more superficial layers, tend to fire most intensely during higher frequency perturbations.

It is our hypothesis that these results reflect (a) a similar order of recruitment from small to larger cells at both cortical and spinal levels, and (b) an interaction of changing central and increased peripheral inputs to both cortical cells and segmental motorneurons. During the voluntary tracking of low frequency perturbations, the task is performed principally by small cells at both levels, which are the first to be recruited by central command structures. At higher perturbation frequencies, the central command changes to a 'clamping' effort, which is associated with a facilitation of input pathways from receptors in the wrist and hand. This input is increasingly effective in modulating the firing rates of large PT cells and motorneurons, but is not reflected in the firing rates of the smaller cells, because they are at saturation levels. (Supported by NIH Grant NS-10183).

1251 ALTERATION OF PHYSIOLOGICAL ACTION TREMOR BY FATIGUE. Paul A. Iaizzo*, Robert S. Pozos, Roger W. Petry*, (SPON: D.J. Forbes) Department of Physiology, University of Minnesota, Duluth, School of Medicine.

An action tremor which accompanies voluntary motion in normal subjects has been called Physiologic Action Tremor (PAT). Since it has been noted that large involuntary oscillations of the limbs can be observed, in extremely fatigued subjects, the present study was undertaken to see if fatigue altered the frequency and/or amplitude of PAT. If so, then PAT might be considered the physiological substrate for the tremors produced by fatigue.

PAT of the ankle was recorded before and after the subject was fatigued by using an AVR-250 accelerometer taped to the patella and biopotential surface electrodes placed on the tibialis anterior and soleus muscles. Acceleration and EMG signals were recorded on a tape recorder and later analyzed for frequency and amplitude changes using a PDP-12 digital computer. The fatigue state was produced by asking the subject to run a distance which the subject considered moderately fatiguing.

Analysis of the data shows that the frequency range of PAT in the non-fatigued subject versus the tremor seen when a subject was fatigued, fall in the same frequency range of 3-8 hz. There was a significant difference ($p < .05$) in the amplitude of the acceleration record as well as the EMG signals in the fatigued states. The mean pooled RMS acceleration values for PAT was 12.0 cm/sec^2 whereas for fatigue altered tremor was 16.4 cm/sec^2.

The data suggests that fatigue induced by running alters significantly the amplitude of PAT. Since PAT, fatigue altered tremor, and clonus have a similar frequency range but varied amplitudes, the alteration of the mechanism for PAT may be an explanation of the other two tremors.

1252 LIMB MUSCLE ACTIVITY IN CHICK DURING LOCOMOTION. Richard Jacobson* (Spon: Paul Grobstein). Dept. of Pharmacolog. and Physiolog. Sci., Univ. Chicago, Chicago, IL 60637.

The intralimb pattern of leg muscle activity during locomotion was determined in recently-hatched chicks. EMG's were recorded from as many as four muscles simultaneously while the animals were walking unrestrained or on a treadmill at various speeds. Some animals were also videotaped in order to relate muscle activity to footfall and joint angle changes. Records have been made from nearly all major muscle groups controlling the hip, knee and ankle joints. The data from 28 chicks provide the following generalizations: (1) No significant differences in the temporal pattern of muscle activity can be observed when free and treadmill walking are compared. (2) Each leg muscle falls into one of two principle categories: those active chiefly during stance, the period when the limb supports and propels the body mass; and those active chiefly during swing, when the limb is lifted off the ground and brought forward. (3) The swing muscles can be further subdivided according to their activity during the flexion or extension of the swinging limb.

We have also correlated this physiological picture of leg muscle activity with the anatomical map of leg motor neuron pools in the lumbosacral spinal cord (Hollyday, unpublished). Perikarya of cells which innervate stance muscles lie in two main regions: a large medial cluster and a smaller dorso-lateral cluster. Perikarya of cells which innervate swing muscles are in motor nuclei which lie between the two main stance clusters.

Two main generalizations arise from this anatomical data. (1) Every segment of the lumbosacral cord has motor pools innervating stance muscles and motor pools innervating swing muscles; thus, the entire step cycle is represented in each segment. (2) There is a longitudinal continuity of motor nuclei that innervate muscles with a similar activity pattern.

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Withdrawn by Author

1254 EFFERENT PROJECTIONS OF THE PONTINE OCULOMOTOR PAUSER REGION IN THE CAT. C.R.S. Kaneko, T. P. Langer* and A.M. Graybiel, Dept. of Psychol., MIT, Cambridge, MA 02139 and Dept. of Physiol. and Biophysics, Univ. of Washington, Seattle, WA 98105.

Two zones in the pontine tegmentum have been identified with distinct oculomotor functions in cats and monkeys on the basis of recordings from single units during eye movements: 1)a paramedian zone containing "burst" and "burst-tonic" units that increase their firing rates shortly before eye movements, and 2) a more medial zone near the level of the VIth nerve rootlets containing "omnipause" units that cease firing before a saccade occurs. We have attempted to distinguish between the efferent connections of these two pre-oculomotor regions by means of the autoradiographic technique. In 3 cats the "pauser region" was identified electrophysiologically and injections of labelled amino acids were made in the vicinity of omnipause neurons. In 3 other cats labelled amino acids were deposited stereotaxically into the paramedian reticular formation near the abducens nucleus.

In all cats labelling could be traced through a broad central expanse of the reticular formation including the nuclei gigantocellularis, pontis caudalis and pontis oralis and extending rostrally into the mesencephalic tegmentum. Regions of the central gray substance,particularly near the IIIrd and IVth nerve nuclei, were variably labelled, somewhat more so in cases of midline than paramedian injection. Labelling of the abducens-periabducens region also appeared but could not be interpreted adequately because the deposits were too close by.

Cases of midline injection were distinguished by a sharp focus of labelling in the medial part of the medial accessory nucleus of the inferior olive. In 1 case the flocculus was labelled. At mesencephalic levels these cases showed denser labelling of the reticular formation, including the nucleus of the posterior commissure and perirubral fields. A striking difference in labelling between the 2 sets of cases appeared at the mesodiencephalic border: all midline injections, but not more lateral deposits, elicited dense labelling of the nucleus of the fields of Forel (NcF), a region known to project directly to the oculomotor complex and implicated in vertical gaze control.

While the anatomical findings cannot definitively establish the projections of functionally defined cell groups, these differences suggest that medial "pauser region" and paramedian tegmentum indeed have distinct efferent connections. In particular, the findings suggest that the medial pauser region of the pons may have direct efferent connections with both vertical and horizontal saccade-generating mechanisms and with cerebellar-precerebellar circuitry as well.

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1255 ORGANIZATION OF REFLEX RESPONSES TO ANKLE DISPLACEMENT IN HUMAN LEG AND ARM MUSCLES. R.E. Kearney and C.W.Y. Chan, Biomedical Engineering Unit and Aviation Medical Research Unit, McGill University, Montreal, Quebec, Canada.

In a previous study (1) we demonstrated that cutaneous stimulation of the human foot evokes systematic reflex changes in muscles of both the ankle and arm. The present study extends this work to consider the nature of the responses elicited by ankle displacement.

Step displacements (0.1 rad in amplitude, 40 ms rise time) of ankle position were applied in either the dorsiflexing or plantarflexing direction by means of a servocontrolled electro-hydraulic actuator. Subjects lay supine and were instructed to maintain a tonic contraction of the relevant muscle (tibialis anterior (TA), gastrocnemius (G), triceps brachii (TB), or biceps brachii (BB)), aided by visual feedback of the smoothed and rectified surface EMG, and not to react to the stimuli. Stimulus-related changes in the tonic EMG activity were detected by means of averaging and Wiener filtering, as a means of assessing reflex activity. Examination of five normal subjects has revealed two principal findings.

First, the responses of the ankle muscles are distinctly asymmetric. Dorsiflexion of the ankle evoked a strong, short latency (40 ms) excitation of G, attributable to the monosynaptic Ia pathway. This was followed by a period of reduced activity, and then a return to normal. The TA displayed an exactly reciprocal pattern of response, in which an early decrease in activity was followed by a period of excitation. In contrast, plantarflexion of the ankle evoked a weaker, short latency excitation of TA followed by a second, often larger, period of excitation lasting from about 80 - 100 ms. Furthermore, the G response did not display any significant reciprocal inhibition. It is possible that the asymmetry of the response reflects differences in the relative importance of cutaneous and muscle afferents in the two muscles. The functional implications of these differences remain to be determined.

The second major finding was that ankle displacement evoked large and systematic reflex responses in TB but not BB. Thus dorsiflexing displacements in the ankle result in a decreased TB activity at a latency of about 70 ms while there was no significant change in BB activity. In contrast, plantarflexing displacements of the ankle evoked a short latency (40 ms) excitation of TB followed by a decrease in activity. Occasionally, small increases in BB activity were also noted. The large size and short latency of the TB responses indicate that they may have important functional roles to play - perhaps in the control of locomotion.

(1) Kearney, R.E., Chan, C.W.Y., & Arrott, A.P., Neuroscience Abstracts, 4: 298, 1978.

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1256 PONTINE CONTROL OF SACCADIC TRAJECTORIES: MICROSTIMULATION AND SINGLE UNIT STUDIES. W.M.King, A.F.Fuchs, W.Becker,and G.Johanson
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Although it has long been thought that saccadic eye movements are preprogrammed, Robinson (1) has recently suggested that the saccadic trajectory is under continual control by pontine circuits. To both test this hypothesis and to determine the neural elements involved in such circuitry, we attempted to interrupt saccades transiently in mid-flight by delivering stimulus trains to various brain stem loci between the abducens and oculomotor nuclei. Cathodal stimulus trains (0.1 msec bipolar pulses at 300 Hz with 10-35 msec train durations) were triggered to occur as early as 3 msec after the onset of randomly selected saccades elicited in the trained monkey. At certain sites near the midline, currents as low as 10-20 μa caused saccades to decelerate within 11 msec of the train onset. Within 11 msec after the train ended, the saccade either resumed its course (perturbed saccade) or was terminated (truncated saccade). A perturbed saccade appeared to be a single eye movement, and in most cases was as accurate as a control saccade to the same target. In contrast, the truncated saccade always fell well short of the target and was followed by a second saccade, often after latencies of less than 50 msec. For long stimulus trains, the second saccade overshot the target necessitating a corrective third saccade. Perturbed saccades occurred if the stimulus train ended during what would have been the acceleratory phase of the eye movement had the stimulus not occurred (computed as the mean acceleratory phase of control saccades to the same target). If the stimulus train ended after the estimated acceleratory phase, the saccade was truncated.

Preliminary reconstructions of successful penetrations indicate that sites where weak stimulation caused saccade interruptions were invariably confined to within ± 1 mm of the midline throughout a core of brain stem which extended from the rostral pole of abducens to a point about 5 to 7 mm ventral to the trochlear nucleus. Single unit recordings obtained through the stimulating microelectrode indicated that sites where omnipause neurons (which ceased firing for saccades in all directions) were encountered always produced saccade interruption at low currents, but interruption was obtained also at sites without omnipause neurons. However, at virtually every effective site some form of saccade related activity was found.

Generally, these results seem consistent with Robinson's "bang-bang" model although modifications will be necessary to account for certain characteristics of interrupted saccades.

1. In: Basic Mechanisms of Ocular Motility and Their Clinical Implications p337 Pergammon Press (1975).

1257 EFFECTS OF DENTATE LESION ON DISCHARGE PATTERNS OF MOTOR CORTEX NEURONS AND REACTION TIME IN MONKEYS. Yves Lamarre, Giuseppe Spidalieri* and Liliane Busby*. Centre de recherche en sciences neurologiques, Faculté de médecine, Université de Montréal, C.P. 6128, succursale A, Montréal (Québec) Canada H3C 3J7.

There is experimental evidence in favor of a role played by the cerebellum in the initiation of some fast movements. It is not known, however, whether dentate provides a true phasic motor command or only a nonspecific tonic facilitation to supraspinal motor centers. In an attempt to answer this question, we examined the effect of electrolytic lesion of the dentate nucleus on the initiation of ballistic flexion and extension of the arm triggered by sensory cues of different modalities. Two monkeys were trained to perform in response to randomly presented light signals, pure tones or small elbow displacements. Unit activity in precentral cortex, angular displacement of the elbow, arm and neck muscle activity, as well as eye movements, were recorded and processed on-line with a PDP-9 computer.

Three average values were calculated: 1) time from onset of stimulus to onset of elbow displacement (RT), 2) time from onset of stimulus to onset of cortical neural changes (RS) and 3) time from onset of cortical neural changes to onset of elbow displacement (RM). The pattern of discharge of each neuron was the same whether the movement was triggered by visual, auditory or somesthetic signals. After dentate lesions, RT and RS increased by about 60 msec for light and sound in the two monkeys, the increase being the same for both flexion and extension movements. The reaction time to elbow displacement was not changed in one animal and was increased by only 20 msec in the other. In both animals, movement parameters, RM values, spontaneous activity and sensory responses to sound and elbow displacement were not changed. These data are in favor of the hypothesis that the lateral cerebellum participates in the phasic motor command for some fast ballistic arm movements, particularly those triggered by teleceptive input signals. (Supported by MRC of Canada)

1258 PROJECTIONS FROM MESENCEPHALON TO THE INFERIOR OLIVARY COMPLEX IN CATS AND MONKEYS. Pierre Langelier*, Raymond Marchand*, René Boucher and Louis J. Poirier, Laboratoires de neurobiologie, Pav. Notre-Dame, 2075 ave de Vitré, Québec, Qué. G1J 5B3.

The autoradiographic method was used to study the fiber projections from the tectum and the mesencephalic tegmentum to differents parts of the inferior olivary complex. Twelve adult cats and four monkeys (Macaca fascicularis and squirrel monkeys) were used in the present analysis. In order to further map the efferents of this area, tritiated leucine was injected unilaterally in the red nucleus, the adjacent tegmentum and different parts of the tectum.

Following injections in the lateral part of the midbrain tegmentum, the labeled material was found in the ipsilateral dorsal accessory inferior olivary nucleus (DAO). In the light of our preliminary results it appears, more specifically in cats, that the neurons of this part of the midbrain tegmentum send a topographically organized projections to the ipsilateral DAO.

In three other cats and one monkey, ^{3}H leucine was injected into the medial portion of the superior colliculus. In agreement with Weber J.T. et al (Brain Research, 144 (1978) 369-377) we found the labeled amino acid into the dorsal part of contralateral caudal medial accessory olive (MAO). However we did not observe any labeled material in the contraleteral MAO with injections in the lateral portion of the superior colliculus.

On the other hand, after injections of tritiated leucine into the rostal red nucleus in cats and monkeys we could observe the distribution of silver grains in the ipsilateral dorsal lamella of the principal nucleus of the olivary complex, as well as along the course of the dorsally situated rubro-olivary pathway.

No evidence was found for a projection to the olivary nucleus with injections in the vicinity of the periaqueductal gray.

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1259 MOVEMENT AND THE MECHANICAL PROPERTIES OF THE INTACT HUMAN ELBOW. J. M. Lanman*and T. A. Zeffiro (SPON: N. Geschwind)
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Recent studies of motor coordination have emphasized the importance of the mechanical properties of muscle in the control of movement. In the present study, we investigated short term changes in muscle properties by measuring the response of the intact human elbow joint to mechanical perturbations. The resistance of the intact joint to such displacements reflects contributions from the muscles and passive tissues spanning the elbow joint. In order to explore the effects of both muscular activation and movement on joint properties we studied three conditions: (1) voluntary isometric contraction,(2) passive movement, and (3) voluntary movement.

In the isometric condition, the subject maintained a constant arm position against various loads. In the passive movement condition the subject's arm was moved at a constant velocity. Finally, the voluntary movement condition combined muscular activation with movement. During all three conditions a small amplitude, high frequency (3-30 Hz) sinusoidal perturbation was applied about the elbow joint. Joint impedance was derived from the resistance of the elbow to this superimposed displacement. We obtained continuous measures of joint impedance, joint angle, and the biceps and triceps EMG's.

During isometric contraction, joint impedance increased monotonically with increasing muscular activation (estimated by EMG). In contrast, during passive arm movements joint impedance decreased markedly. This was true even for very slow passive movements when no change in muscular activation could be detected. Finally, voluntary movements resulted in changes in joint impedance which were consistent with the combined effects of muscular activation and movement.

We attribute these changes in joint impedance to variations in the properties of the muscles acting about the joint. We conclude that length changes (whether active or passive) and neural activation have opposing effects on the mechanical properties of muscle. These results will be discussed in relation to the well-characterized effects of length change and activation on the mechanical properties of isolated muscle.

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1260 LONG LATENCY VERSUS LONG LOOP REFLEXES: DEPENDENCE ON THE TEMPORAL CHARACTERISTICS OF THE IMPOSED DISPLACEMENT. R.G. Lee and W.G. Tatton. Neurosc. Research Group, Univ. of Calgary, Calgary, Alberta and Playfair Neurosc. Unit, Univ. of Toronto.

The reflex EMG response to perturbation of an extremity consists of an early component (M1) and one or more late components (M2, M3). The origins of the late components have been the subject of much recent controversy. Several lines of evidence, including their long latencies, support the concept that the late components are at least partially mediated by a transcortical feedback loop. However, assigning specific latencies to these responses requires the assumption that the effective input occurs over a limited time interval at or near the onset of the displacement. The mechanical properties of the torque motor systems used to study these responses in humans are such that the displacement continues for 100 msec. or longer even when a step-change in load is applied. The present study was designed to examine the late components of the EMG response when the displacement was arrested at varying times following the onset of the perturbation. Recordings were carried out on 12 normal human subjects using a computer controlled torque motor to generate randomly timed displacements of the wrist joint. EMG responses from the wrist flexors were rectified and averaged and the integrated EMG activity was measured over the M1 interval (30-55 msec. following onset of the imposed load) and over the M2-M3 interval, (55-90 msec.). When the displacement was stopped prior to 45 msec. there was a well defined M1 response but the late EMG components beyond 55 msec. were absent. As the duration of the displacement was increased beyond a critical interval between 50-55 msec. there was a rapid increase in the size of the late responses. Further, the duration of the late components was monotonically related to the duration of the imposed displacement beyond the critical period.

These results raise the possibility that the late EMG components are entirely independent of long loop mechanisms activated near the onset of the perturbation but rather result from segmental reflexes activated later in the course of the displacement. Yet the timing of the critical interval (50-55 msec.) and the dependence of the duration of the late components on the time course of the displacement makes two other possibilities more likely: 1) The abrupt termination of the handle displacement introduces inhibitory influences on motoneurons which prevent them from responding to descending excitatory inputs from long loop pathways. 2) Continued stretch or displacement provides an essential convergent input to motoneurons or to interposed interneurons in polysynaptic pathways which is required for the motoneurons to respond to long loop inputs.

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1261 DISCHARGE PATTERNS OF CONCURRENTLY ACTIVE MOTOR UNITS. Ronald S. Le Fever* and Carlo J. De Luca. Dept. of Orth. Surg., Children's Hosp. Med. Ctr., Harvard Med. Sch., and M.I.T., Boston, Ma..

The discharge patterns of concurrently active motor units have been investigated in three muscles of four normal adult subjects. Both constant-force and force-varying isometric contractions have been studied in the deltoid, biceps and first dorsal interosseous (FDI) muscles, at levels up to 80% of the maximal voluntary contraction. These firing patterns have been obtained through the use of an expanded version of the technique for the separation of superimposed action potential trains reported at the 8th Annual Meeting of the Society for Neuroscience.

Three channels of EMG signal, one bipolar and two monopolar, are recorded from a modified DISA bipolar needle electrode. Analog and digital filtering of the EMG signal is employed to reduce the duration of the action potentials, and consequently the degree of superposition. The EMG signal is decomposed into its constituent action potential trains using a visually assisted computer algorithm. Using this technique, the firing patterns of typically three to six motor units can be obtained throughout an entire contraction with usually less than 2% error.

Statistical analysis performed on these firing patterns indicates that concurrently-active motor units tend to rapidly modulate their firing rates in unison with corresponding small force fluctuations. The magnitude of these rapid firing rate changes compared to the small fluctuations in force is considerably larger than the overall change in firing rate due to an overall change in the force level. This analysis also indicates that motor units in the FDI increase their firing rates to a greater extent than motor units in deltoid or biceps when the overall force level is increased. Also, in the FDI of one subject, three motor units were observed to lock into exact synchronization for a period greater than 1.5 seconds during an isometric contraction at 60% of maximal voluntary contraction. When these motor units were not firing synchronously, however, both their firing rates and modulation of these rates were similar.

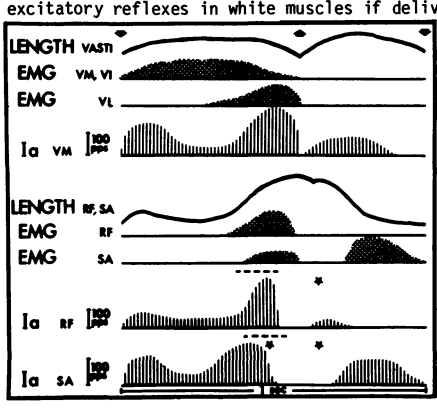
Quite often the motor units recruited at the lowest force levels exhibit the greatest amount of firing rate modulation at high force levels. This discharge behavior of motor units suggests that the number of motor units recruited and their overall firing rates supply the bias of a contraction level; and that small fluctuations about this force are achieved through the simultaneous changes in the firing rates of all active motor units. This scheme has great appeal from a systems control viewpoint. (Supported in part by NIAMDD grant #AM 19665, and by a joint grant from the United Cerebral Palsy Res. and Ed., the C.A. Dana and the Hearst Foundations.)

1262 MODIFICATIONS UNDERLYING ADAPTIVE PLASTICITY OF THE PRIMATE VESTIBULO-OCULAR REFLEX ARE POST-SYNAPTIC TO THE MEDIAL VESTIBULAR NUCLEUS. S. G. Lisberger and F. A. Miles. Lab. Neurophysiol., NIMH, Bethesda, MD 20205.

We have been attempting to localize the site(s) in the vestibulo-ocular reflex (VOR) pathways where changes underlying long-term adaptive plasticity of the VOR might occur. Recordings were made in the medial vestibular nucleus (MVN), which contains the secondary neurons in the horizontal VOR pathways. Half of the sample ("high-gain") was recorded in monkeys adapted to 2.0X telescopic spectacles: VOR gain measured in darkness was greater than 1.5. The rest of the sample ("low-gain") was recorded in monkeys adapted to goggles providing a visual field fixed with respect to the head: VOR gain was less than 0.4. For pure-vestibular and vestibular-plus-saccade cells, sensitivity to vestibular inputs was measured from sinusoidal whole-body rotation with the animal's head in the normal stereotaxic plane. Pitch tilting of the whole animal was used to identify vertical canal inputs. Most cells (96%) having increased firing rates during ipsilateral head rotation (VI) received inputs from the horizontal canals. On average, cells in the high-gain sample had slightly greater sensitivity to head velocity (0.78 spikes/s/°/s, n=130) than cells in the low-gain sample (0.64 spikes/s/°/s, n=129). Most cells (82%) having increased firing rate during contralateral head rotation (VII) received inputs from the vertical canals. Again, cells in the high-gain sample had slightly greater sensitivity (0.51 spikes/s/°/s, n=129) than those in the low-gain sample (0.44 spikes/s/°/s, n=146). Grouping individual cells according to various response properties (sensitivity, phase shift, discharge during saccades) failed to reveal any sub-population displaying large changes that would have been obscured by a large, unchanged fraction of our sample. In addition, there were no differences in phase shift and only small differences in resting rate. For a small sample of vestibular-plus-position cells (VI,EII), sensitivity to vestibular inputs was estimated as the value of K_2 obtained by fitting firing rate during rotation in the dark with the equation FR = DC + K_1 x (eye position) + K_2 x (head velocity). Again, cells in the high-gain sample had slightly greater sensitivity (0.97 spikes/s/°/s, n=22) than those in the low-gain sample (0.78 spikes/s/°/s, n=15). The differences we have found in the sensitivity of MVN cells are much too small to account for the 4-fold difference in VOR gain. We conclude that the changes underlying plasticity occur either at the terminals of MVN cells or in other VOR pathways post-synaptic to the MVN.

1263 UNIT ACTIVITY OF CAT KNEE EXTENSOR SPINDLE AFFERENTS DURING NORMAL AND PERTURBED WALKING. G.E. Loeb and J.A. Hoffer. Lab. of Neural Control, NINCDS, NIH, Bethesda, MD 20205.

Floating microelectrode wires (Loeb and Duysens, J. Neurophysiol. 42:420, 1979) were used to record from cells in the L5 dorsal root ganglion which innervate the five anterior thigh muscles (quadriceps plus sartorius). The unit records were used to spike-trigger average the delayed signal from a femoral nerve cuff electrode to obtain conduction velocity. Stimulation through implanted bipolar EMG electrodes identified spindle afferents and their muscles of origin. Two implanted length gauges recorded the length of the pure knee extensors, vastus medialis (VM), intermedius (VI), and lateralis (VL), and the hip-flexing knee extensors, rectus femoris (RF) and sartorius (SA). Representative activity patterns during slow walking are shown schematically. VM and VI (mainly red muscles) were active throughout stance whereas VL and RF (white) contributed mainly to push-off. SA (white) also provided some late stance thrust but was used mainly as a flexor. Three spindle Ia's shown were active during stance, with onset bursts reflecting dynamic stretch sensitivity and/or fusimotor activity coincident with the onset of quadriceps activation as a whole. The late stance bursts might reflect similar dynamic sensitivity for RF and SA Ia's, but not for VM, where shortening must be overcome by γ-static activity coincident with the white muscle burst (not seen in VM). We interpret swing phase activity in the VM Ia as due to passive stretch; in SA Ia, to α-γ coactivation. Single 0.1 msec shocks to sural nerve or foot dorsum skin elicited excitatory reflexes in white muscles if delivered just before foot lift (dotted lines). Brisk spindle discharge then followed but only when the muscle was elongating (at *'s), suggesting γ-dynamic activation. Light taps to various skin areas of the limb during quiet standing elicited brief (~50 msec) bursts (~200 pps) of Ia firing at 30-50 msec latency without EMG activation of these muscles, a pattern not produced by electrical stimuli during walking.



1264 ACTIVITY OF MULTIPLE INDIVIDUAL MOTOR UNITS IN CATS DURING LOCOMOTION. W.B. Marks. Lab. of Neural Control, NINCDS, NIH, Bethesda, MD 20205.

Arrays of wires are implanted in the medial gastrocnemius muscle of the cat's hindlimb. Recording tips are spaced closely enough that any unit impulse is seen in several leads, and far enough apart such that, for different units, the ratios of amplitudes in the leads differ. The number of active units is held within a resolvable range by limiting the speed of locomotion. A ring of uninsulated probes forms a cylinder around the recording leads in order to attenuate invading fields from remote units and to provide a common indifferent signal for the recording channels. Motor unit waveforms are distinguished by their pattern of amplitudes across several leads as well as by their time courses in each lead. Recording multiple units with multiple leads appears to be less sensitive to muscle movement than differential recording from pairs of leads. In the former, the recording points are spaced farther apart and the multichannel unit patterns are distinguishable in spite of changes in their waveform in a few channels caused by movement. In addition, the lead array is stabilized by attaching it to the surface tendon; the leads terminate just below the surface, the origin of the fibers. When two or more unit waveforms overlap in time and superimpose, they are resolved using a digital multichannel filter (Roberts & Hartline, Brain Res. 94:141, 1975) which forms linear combinations at multiple delays from all the channels, each combination orthogonal to all but a chosen multichannel unit waveform. Multi-lead recording appears to be functional at high levels of muscle activation, since the extra channels enable the filter to resolve more overlapping impulses.

Thusfar we have followed the activity of up to five motor units of the medial gastrocnemius muscle during walking, using 8 leads spaced about 300 microns apart and implanted in the dorsal margin of the muscle where high oxidative (S & FR) fibers, used during walking, are sparse (Burke et al., J. Neurophysiol. 40:667, 1977). We will attempt to derive from such multiunit data the number of different signals impinging on the motor neuron pool. If for example the firing rate of some units is rising while that of others is falling, or if in general all the recorded firing rates or recruitment order cannot be explained from the time variation of a single number, the 'muscle activation', then additional influences on the pool must be present and can be calculated.

1265 INTERACTION OF SACCADES INDUCED BY VISUAL STIMULI AND ELECTRICAL STIMULATION OF THE SUPERIOR COLLICULUS. Lawrence Mays and David Sparks. Neurosciences Prgm. & Dept. of Psychology, Univ. of Al. in Birmingham, Birmingham, AL 35294.

It has often been assumed that saccades elicited by visual stimuli are retinocentrically organized ballistic eye movements. This view implies that a retinal error signal is translated into a command to the saccadic system to move the eyes in a certain direction for a certain distance. In contrast, another model of the saccadic system states that saccades are spatially organized and not ballistic (Zee et al., Arch Neurol. 33, 1976). A spatially organized system requires that the eyes be driven to a particular position in the orbit rather than be preprogrammed for a certain vector.

These two models predict different effects of saccades induced by electrical stimulation of the superior colliculus upon visually elicited saccades. Electrical stimulation of the deeper layers of the monkey superior colliculus produces short latency (e.g., 25 msec) saccades of an amplitude and direction determined primarily by the stimulation site. Macaques were trained to make accurate saccades to briefly presented visual targets for a water reward. Eye position was measured using the electromagnetic search coil technique. Between the signal to make a saccade to a target and the beginning of the saccade, the target was turned off and a brief (40 msec) electrical stimulus train was delivered to a site in the superior colliculus through a microelectrode. Thus, after the signal to look to the target, but before a saccade could be made, the eyes were driven to another position. The electrically induced saccades were usually followed by another short (or zero) latency saccade. This second saccade was an attempt to acquire the recently presented visual target. If saccades are preprogrammed in a retinocentric coordinate system then this second saccade should have the same vector as if no electrical stimulation occurred. Since the eyes were forced to move by the electrical stimulation, the final eye position should miss the actual target position by an amplitude and direction equal to that produced by the electrical stimulation. If, on the other hand, saccades are programmed to take the eyes to a specific location in the orbit (or look to a specific location in space) then the amplitude and direction of the visually elicited saccade should compensate for the intervening electrically-induced saccade. This latter result, consistent with the spatial model of saccade generation, was reliably observed. Even if the electrical stimulation was timed to interrupt the visually elicited saccade, the stimulation-induced movement was followed by a short (or zero) latency saccade to direct the eyes to the location of the recently extinguished visual stimulus.

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1266 INTERCONNECTIONS BETWEEN VESTIBULAR NUCLEI AND THE NUCLEUS PREPOSITUS HYPOGLOSSI. R.A. McCrea and R. Baker. Dept. Physiol. & Biophys., New York Univ. Med. Ctr., 550 First Ave., New York, N.Y. 10016.

Previous studies have shown that neurons in the nucleus prepositus hypoglossi receive monosynaptic inputs bilaterally from the vestibular nuclei (Baker and Berthoz, 1975) and that prepositus neurons in turn project back to the vestibular nuclei (Pompeiano, Corvaja and Mergner, 1978). The aim of the present study was to examine in detail the specific morphological and physiological characteristics of these pathways. When ^{3}H Leucine was injected into the region of the prepositus, the medial and descending vestibular nuclei were heavily labeled bilaterally. Intracellular injections of HRP into single prepositus neurons revealed that axons of medium sized, 'principal', cells sent collaterals to both the medial and descending vestibular nuclei. These prepositus neurons terminated exclusively on either the ipsi- or contralateral side of the brainstem. The latter pattern appeared to be correlated with the vestibular input because collateralization usually occurred on the side from which vestibular excitation was received. When HRP was injected extracellularly into the prepositus, many neurons were labeled throughout the medial and descending vestibular nuclei and to a lesser extent in the superior and ventral lateral vestibular nuclei. On the other hand, intracellular injection of HRP into axons of single secondary vestibular neurons in the descending and medial vestibular nuclei in acute and alert cats revealed that neurons which terminated in the prepositus also sent collaterals towards the spinal cord in the medial vestibulospinal tract. In many cases, the same vestibular neuron also terminated in the abducens nucleus. Similar collateralization may also exist for other secondary vestibulo-ocular neurons because many were antidromically activated following stimulation of both the prepositus and oculomotor nuclei. Those vestibular neurons that sent collaterals to the spinal cord, prepositus and abducens nucleus did so either ipsi- or contralaterally, exclusively. These data indicate that prepositus neurons receive some of the same vestibular signals as extraocular and neck motoneurons. We conclude that the above connections suggest the prepositus nucleus is intimately involved with both eye and head movement. The extensive reciprocal connections between prepositus and vestibular neurons may be important for the generation of both eye and head position and, in fact they may be necessary for providing gaze signals to other neurons, including motoneurons. Supported by USPHS Grants NS-13742, EY-02007 and Fellowship NS-05857.

1267 TWO MACRO-REPRESENTATIONS OF THE FACIAL MUSCLES IN THE PRECENTRAL GYRUS OF MACAQUE MONKEYS. EveLynn McGuinness*, Dave W. Sivertsen*, and John Allman. Vanderbilt University, Nashville, TN 37240 and California Institute of Technology, Pasadena, CA 91125.

Using a chronic preparation we stimulated with microelectrodes approximately 2000 responsive sites in the face region of motor cortex in 6 macaque monkeys (4 Macaca mulatta, 2 Macaca fascicularis). Current levels used were always less than 25 μA. The modal threshold for mimetic muscles was between 2.5 and 5 μA. Responses were extremely discrete, the usual response at threshold current levels was a small focus of movement in part of a muscle. Facial muscles cluster together into two macro-representations or domains in the posterior and anterior portions of the face region of the precentral gyrus with tongue movements represented in the intervening portion and along the lateral extent. Eyelid movements are represented at the medial edge of the face representation adjoining the representation of shoulder muscles. There is a diagonal band of jaw responses which begins laterally at the central sulcus and cuts across the surface of the gyrus in the anteromedial direction. Within both anterior and posterior domains there is local re-representation of muscle movements. This microrepresentation has been described previously (McGuinness and Allman, 1977, Neuroscience Abstracts; Kwan et al., 1978, J. Neurophysiol.). Although the domains cannot be characterized as distinct topographical representations of the entire face on the basis of the data we now have, there is a tendency for adjacent muscles to occur together and the representations may be roughly topographical within the limits set by the morphological structure of the muscles themselves. We feel that the basic microorganization is columnar; however, the columns could be either roughly cylindrical or take the form of narrow curving bands, running medio-laterally across cortex. On the basis of microstimulation alone it is difficult to differentiate between patterns produced by stimulation of frequently repeated non-contiguous zones devoted to the same muscle or narrow bands which are intersected by electrode penetrations at different points in cortex. Our results might best be characterized as two complete but not strictly topographical representations of the facial muscles which are roughly mirror-symmetrical around the tongue and possibly the eyelid. Strick (Brain Res., 1978) has reported a dual representation in the hand region of motor cortex in the squirrel monkey. Although the pattern he finds differs from ours in detail (alternation rather than reversal) the basic principle is similar.

This research was supported by NICHD-00973, J.F. Kennedy Center of Research, George Peabody College and NS-00178.

1268 ELECTROPHYSIOLOGY OF MYOTONIC DYSTROPHY IN CULTURE. Michael Merickel, Richard Gray*, Priscilla Chauvin*, and Stanley Appel. Dept. of Neurology, Baylor Col. Medicine, Houston, TX 77030

Myotonic muscular dystrophy (MyD) is an inherited disease (autosomal dominant) which involves progressive muscular weakness and muscle degeneration. An inborn error of metabolism is expected to underly MyD. However, the specific defect has not been identified in this disorder or in any of the dystrophies. We have approached the study of MyD by utilizing electrophysiological techniques to investigate the membrane properties of muscle fibers from normal and MyD patient biopsies which are grown in a primary tissue culture system. Other investigators have attempted unsuccessfully to find morphological abnormalities in cultured MyD muscle at the EM or light microscope level which makes the investigation of possible electrical abnormalities particularly important. The cultures were prepared from muscle biopsy specimens which were trypsin dissociated using previously reported techniques (Yasin et al, J. Neurol. Sci.32:347-360 1977). Cultures were chosen for electrophysiology on the basis of morphological criteria, such as the appearance of striations, and were studied between three to five weeks after plating.

Our results have demonstrated that myotubes cultured from MyD patient biopsies have abnormalities in some of their fundamental electrical properties compared to myotubes from normal, control biopsies. However, no morphological abnormalities were observed at the light microscope level. Abnormalities in the electrical properties of MyD myotubes include: 1) significantly decreased average resting potential (-32.4 ± 2.1 mV for myotonic vs -45.2 ± 2.2 mV for control); 2) repetitive firing of action potentials when slowly released from membrane potentials hyperpolarized with respect to rest (eg., slow depolarizing ramp) while control myotubes fire only a single action potential; 3) significantly decreased outward-going, delayed rectification compared to control myotubes based on observations of steady-state current voltage plots. The decreased delayed rectification is being investigated as a cause of the repetitive firing behavior described in 2). A decreased resting potential has also been observed in intact muscle preparations by other investigators. This information in consort with our observations described above suggest that the MyD disease process is propagated in primary culture, even though it may be modified by the culturing process to some extent. Propagation of the MyD disease process in culture is particularly important because it will permit detailed electrophysiological studies to be carried out to further characterize the abnormality (ie., determination of ionic basis of resting potential decrease) as well as being an excellent system for future biochemical studies of the specific inborn error of metabolism.

1269 EFFECT OF LONG-TERM ADAPTATION TO ORAL RESPIRATION ON ELECTROMYOGRAPHIC DISCHARGE OF CRANIOFACIAL MUSCLES. Arthur J. Miller and Karin Vargervik*. Ctr.Craniofacial Anomalies, UCSF, San Francisco, California 94143.

Bilateral blocking of the nasal airway in rhesus monkeys altered the neuromuscular activity of several craniofacial muscles. Electromyographic (EMG) activity was recorded from 16 craniofacial muscles in 26 paired animals (13 experimental) during the first 6 months of adaptation to oral respiration. The craniofacial muscles demonstrated two types of EMG patterns: 1)rhythmicity with respiratory pattern; and 2) an increase in spontaneous, uninterrupted discharge (i.e., tonic).

The presence of a rhythmic discharge, its sequence within the respiratory cycle and its mode of developing its maximum discharge were assessed by rectifying and averaging the mean voltage for each craniofacial muscle and a primary respiratory muscle. Five craniofacial muscles were rhythmically active in the normal monkeys: genioglossus (5% of recorded time), dorsal fibers of the tongue (3%), levator labii superioris proprius (10%), elevator of the upper lip (13%), and dilator naris (68%). The experimental animals showed significant increase in the rhythmicity in the tongue and lip elevator muscles (non-parametric Sign Test: $\alpha < .05$). Oral respiration induced rhythmic activity in several other craniofacial muscles: geniohyoid (14%), digastric (7%), anterior temporalis (14%), and lateral pterygoid (10%). The medial pterygoid, zygomaticus and caninus were rhythmically active in 1-2 experimental animals.

Tonicity was determined by rectifying and integrating EMG activity with automatic resetting of the integrator for 10 trials over 2 hours. The intervals between resets were averaged by a digital counter for 1000 intervals, displayed on a scope raster, and computed for a first order histogram. In the control animals, tonicity was present in seven muscles: geniohyoid (45%), digastric (19%), genioglossus (57%), lip elevator (26%), caninus (37%), mentalis (63%), and anterior temporalis (53%). In the experimental animals tonicity in these muscles was not changed significantly but tonicity was induced in several other craniofacial muscles: dorsal fibers of the tongue (35%), superior orbicularis oris (25%), inferior orbicularis oris (32%), medial pterygoid (19%), and lateral pterygoid (14%). The platysma, zygomaticus and buccinator were tonically active in 1-2 experimental animals. (Supported by NIH Grant # DEO 2739).

1270 CLASSICAL CONDITIONING AND SENSITIZATION OF FLEXOR MOTOR UNIT ACTIVITY IN THE SPINAL CAT. K.E. Misulis* and R.G. Durkovic, Dept. Physiol., Upstate Med. Ctr., Syracuse, NY 13210

Decerebrate cats were made spinal by a T10 cord transection. Classically conditioned flexion reflex facilitation was elicited in the manner of Durkovic (Physiol. and Behav. 14:297, 1975). The conditioned stimulus (CS) was electrical stimulation of the cutaneous saphenous nerve at 10/sec for 1.5 sec. The unconditioned stimulus (US) was electrical stimulation of the cutaneous portion of the superficial peroneal nerve at 30/sec for 0.5 sec. Nerve stimuli recruited Aα and Aδ fibers but were below C fiber threshold. Conditioned and unconditioned responses were flexion reflexes measured by an isometric tension transducer attached to the tibialis anterior (TA) muscle tendon. In addition, single motor unit EMG was recorded by means of fine needle electrodes inserted into the free belly of the TA muscle. For conditioning animals, the US was presented during the last 0.5 sec of the CS train. For sensitization animals, the US followed the CS onset by 30 sec. In both paradigms the interval between CS presentations was 60 sec. Five pre-acquisition CS alone trials were presented initially and used as a baseline for comparison of behavior during 15 acquisition trials for both conditioning and sensitization groups. Selection of a motor unit for recording during conditioning or sensitization was based upon the discharge characteristics of the unit in response to the CS before acquisition. The criteria were such that each unit must have fired at least once during the five pre-acquisition trials, in response to any of the first 10 pulses of the CS train. Also, each unit must not have fired to more than about 50% of these stimuli. One unit was examined in each of the 20 conditioning and 20 sensitization animals.

During conditioning, tension in response to the first 10 CS pulses increased an average of 33% compared to pre-acquisition levels. The increase in tension during CS presentations for sensitization animals was 5%. Motor unit activity in response to the CS increased an average of 48% during conditioning and 8% for sensitization animals. The difference between conditioning and sensitization results was highly significant for both tension and unit recordings. For individual motor units there was no evidence of the occurrence of multiple discharges in response to any pulse of the CS train. Thus, a variation in inter-spike interval of doublets or triplets does not appear to play a role in conditioned facilitation in this preparation.

The results indicate that during classically conditioned facilitation of the flexion reflex in the spinal cat, TA alpha motoneurons have an increased probability of firing single action potentials in response to each CS pulse.

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1271 MORPHOPHYSIOLOGY OF THE SUPERIOR VESTIBULAR NUCLEUS IN THE CAT AND RABBIT. AN INTRACELLULAR HRP STUDY. A. Mitsakos*, H. Reisine, and S.M. Highstein.Dept.Neurosci. Albert Einstein Col. Med.,Bronx, N.Y. 10461.

The superior vestibular nucleus (SVN) was explored with HRP loaded microelectrodes in anesthesized, paralyzed cats and rabbits. Stim. electrodes were placed in IIIrd nucleus,and on ipsilateral(Vi) and contralateral(Vc) vestibular nerves.Intracellular responses to the above stims. were obtained, HRP was injected.Animals were perfused, frozen sections reacted with $CoCl^3$-diaminobenzidine, and neurons reconstructed with the aid of a drawing tube.

Neurons responding antidromically to IIIrd nucleus stim. are central or dorsal in SVN.They receive monosynaptic EPSPs from Vi and are either unresponsive to Vc or receive disynaptic IPSPs.Axons of these cells ascend, without collaterals, in the MLF or brachium conjunctivum. These are presumably the relay neurons to IIIrd nucleus subserving vestibulo-ocular reflexes. We hypothesize that neurons receiving monosynaptic EPSPs from Vi are functionally Type 1 but should not be as deeply modulated by natural stimuli as are Type 1 cells receiving Vi-EPSPs and Vc-IPSPs.

Presumed vestibular commissural neurons are central or ventral in SVN and send their axons,without collaterals, across the midline toward the contralateral vestibular nuclei. These neurons receive Vi-EPSPs.

Central SVN neurons also project their axons, without collaterals,to the cerebellum via the brachium pontis.These neurons receive Vi-EPSPs and Vc-IPSPs.

Some neurons, ventral in SVN send their axons ventrally to collateralize in the reticular formation. These neurons receive IPSPs from Vi and Vc and are presumed to be functionally Type IV.

To date, no SVN neuron monosynaptically activated from Vi has an axon collateral within the SVN.This suprising finding implies that the SVN components of the vestibulo-ocular reflexes are organized by descrete pathways straight through the nucleus and shifts the presumptive sites for the integration of information from different planes of space to sites other than the SVN.

1272 THE MOTONEURON AS AN ENSEMBLE AVERAGER OF SPINDLE PRIMARY AFFERENT SPIKE TRAINS. G.P. Moore, R.A. Auriemma and D.G. Stuart
Dept. Biomed. Eng., USC, Los Angeles, CA 90007 and Dept. Physiology, Univ. Arizona, Tucson, AZ 85724.

The intracellular potential of medial gastrocnemius (MG) motoneurons of deeply anesthetized cats was recorded while the MG muscle itself was subjected to continuous band-limited (0-100 Hz) random passive stretches by a servo-puller connected to its tendon. Stretch amplitude had a Gaussian distribution whose rms value was approximately 100 μ. A time-averaged relationship (cross-correlation function) between the imposed length change and the afferent spike train was computed for several different spindle primaries. This function is proportional to the averaged length signal preceding a spike and invariably includes a shortening-lengthening sequence. Its transform in the frequency domain is suggestive of a system sensitive to rate of change of muscle length. Since MG motoneurons are known to receive monosynaptic input from a large percentage of MG primary spindle afferents, the motoneuron potential might be expected to reflect the instantaneous behavior of the ensemble of primary afferent discharge. When the transmembrane potential of MG motoneurons was correlated with the random length signal the resulting functions were nearly identical in waveform to the correlation functions obtained from individual afferents using the same input. Given the characteristics of the input signal, the correlation function provides an estimate of the average change in afferent firing probability and in motoneuron membrane potential which would follow an impulse change in MG length, an input roughly equivalent to a tendon tap. Under these particular experimental conditions, therefore, the motoneuron appears to act as a constant gain element whose input-output gain characteristics are largely derived from the frequency characteristics of spindle primary afferent discharge (a contribution from spindle secondaries with similar characteristics cannot be excluded). We have demonstrated, therefore, that the response of a single motoneuron averaging an ensemble of spindle primary afferent spike trains from its own muscle can be almost indistinguishable from the time average of the response of a single primary afferent.

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1273 EXCITABILITY CHANGES IN SPINAL CORD MOTONEURONS DURING ACTIVE SLEEP. Francisco R. Morales* and Michael H. Chase. Departments of Physiology and Anatomy and the Brain Research Institute, UCLA, Los Angeles, CA 90024.

During active sleep there is suppression of the tone of the somatic musculature. We have observed that this hypotonia (or atonia) is accompanied by sustained membrane hyperpolarization in antidromically identified lumbar motoneurons. In the present study we examined changes in excitability of these motoneurons during active sleep as compared to quiet sleep. Experiments were carried out in undrugged, normally respiring cats employing the technique described by Morales and Chase (Intracellular Recording of Lumbar Motoneuron Membrane Potential during Sleep and Wakefulness, Exp. Neurol., 62:821-827, 1978). The excitability of lumbar α-motoneurons was evaluated (1) by studying the degree of soma-dendritic invasion of antidromically propagated action potentials, and (2) by examining the response of these cells when depolarizing currents were passed through the intracellular microelectrode. Antidromic invasion was studied in 15 cells by delivering short duration, low intensity stimuli to a hind limb peripheral nerve. Intrasomatic records showed that the B (soma-dendritic) spike was either delayed or completely blocked during active sleep. The A (initial segment) spike occasionally exhibited phasic reductions in amplitude. In 11 cells there was an average increase in rheobasic current of 255% (range: 160 to 380%) when active sleep was compared to quiet sleep. This elevation in threshold for unitary spike generation was paralleled by a corresponding increase in threshold for repetitive discharge. These findings indicate the presence of potent postsynaptic inhibition of final common pathway motoneurons during active sleep.

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1274 CHANGES IN SHORT AND LONG LOOP REFLEXES BEFORE VOLUNTARY MOVEMENTS IN MAN. James A. Mortimer, David D. Webster* and Thomas G. Dukich*. Geriatr. Res. Educ. Clin. Ctr., VA Med. Ctr., and Dept. Neurol., Univ. of MN, Minneapolis, MN 55417.

Changes in the magnitudes of short and long latency EMG responses to step increases in load prior to the initiation of a ballistic flexion movement were studied in 8 persons with normal motor function (23-35 yrs). Torques were applied to subjects' forearms through a horizontal support coupled to a pair of DC torque motors. EMG was recorded from bipolar surface electrodes over the biceps and triceps muscles. Subjects were asked to maintain a 90° elbow flexion against a steady 2 Nm load tending to extend the forearm, and instructed to respond to a 2.9 KHZ tone by flexing the forearm as quickly as possible. Torque pulses of 2 Nm amplitude and 500 ms duration tending to extend the forearm were presented at 8 different "delays" relative to the tone (50 and 25 ms prior, at the same time, and 25, 50, 75, 100 and 125 ms following tone onset). For each delay 20 torque pulses were given, and the tone was presented without torque perturbation on 20 trials. EMG responses corresponding to the 9 modes of stimulus presentation were sorted and averaged by computer with torque and position. The average EMG response to the tone in the absence of torque pulses was subtracted from the average EMG responses for each time delay to obtain the response to the torque pulse at that delay. Motor response indices were then calculated by integrating the rectified EMG waveforms from 25 to 50 ms and 50 to 75 ms following the onset of the torque pulse. The two indices thus obtained correspond approximately in time to the M1 and M2 components described by Tatton and Lee.

Reaction times measured from the onset of the tone to the earliest change in the EMG averaged 110 ms. No substantial change was seen in the biceps M1 or M2 index for delays prior to 50 ms following the tone. A large increase in the M2 index occurred at a torque pulse delay of 50 ms and persisted approximately 75 ms. Since the M2 index is computed on the basis of activity beginning at 50 ms, the increase in its magnitude occurs 100 ms following the tone or approximately 10 ms preceding the EMG response to the tone. By contrast, the earliest average change in the M1 index was not seen until 125 ms following the tone or approximately 15 ms after the beginning of the voluntary EMG response. This difference in latency for changes in the M2 and M1 indices was evident in 6 of 8 subjects. In the other 2 subjects, no increase in the M1 or M2 indices occurred before 150 ms following the tone.

The results are consistent with modification of the gain of a pathway mediating the M2 response at a level higher than the spinal cord. Changes in the M1 index may be related to motoneuronal facilitation accompanying the voluntary EMG response.

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1275 FRONTAL LOBE INPUT TO PRIMATE MOTOR CORTEX. Kamel F. Muakkassa* and Peter L. Strick. V.A. Med. Ctr. and Depts. of Neurosurg. and Physiol., SUNY-Upstate, Syracuse, NY 13210.

There is both controversy and confusion concerning the location of "premotor" areas in the frontal lobe which have direct access to the motor cortex. Our experiments sought to define more clearly the origin of frontal lobe inputs to face, arm and leg areas of the primate motor cortex (area 4). Small single or multiple injections of 30% HRP were made into the face, arm or leg areas in the motor cortex in 11 monkeys (M. mulatta and fascicularis). In 5 of these animals the injections were placed after arm, face or leg areas were mapped using intracortical microstimulation.

HRP injections into area 4 (face, arm or leg) resulted in retrograde labeling of neurons in four spatially separate regions of the frontal lobe. For example, HRP injections into the leg area of motor cortex resulted in labeling of neurons in: 1) the superior limb of the arcuate sulcus (caudal bank), 2) the medial bank of the superior precentral sulcus, 3) the supplementary motor area (SMA), caudally, and 4) the ventral bank of the cingulate sulcus, caudally. Labeled neurons were found in both deep and superficial cortical layers. The size of labeled neurons in layer III, where the highest density of labeled neurons was found, varied considerably and included small and large pyramidal cells.

Each of the four "premotor" areas in the frontal lobe is somatotopically organized. HRP injections into the face area of motor cortex resulted in labeling of neurons in: 1) the inferior limb of the arcuate sulcus (caudal bank), 2) the lateral bank of the inferior precentral sulcus, 3) the SMA, rostrally, and 4) the ventral bank of the cingulate sulcus, rostrally. HRP injections into the arm area of the motor cortex resulted in labeling of neurons in the four "premotor" areas between the regions labeled following the face and leg area injections.

Of the four "premotor" areas the greatest density of labeled neurons was always found in the arcuate and SMA regions. Labeled neurons were also seen in the motor cortex and the four "premotor" areas contralateral to the injection sites.

Thus, our results demonstrate four somatotopically organized "premotor" areas in the frontal lobe which project directly to the motor cortex of both hemispheres.

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1276 ABNORMAL SPINAL REFLEXES CONTRASTED IN CEREBRAL PALSY AND ADULT CNS INJURIES. Barbara M. Myklebust*, Richard D. Penn, Gerald L. Gottlieb, Gyan C. Agarwal*. (SPON: RD Penn). Dept. Neurosurg., Rush Med. Coll., Chicago, IL 60612.

The EMG activity evoked by sudden perturbations at the ankle differ in normal subjects, cerebral palsy (CP) patients, and patients with adult-onset damage to the CNS. In the normal adult, dorsiflexion produces a stable myotatic reflex in the stretched triceps surae (TS) muscle at 40-45 ms, while the anterior tibial (AT) muscle remains electrically silent. Plantarflexion evokes a much more variable response in the stretched AT muscle at 80-120 ms, while the TS group is silent.[1]

Thirteen of 14 patients (ages 7 to 27 years) with spastic CP with or without athetosis showed simultaneous responses, at the latency of the myotatic reflex, in the antagonistic AT and TS muscles following forced dorsiflexion. Plantarflexion evoked an abnormally early response (40-45 ms) in the AT muscle. The one patient who did not show this pattern was the only subject who was independent in ambulation and able to participate in sports.

Eight patients (ages 18 to 57 years) were tested who had adult-onset injuries to the CNS, including multiple sclerosis, stroke, incomplete spinal cord transsection, and head trauma. As with the CP patients, clinical evaluations demonstrated hyperreflexia and increased tone in all cases; clonus was frequently present. Seven patients demonstrated an abnormally early response in the AT muscle with plantar stretch, but they did not have co-activation of AT and TS muscle following dorsal stretch. In one patient who had several strokes during the 25 years prior to testing, dorsiflexion elicited coactivation of antagonists.

These results confirm that patients with spasticity have abnormal spinal reflexes; while the clinical signs may be similar in patients with cerebral palsy and adult-onset CNS injuries, the evoked spinal reflexes are different in these two types of patients. The time of onset of injury with respect to the maturation of the CNS may be a significant factor in the development of coactivation of antagonistic muscle groups. Finally, cerebral palsy is a disorder of the brain and of the spinal cord, which may involve abnormal spinal circuitry resulting from a CNS insult during the perinatal period.

Supported by United Cerebral Palsy Education and Research Foundation.

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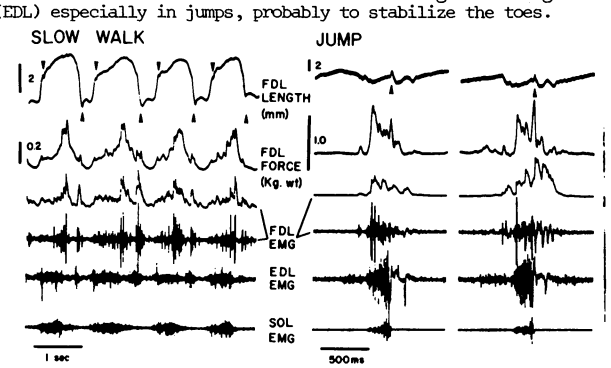
1277 THE INTEGRATION OF BALANCE POSTURAL ADJUSTMENTS INTO THE WALKING ACTIVITIES OF NORMAL HUMAN SUBJECTS. Lewis M. Nashner. Neuro. Sci. Inst., Good Samaritan Hosp. & Med. Cntr., Portland, OR 97209.

This experiment is the first of a series which will examine the neural processes integrating stabilizing postural adjustments into the locomotor activities of human subjects. The six degree-of-freedom platform (used previously for stance postural studies) has been incorporated into a walkway so that the trajectory of the support leg could be perturbed unexpectedly in a number of different ways and at different phases of the step cycle. The EMG adjustments occurring within the interval 100-175 ms after the onset of perturbations were found by calculating the difference between the EMG waveforms of perturbed and unperturbed trials.

The patterns of EMG adjustments of four leg muscles, gastrocnemius and tibialis anterior of both legs, were statistically consistent among six subjects tested. The organizational characteristics of them can be summarized as follows: Stabilizing adjustments elicited by perturbing the support leg during the mid-stance phases of the step cycle (the leg carries the full vertical load) were organizationally similar to those elicited by the imposition of comparable platform perturbations during quiet stance. In contrast, only perturbations imposed upon the leading leg elicited EMG adjustments during the double-support phases of the step cycle, and the adaptive characteristics of those adjustments were quite different than those evident during quiet stance or the mid-stance phases of locomotion.

1278 FORCE PRODUCTION, LENGTH CHANGES AND EMG ACTIVITY IN FLEXOR DIGITORUM LONGUS (FDL) MUSCLE DURING WALKING AND JUMPING IN UNRESTRAINED CATS. M. J. O'Donovan*, R. P. Dum and R. E. Burke. (Spon: K. Frank) Lab. of Neural Control, NINCDS, NIH, Bethesda, MD 20205. The FDL muscle in cat acts on the distal phalanx to plantar flex the toes and protrude the claws. Since its motor unit organization (Dum et al., Neuroscience Abstr. 4:294, 1978) differs somewhat from the ankle extensors for which force data have been reported (Walmsley et al., J. Neurophysiol.41:1203, 1978), we used similar methods to study FDL output in intact cats. In addition to force and EMG recordings (ibid.), we measured length of the FDL proper with a mercury-in-silastic transducer attached to the tibia near the origin of FDL fibers, with the distal end sewn onto the FDL tendon of insertion. All leads came to a connector on the cat's back and data was recorded on FM magnetic tape synchronized to videotape records of the animal's movements. Gauges were calibrated in situ under anaesthesia. During walking (below left), FDL force peaks at less than 20% of maximum tetanic output (1.5 - 2 kg) during the last 1/3 of stance, after a period of active lengthening. A 2nd burst of EMG activity often occurs at the end of stance as FDL rapidly shortens,producing positive work around toe liftoff (upward arrow; downward arrow denotes toe touchdown). Jumping involves both larger force peaks from FDL (1 kg; below right) and smaller length changes than during treadmill walking. FDL force suddenly rises as the foot begins to extend (start of soleus(SOL) burst) and peaks again as the toes lift off (arrow). There is co-contraction of FDL and extensor digitorum longus (EDL) especially in jumps, probably to stabilize the toes.



1279 VISUALLY INDUCED ADAPTIVE CHANGES IN OCULOMOTOR CONTROL SIGNALS. L. M. Optican* and F. A. Miles. Lab. Neurophysiol., NIMH, Bethesda, MD 20205.

The neural commands underlying saccadic eye movements have two components: a dynamic component, or pulse (p), which rapidly moves the eye to a new orbital position, and a static component, or step (s), which then holds it there. If these two components are not appropriately matched the eye drifts away from the new fixation point. Such post-saccadic drift is evident in patients with VIth nerve palsies of recent origin (Kommerell et al., Invest. Ophth., '76) and in monkeys recovering from surgical detachment of the extraocular muscles (Optican & Robinson, Soc. Neurosci., '77), but is corrected over a period of days. This has led to the suggestion that an appropriate match between the pulse and step components is maintained by an adaptive mechanism which responds to the post-saccadic slip of the retinal image. We sought to determine whether post-saccadic retinal slip alone was sufficient to elicit this adaptation. Experimental subjects were two rhesus monkeys. Each was seated, with its head fixed, before a translucent screen (subtending 100° x 100°) on which a densely featured image was back-projected. A servo-controlled mirror galvanometer in the projector's light path allowed the scene to be moved horizontally under computer control. The animals' eye movements were monitored with the Robinson search coil method and fed into the computer. Immediately after each saccade, the computer caused the scene to drift horizontally, with an exponential time course (40 ms time constant), by an amount equivalent to 45% of the amplitude of the horizontal component of the saccade. In some experiments the scene was made to drift in the same direction as the saccade and, in others, in the opposite direction. After several hours of this optically-imposed post-saccadic slip, both monkeys developed post-saccadic ocular drift which was always in the same direction as the imposed visual drift and was evident both in the dark and when the animals viewed a stationary visual scene. The pulse-step mismatch (psm), defined as $[(p-s)/p]100\%$, was used to provide an estimate of the animals' post-saccadic drift: positive values indicate backward drift, negative values, forward drift. Before exposure to the stimulus, the mean psm was -0.3 $\pm$ 1.9% (N=48). After 8 hours of exposure to opposite-direction slips the mean psm was 5.5 $\pm$ 2.3% (N=58, range 0.6 to 10.3%) and the mean psm following same-direction slips was -10.0 $\pm$ 4.6% (N=60, range -2.8 to -23.1%). The time constant of the ocular drift was similar in both situations, with a mean of 69 $\pm$ 25 ms (N=39). We conclude that the step components of saccadic eye movements are matched to the pulse components by adaptive mechanisms which, at least in part, sense post-saccadic retinal image slip.

1280 VESTIBULO-OCULAR REFLEX (VOR) IN THE SQUIRREL MONKEY: EFFECTS OF HORIZONTAL CANAL (HC) INACTIVATION. G. Paige* (Spon: J.M. Goldberg.) Dept. Pharmacolog. & Physiolog. Sci., Univ. Chicago, Chicago, IL 60637.

Eye movements in squirrel monkeys were recorded, in darkness, before and after unilateral HC inactivation, obtained by a plugging procedure (Money & Scott, 1962). Monkeys were rotated sinusoidally in the HC plane, within a bandwidth of .01-4.0 hz, and at constant amplitude (40^o/sec). Amplitude intensity series ($40-360^o$/sec) were presented at .02 and .2 Hz.

In normal monkeys, VOR gain is relatively flat and averages .8 over the recorded bandwidth. Phase lead (re velocity) is near 0^o from 4. to .1 Hz, increasing to near 45^o at .01 Hz. The effective time constant (T_e) of 17 sec is 2-3 times greater than that typical of peripheral vestibular afferents (Goldberg and Fernandez, 1971). Intensity series data display gain and phase linearity at .2 Hz. Phase lead at .02 Hz decreases from ~25^o at 40^o/sec to ~12^o at 120^o/sec, with little further change at higher amplitude. No gain change accompanies this effect.

Recordings made within 2 days of HC plugging indicate that VOR gain is near 50% of normal. Gain and phase linearity is normal. Spontaneous nystagmus (SN), with slow phase ~10^o/sec to the plugged side, is always present.

During the month following HC plug, three signficiant modifications of the VOR are observed: a) generalized gain recovery (to ~75% of normal); b) increased low frequency phase lead (.01-.05 Hz), usually accompanied by gain reduction, such that T_e is reduced to near that of peripheral afferents; and c) SN is greatly reduced.

Animals recovering from HC plug in a normal cage environment display most of the observed modifications during the first week. If either vision or head movement is prevented during this time, gain remains virtually fixed. However, reduction in T_e is always observed. SN is still present and is often enhanced or erratic.

Recordings from peripheral afferents of HC-plugged monkeys are consistent with the notion that the canal plug effectively eliminates canal function, leaving tonic vestibular input intact.

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1281 STABLE SIMULTANEOUS SINGLE UNIT RECORDINGS FROM GROUPS OF MOTOR CORTICAL NEURONS IN THE UNANAESTHETIZED AND UNRESTRAINED CAT. C. Palmer*, M.J. Bak, G.M. Dold* and E.M. Schmidt. Lab. of Neural Control, NIH, Bethesda, MD 20205.

Simultaneous recordings of nearby single neurons in the forelimb area of cat motor cortex have been obtained on closely spaced microelectrodes in the freely moving animal during a variety of movements and manipulations. The microelectrodes, modified from a previous chronic electrode design in use in this laboratory (Salcman, M., Bak, M.J. Med. Biol. Eng. 14:42-50), consisted of prefabricated groups of 2 or 3 electrodes with intershank distances of from 200 μ to 75 μ and lengths of 1.1 to 1.5 mm to record in laminar V. The location of the forearm area was established during surgery using cortical surface stimulation. Stimulating electrodes were implanted in the pyramidal tract to identify PT neurons. All the electrode locations were later histologically confirmed. Unit activity was monitored with muscle electrical activity (EMG) from four forelimb muscles, biceps brachi (BIC), triceps brachi (TRI) palmaris (PAL) and extensor digitorum communis (EDC), the elbow angle was recorded with a saline filled length gauge. The data was recorded on tape synchronized with videotape records of movements. The animals were also implanted with indwelling intra-jugular cannulas, so that each unit's sensory field could be confirmed under light barbiturate anaesthesia. Fig. 1 shows 2 units recorded on microelectrodes 120 μ apart, during free fall from a height of 40 cm. Foot contact was obtained with a plate containing a piezoelectric crystal. Both units had cutaneous input from the lateral margin and dorsum of the foot pad yet the units were relatively silent during foot contact indicating that in certain movements there may be gating of sensory input to the motor cortex.

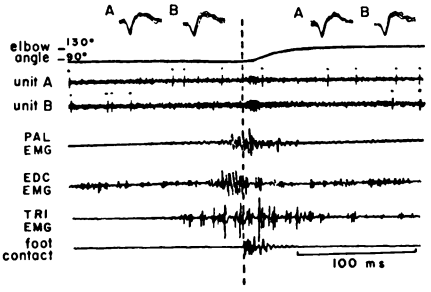

Fig. 1. 2 cortical units A and B, at the top of each section shown unit shape is before and after foot contact (----).

1282 EFFECT OF MIDBRAIN LESIONS ON VESTIBULO-NECK REFLEXES. Costas Pappas* and John H. Anderson. Depts. Phsl., and Otolaryngol., Sch. Med., Univ. of Minn., Minneapolis, MN 55455.

The control of head posture is important for stabilizing gaze and whole body posture relative to gravity. For this, vestibular reflexes which are dependent upon stimulation of the vertical semicircular canals and otolith organs play a major role. In order to quantitate the vestibular contributions, one can define the relation between input accelerations and the neck muscle (EMG) responses in restrained animals. Recently, Anderson and Pappas (Soc. Neuro. Abst., 1978) have attempted this, more specifically to characterize the overall reflex dynamics in alert, restrained cats during sinusoidal roll and pitch rotations. The results indicated a) both vertical canal and otolith inputs make significant contributions to the motor responses and b) both "direct" and "indirect" pathways must be involved. Regarding the latter, it should be noted that after chronically sectioning the descending MLF (2-8 day prior to recording), it has been found that there is a profound disturbance of the reflex dynamics: For low frequency (less than 0.15 Hz) pitch rotations the phase of the muscle response regarding angular acceleration shows much less of a lag, approaching that of the canal afferents themselves (Anderson and Pappas, European Neuro. Soc. Abst., 1979). Compared to normal, this indicates that a response to the otolith inputs and to a neural integration of the canal inputs was reduced.

To account for this we postulate that the midbrain reticular formation, i.e., the region of the interstitial nucleus of Cajal (INC) and the rostral interstitial nucleus of the MLF, is necessary for the proper convergence of otolith and canal inputs and for the neural integration of vertical canal inputs (Pola and Robinson, J. Neurophysiol. 41:245-259, 1978). The processed signals could possibly then be carried over the interstitial-spinal or MVST pathways in the MLF. As a first test of this, we made bilateral, electrolytic lesions in the region of INC in 4 cats. 2-8 days thereafter we recorded the neck EMG responses of the biventus cervicus muscle when that cats (alert, restrained, and blindfolded) were subjected to sinusoidal pitch rotations. The results from all these animals (throughout the 8 day period) were similar to that of the MLF lesioned cats: For low frequency the EMG showed much less of a lag, e.g., 80-110 deg at 0.15 Hz instead of 130-140 deg in the normal. At the higher frequency (1.0-3.0 Hz), the phases were approximately normal. These data thus do provide some support for the proposed role of the midbrain nuclei. To further test this, experiments are now in progress whereby we are attempting to selectively destroy only the neuron populations, leaving fibers in passage undisturbed, by using the neurotoxic agent, kainic acid.

1283 DOUBLE REPRESENTATION OF THE DISTAL FORELIMB IN CAT MOTOR CORTEX: ANATOMICAL AND PHYSIOLOGICAL DEMONSTRATION, C.L. Pappas* and P.L. Strick, (SPON: J.B. Preston) Depts. of Physiol. and Neurosurg., SUNY-Upstate and V.A. Med. Ctr., Syracuse, N.Y. 13210

The representation of body parts in the motor cortex has been traditionally expressed as a distorted map of the body displayed on the cortical surface. In this map, each body part is represented only once. The results of recent experiments have questioned the concept of a single representation of body parts.

Using intracortical microstimulation (ICMS), we have found two spatially separate representations of the distal forelimb in area 4γ of the cat motor cortex. The two representations were most clearly seen in maps of digit responses. Two "digit zones", where ICMS at threshold evoked contractions limited to digit musculature, were seen in all animals tested. Although their absolute location varied, the two "digit zones" were always separated by a field from which responses of more proximal musculature were evoked. In some animals separate wrist zones also could be demonstrated adjacent to each "digit zone". Shoulder representation tended to surround the areas where more distal responses were evoked. EMG monitoring during ICMS demonstrated that the same digit muscles could be represented in both "digit zones".

We next sought to anatomically demonstrate the two digit representations. A number of anatomical studies have demonstrated that callosal connections originate from all areas of the motor cortex except those which contain the representation of distal musculature. We reasoned that the shape and location of distal forelimb representation in area 4γ ought to be revealed by the absence of callosal connections. We reexamined the origin of callosal connections in area 4γ of the cat motor cortex using retrograde transport of HRP. Two spatially separate "callosal holes", where labeled neurons were either absent or of markedly low density, were found within the forelimb region of area 4γ. While the absolute location of the "callosal holes" varied among animals, the holes were consistently separated and surrounded by areas with many labeled neurons.

To provide direct evidence that the "digit zones" fall within "callosal holes", we combined ICMS and EMG recording with retrograde transport of HRP in 3 animals. A small lesion was made in each physiologically defined "digit zone" in cats previously injected with HRP into the contralateral motor cortex. The lesions were found within the anatomically defined "callosal holes".

In summary, a double representation of the distal forelimb can be defined in area 4γ of the cat motor cortex using both anatomical and physiological methods.

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1284 MOTOR UNIT PROFILE OF MOUSE SOLEUS. David J. Parry* and Donald M. Lewis* (SPON: K. C. Marshall). Dept. Physiol. Univ. of Bristol, England.

Studies on single motor units have revealed a rather good correlation between mechanical properties and histochemical fibre type. (Edstrom & Kugelberg, 1968; Burke et al., 1973) The mouse soleus does not show the fibre type composition characteristic of this postural muscle in other species. It contains only about 40% of fibres exhibiting staining properties of type I fibres i.e. low alkali-stable myofibrillar ATPase coupled with high oxidative enzyme activity. The remaining fibres are also high in oxidative capacity but stain darkly for ATPase. These latter fibres have variously been labelled red, C, IIA, FOG, or II_{ox}, and appear at least in the cat, to be relatively fatigue resistant, fast-twitch fibres. (FR type of Burke et al., 1973.) We have examined the motor unit profile of soleus muscles of normal mice of the strain C57 Bl/6J, employing conventional ventral root splitting techniques. Mean motor unit size, expressed as a % of whole muscle twitch tension was about 5% (range 2.2 to 10.5%), suggesting that the muscle contains about 20 motor units. No correlation was found between the size of units and their times to peak tension. The time to peak tension of whole soleus muscles range from 13.1 to 21.6 msec and in 45 motor units a flat distribution with range of 7 to 37 msec was obtained. No evidence of a division into two groups of fast and slow units as observed by Close (1967) in rat soleus was observed. 32 of the 45 units (i.e. about 70%) had times to peak of less than 20 msec, the upper limit of the faster group of units seen by Close in rat soleus.

Burke, R. E., Levine, D. N., Tsairis, P. and Zajac, F. E., (1973). J. Physiol. 234, 723-748.
Close, R. I. (1967). J. Physiol. 193, 44-55.
Edstrom, L. and Kugelberg, E. (1968). J. Neurol. Neurosurg. Psychiat. 31, 424-433.

Supported by grants from the Muscular Dystrophy Group of Great Britain and the Muscular Dystrophy Association of Canada. D.J.P. was the recipient of a Canadian MRC Visiting Scientist Award and was on leave of absence from Dept. Physiol. Univ. of Ottawa, Ontario, Canada.

1285 SOMATOTOPY IN DEEP CEREBELLAR NUCLEI REVEALED BY ACTIVE MOVEMENT OF INDIVIDUAL JOINTS. J. Gavin Perry* and W. Thomas Thach. Dept. Anat. & Neurobiol., Wash. Univ. Sch. Med., St. Louis, MO 63110.

A method was developed for examining somatic representation in motor systems. Monkeys were trained to make rapid alternating movements of the lower leg and each of four joints of the arm. Every eight alternations, the monkey was rewarded with juice and his mouth would perform a sixth (licking) movement. Colored lights informed the monkey as to which joint to move and when. Monkeys were over-trained (10 months) and therefore made the minimum movement (as determined by EMG) necessary to obtain a reward. EMGs were recorded from 30 muscles over one side of the body while the monkey performed the task. This was done before and after a three month period of unit recording. The EMG data showed that the different movements involved different muscles and that the movements were constant from day to day except for a slight slowing towards the end of the experiment.

Single units of both the dentate and interpositus nuclei were recorded while the monkey performed the task. Over a three month period nearly 640 units were recorded in 63 penetrations in one monkey. Discharge from every unit large enough to isolate was recorded on tape along with electrical signals representing flexion and extension timing for each movement.

Of 22 penetrations through the dentate nucleus, 9 contained units related to mouth movements. These were all clustered in the posterior half of the nucleus. Two penetrations in the anterior third of dentate contained only leg related units. Another 5 penetrations in the anterior third contained some units related to leg only, and others to arm and leg movements. The remaining penetrations across the middle third of the nucleus contained units related to the various arm movements. Although clusters of units relating to a specific joint were seen, there was no obvious somatotopic mapping of the proximo-distal dimension in this experiment.

Interpositus was similar to dentate, with mouth units posterior and leg and arm units anterior. Further experiments with better control of movements may reveal finer grain somatotopic representation in these nuclei.

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1286 INTERACTION OF VESTIBULAR AND NECK REFLEXES IN CONTROL OF NECK MUSCLE ACTIVITY. B. W. Peterson, J. H. Fuller, G. Bilotto and V. J. Wilson. The Rockefeller University, New York, N.Y. 10021.

Reflex stabilization of the head during angular rotations in the horizontal plane was studied in precollicular decerebrate cats with their heads tilted 28° forward from the stereotaxic plane in a holder that allowed rotation of the head about a vertical axis passing through the C_1-C_2 joint. Head angular rotation or torque about this axis was measured together with EMG activity of neck muscles that produce horizontal head movement (obliquus capitis inferior, splenius, complexus). Rotational movements were modulated either by single sinusoids or by sums of 8 sinusoids with frequencies ranging from 0.01-2 Hz.

Two reflexes were found to contribute to stabilization of the head in the horizontal plane: the vestibulocollic reflex (VCR) and a neck-neck reflex (NNR) in which neck rotation induced compensatory contraction of neck muscles. When the VCR was elicited alone by whole body rotation with the head fixed, it caused EMG activity like that observed by earlier workers (eg: 114° lag between leftward angular acceleration and peak activity of right muscles at 0.2 Hz) while head torque lagged 10° behind EMG. When the head was allowed to move during body rotation, compensatory head rotations occurred which lagged 162° behind acceleration at 0.2 Hz. Although the amplitude of head rotation was always less than half that of the turntable stimulus, EMG amplitude decreased by as much as 80%.

The NNR, measured in isolation by rotating the body upon a stationary head, behaved as a first order lead system. At 0.2 Hz and below, maximum activity of right neck muscles was in phase with peak leftward deviation of the neck (ie: rotation that lengthened the right neck muscles). Above this frequency phase advanced and gain increased. The NNR therefore acts as a "neck stretch reflex" which in the present situation counters movement of the head relative to the body. A stretch reflex of the neck musculature has been described by Bizzi et al. (J. Neurophysiol. 41:542-56, 1978) in the alert monkey where, however, it made only a minor contribution to compensation for loads applied to the moving head. In our preparation the NNR was an important factor in the large decrease seen in neck muscle activity during body rotation with the head free. It also appears to contribute significantly to opposing movement of the head on a fixed body where the NNR and VCR work together. The high reflex resistance to movement in this latter situation suggests that the combined action of the NNR and VCR may be important to compensate for inertial and mechanical instability of the head-neck system.

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1287 PATTERNS OF POSTURAL SUPPORT DURING LIMB MOVEMENT. A. Polit* and J. Massion*(SPON: E. Bizzi)C.N.R.S. I.N.P. Marseille, France

When a standing quadruped makes a limb movement, a change in posture occurs which permits the execution of the movement without loss of balance. Previous work has shown that when the limb movement is triggered by a tactile/proprioceptive stimulus on a leg the weight supported by both the stimulated forelimb and the contralateral hindlimb decreases while that in the other diagonal pair increases. This pattern has also been observed in dogs making hindlimb or forelimb movements[1] and has been observed during stimulation of motor cortex in cats.[2] Several lines of reasoning suggest that this pattern is organized at bulbo-spinal levels as are those for locomotion.

Five cats were used in these experiments. The animals were trained to stand quietly with their paws on platforms which measured vertical force. Moveable platforms, right and left, were used to elicit a placing reaction by a forelimb. The animals were also conditioned to lift either forelimb on command using a preparatory tone followed by a beeping tone and flashing light to trigger the movement. The pattern of postural adjustment during this type of movement was different from that during placement: weight transfer was primarily between the forelimbs, although there was sometimes a symmetrical increase in weight under the hindlimbs. Placement movements elicited after training the cats to lift on command tended to be less diagonal and approached the pattern observed for conditioned movements.

In 3 cats the motor cortex was electrically stimulated shortly after the signal eliciting a movement was presented. Cortical points in the forelimb area were used. The pattern of postural support accompanying the stimulation-induced movement was less diagonal when the lifted limb was the one that was to be used in the conditioned movement than when the stimulation-elicited lift involved the contralateral limb. This response asymmetry disappeared during control sessions in which the cat was required only to stand quietly.

These experiments suggest that postural commands accompanying movement target final positions not only for the cat's center of gravity, but also the torsion on the vertebral column, and that a "tuning" of the output circuits preceeds overt movement. In addition, the use of conditioned movements in a postural context provides an interesting approach to the study of mechanisms by which new patterns of postural support are acquired.

1) Ioffe, M. and A. Andreyev (1969) Zh. Vyssh. Nerv. Deyat. Pavlova,19,557-565.
2) Gahery, I. and A. Nieoullon (1978) Brain Res., 149, 25-37.

1288 CONTRIBUTION OF INTRAFUSAL VISCOELASTICITY TO MUSCLE SPINDLE ADAPTATION. R. E. Poppele, D. Quick*, and W. R. Kennedy. Lab. of neurophysiology and Dept. of Neurology, University of Minnesota, Minneapolis, MN 55455

Adaptation of muscle spindles to stretch is thought to be largely due to a viscoelastic relaxation of the intrafusal muscle bundle. Indeed, it has been shown that dynamic bag fibers (bag_1) in particular do exhibit such viscoelastic relaxation following a quick stretch. This is a mechanical adaptation which will result in a phase lead in the response of the receptor to sinusiodal stretches, a fact which provides an opportunity to determine whether the mechanical adaptation is quantitatively adequate to account for receptor adaptation. Measurements of the phase lead of responses to small amplitude sinusiodal stretches were made in isolated cat tenussimus spindles. Strain (or the change in length per unit length) across the primary sensory area of bag fibers was plotted as a function of time along with the primary afferent discharge rate for applied stretch frequencies from 0.05 to 2 Hz. The results suggest that only a portion of the phase lead exhibited by the primary afferent can be accounted for by muscle viscoelasticity. The maximum phase lead of sensory strain occurs in the neighborhood of 0.2 Hz where it is still approximately 30^{o} less than the phase lead exhibited by the primary output. At 1.0 Hz, the sensory strain is in phase with the applied stretch, whereas the primary ending output has a phase lead of 90^{o} (at 25^{o} C). Therefore if adaptation in spindle receptors is to be quantitatively accounted for by mechanical factors, these must be associated with structures within the sensory area and not with the viscoelasticity of intrafusal muscle poles.

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1289 EVIDENCE FOR SKELETOFUSIMOTOR (BETA) EFFECTS DURING CHANGES IN MUSCLE LENGTH AND FORCE IN THE DECEREBRATE CAT. E.M. Post*, W.Z. Rymer, Z. Hasan*, (SPON: J. Horel). Dept. Neurosurgery, SUNY-Upstate, Syracuse, N.Y. 13210.

Last year we presented evidence that reflexively-induced increases in isometric spindle receptor discharge rate occurring after extrafusal threshold was reached could be attributed to beta fibers. To better appreciate the nature of beta effects, an understanding of their action during changing muscle length and force is necessary. The purpose of this present study was to identify possible beta influence on spindle receptor discharge during constant velocity stretch and isotonic shortening and to correlate these results with those isometric responses presumed to be of beta origin.

We recorded from triceps surae spindle receptor afferents in isolated dorsal root filaments of 13 decerebrate cats. Decerebrates were used so that variations in initial muscle force could be induced in a quasi-physiological manner via crossed extensor stimulation (CES). The receptor-bearing muscles were subjected to stretch with and without CES, and to reflexively-induced isotonic shortening with the muscle stretcher providing an electronically simulated load.

Of the 60 units examined with ramp stretch, 36 showed additional increases in discharge during CES. 3 response patterns could be discerned: 1) an increase in discharge during the ramp (11/36) termed dynamic type, 2) an increase in discharge during the pre- and post-ramp periods only (16/36) termed static type and 3) a combination of 1 and 2 (9/36), static-dynamic type. 10 of these 36 units showed a late rate acceleration during stretch not paralleling the unstimulated response. Since gamma fibers are relatively unresponsive to stretch, these units seemed to be the most likely candidates for beta activity. Supportive evidence for a beta contribution was provided in that 7 of these 10 units showed isometric acceleration.

Isotonic shortening was studied in 28 units. The isometric responses of these units proved to be an accurate predictor of their behavior during shortening; 6 of the 7 units that decreased their rate with increases in isometric force also showed a diminution in rate (x=8.65 ips/mm, 3.4-20.9) with shortening while 8 of the 11 units that isometrically accelerated increased their rate (x=9.38 ips/mm, 1.1-32.4) with shortening. Because beta action should induce parallel activation of intra-and extrafusal fibers, a limited decline or even an acceleration in rate during shortening would be anticipated.

It appears that identification of beta fibers may be possible by discerning a constellation of response patterns during isometric and dynamic states.

1290 RESPONSES OF ASCENDING TRACT OF DEITERS' (ATD) NEURONS TO NATURAL AND ELECTRICAL STIMULI. H. Reisine and S.M. Highstein. Dept.Neurosci.,Albert Einstein College of Medicine, Bronx, New York,10461.

The ventro-lateral vestibular nucleus (VLV) was explored with intra- and extracellular microelectrodes in anesthesized, paralyzed cats. Stimulating electrodes were placed in the IIIrd nucleus and on the ipsi-(Vi) and contralateral (Vc) vestibular nerves. The MLF and brachium conjunctivum (BC) were acutely severed in the pontine tegmentum. Neurons in the VLV,antidromically activated by IIIrd nucleus stimulation received monosynaptic EPSPs from Vi and disynaptic IPSPs from Vc. Neurons with the above profile of responses were injected with HRP and subsequently were invariably found to lie in the VLV.

Antidromic activation of VLV neurons was signalled by an antidromic field potential in VLV. This potential was abolished by lesions 1 mm wide between the MLF and BC in the pontine tegmentum indicating the course of the ATD.

In a second set of experiments decerebrate cats were rotated about a vertical axis in the plane of the horizontal semicircular canal. Stimulating electrodes were implanted as above. VLV neurons were isolated extracellularly and identified as ATD neurons by their antidromic responses to IIIrd nucleus and orthodromic (monosynaptic) responses to Vi stimulation. Responses of these neurons are in phase with table (head) velocity in the range of $\pm$ 30^{o}/sec. at 0.4 - 0.65 Hz increasing to ipsilateral and decreasing to contralateral rotation (Type 1 responses). This head velocity signal should be transmitted directly to medial rectus motoneurons by the ATD and undoubtedly contributes to the horizontal vestibulo-ocular reflex in intact cats. The remaining reflex following bilateral MLF lesions may be accounted for, in part, by the head velocity signal carried by the intact ATD.

1291 AVOIDANCE TRAINING OF A SUSTAINED RESPONSE AFTER DORSAL RHIZOTOMY IN RATS. Barbara E. Rodin* and Doreen Berman. Dept. Psych., Queens College of CUNY, Flushing, N.Y. 11367

It has been suggested that acquisition of movement, without sensory feedback, may occur by pairing efferent commands with feedback from environmental contingencies. However, the types of instrumental movements previously reported in dorsal rhizotomized (DR) limbs have been discrete and of short duration. Although the efferent command system alone may be adequate for the acquisition of any number of discrete movements, it is hypothesized that it would be insufficient for the acquisition of sustained responses. These presumably require a mechanism for detection of passive return of the limb to resting position. To test this, rats were trained in a shock avoidance task requiring sustained alteration in baseline limb position. Performance of unilateral DR limbs was compared with that of contralateral intact limbs.

Two groups, each consisting of eight surgically treated (T13-L6, left) rats, were used. One group was trained preoperatively with both limbs and retested a month after surgery; the other group, without preoperative experience, received initial training a month postoperatively. Half the animals in each group were trained first with the left and half with the right limb. During training, animals were suspended over an electrolyte solution, without vision of the hind limbs. An electrode for monitoring limb position was attached to one hind-foot. Whenever the electrode entered the solution, escapable shock was delivered to the trunk. Avoidance could be achieved by maintaining the position of the monitoring electrode above the surface of the solution. Number of insertions and time in solution were recorded, and animals were run to a predetermined criterion. Training sessions were twenty minutes long.

With sensate limbs, sustained alteration in limb position was acquired rapidly both before and after surgery. With DR limbs, however, acquisition of the sustained response failed to occur under all conditions but one. The appropriate response was achieved in DR limbs only when order of postoperative testing or training (to criterion) was intact limb: DR limb. Acquisition was more rapid when there had been preoperative experience in addition to contralateral limb training. However, comparing the DR with the intact limb, many more sessions were required for acquisition, and a different response topography was found. When avoidance was not achieved, animals escaped shock with the DR limb via a series of rapid discrete movements.

Sensory feedback is apparently necessary for the acquisition of sustained movements of this type in rats, unless there has been recent performance of the response by the contralateral intact limb.

1292 THE ACTIVITY OF RETICULO-SPINAL CELLS DURING LOCOMOTION IN CATS. S. Rossignol and J. Parent*. Centre de Recherche en Sciences Neurologiques, Département de physiologie, Université de Montréal Montréal, Québec, CANADA, H3C 3T8.

Reticulo-spinal cells were recorded extracellularly at the medullary and pontine levels during treadmill locomotion in precollicularly decerebrated cats. Cells were identified by high frequency antidromic stimulation through a thin silver strip inserted under the spinal cord at the level of L1. EMGs of the Quadriceps and the Triceps Radialis were recorded on both sides. The cervical vertebrae were solidly fixed and all 4 limbs were allowed to move. A good number of cells were kept for several hundred cycles allowing to record their activity at different walking speeds (walk and trot) as well as during manual blockade of one or more limbs. Of the 31 cells recorded, 27 (87%) were modulated while 4 (13%) remained silent or were silenced during locomotion. Cells discharging in distinct bursts showed a positive correlation between the burst duration and the duration of the step cycle at various speeds, indicating a relation to a component of the step cycle which changes with speed namely the stance phase, the swing phase being considered practically constant at various speeds. The peak firing of different cells in relation to the step cycle was found to vary. The stability of the firing pattern of a given cell was tested by varying the walking speed which in a number of cases changed the phase relationship between fore- and hindlimbs as well as by blocking the limbs manually. In some cells carefully analyzed by computer it was possible to show, when triggering the analysis on the EMG of one forelimb, that the firing pattern was invariant when the forelimbs were walking alone or with the right or the left hindlimb and even when the coupling changed between fore- and hindlimbs. This strong dependence of the cell discharge on the forelimbs could be seen even more clearly in some cells when blocking the forelimbs which stopped the firing although the hindlimbs continued walking. At times when the animal initiated a period of locomotion with the hindlimbs, the cells started firing only later when the forelimbs also walked. Although cells could discharge with only the forelimbs walking, the frequency of discharge often changed when one or both hindlimbs walked. The strong dependence on forelimbs of the discharge of reticulospinal cells with axons identified in the lumbar cord might suggest that they play a role in the coordination of fore- and hindlimbs during locomotion.

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1293 **Absence of Force-Feedback Contributions to the Stretch Reflex of the Decerebrate Cat.** W.Z.Rymer, Northwestern University Medical School,Chicago, Ill. and Z.Hasan, SUNY-Upstate, Syracuse, N.Y.

The reflex force evoked by stretch of the soleus muscle in the decerebrate cat is proportional to the length increment, and is largely independent of stretch velocity. It has been suggested that this spring-like behaviour arises from the combined effects of force and length feedback, derived largely from tendon organ and spindle receptor input respectively[1].

We examined the contributions of force-feedback to stretch reflex action with the help of Dantrolene sodium, an agent known to reduce contractile force via excitation-contraction decoupling. Experiments were performed in the soleus muscle of 12 decerebrate cats. The calcaneal insertion was severed, and attached to a servo-regulated muscle stretcher. Emg signals were recorded differentially using fine wires immplanted in the muscle, and the emg was rectified and filtered with 25 msec. time constant. Force and length were also measured.

In each animal, a series of stretches of constant amplitude and velocity were used to record force amd emg responses, over a range of initial forces. Initial force variations were induced via the crossed extensor reflex. The animal was then given successive 1.0 mg doses of Dantrolene intravenously, until peak twitch tension was reduced by at least 30%. The stretch reflex responses were then reexamined, again using crossed extensor stimulation to vary initial force levels. In every case, reflex stiffness was markedly reduced, yet there was no significant increase in the emg incremental response. Moreover, the lack of emg change was not a result of Dantrolene effects on muscle spindles-- the monitored response of single spindle receptor afferents showed no significant alteration until much larger cumulative doses of Dantrolene were achieved (typically greater than 5mg/kg). Were force-feedback to contribute to the spring-like behaviour, a reduction in contractile force should have been followed by an increase in emg. Since no such increase occurred, it is concluded that force-feedback contributions are insignificant in this preparation.

1 Nichols, T.R. and Houk,J.C. 1976: The improvement in linearity and the regulation of stiffness that results from actions of the stretch reflex. J.Neurophysiol. 39: 119-142

1294 EFFECT OF TOPICAL ANESTHESIA TO VARIOUS SKIN AREAS ON THE H-REFLEX. Mohamed A. Sabbahi*, Whitney R. Powers*, and Carlo J. De Luca (SPON: J.V. Basmajian) Dept. of Health Sciences, Sargent College, Boston University, Boston, Ma.

The H-reflex was recorded every 5 seconds with surface electrodes located over the soleus muscle, using the method of Sabbahi Awadalla (1976). The achilles tendon reflex (ATR) was elicited by an electrically activated solenoid plunger which produced strikes of equal force to the tendon, and the reflex response was recorded every 10 seconds via the same electrodes. Topical anesthetic (20% Benzocaine) was sprayed to the appropriate skin areas for 10-20 sec. and recordings were taken at time intervals for a total of 40 minutes. Anesthetic was applied to the skin overlying the calf, tibial, quadriceps and hamstring group of muscles in twelve normal subjects, and to the skin of the whole lower limb in three other subjects. The H-reflex response was recorded before (control) and after the application of the anesthetic.

The H-reflex was significantly increased in amplitude after application of the anesthetic to the skin overlying the calf, hamstring and quadriceps muscles. This reflex facilitation continued to increase for the duration of the experiment. Thirty minutes after the anesthetic was applied to the skin overlying the calf muscles the peak-to-peak amplitude of the H-reflex increased by 76%; when applied over the hamstring muscles, by 200%; and when applied over the quadriceps muscles, by 167%. The degree of reflex facilitation varied from one subject to another; in one subject it was more than 600% of the control value after 40 min. of the application of the anesthetic.

When the anesthetic was applied to the skin overlying the anterior tibial muscle, reflex inhibition was recorded. After 30 minutes, the peak-to-peak amplitude of the H-reflex was 60% of the control value. However, the degree of inhibition was less significant in the first 15 min. after application of the anesthetic.

When the whole lower limb was sprayed with anesthetic, the reflex facilitation occurred. The amplitude of the H-reflex increased by 111% after 30 minutes.

Neither the M-response nor the ATR showed significant changes after the application of the anesthetic. These results do not support the findings of Hagberth et al., 1963, but are consistent with our previous work (Sabbahi Awadalla, 1976). These results in association with the results of other studies of the H-reflex recovery curves currently in progress in our laboratory, indicate that the skin receptors modulate the H-reflex via a central mechanism. All of the skin areas of the lower limb except that directly over the antagonist muscle (Tibialis anterior), supply inhibitory inputs to the motorneurons of the calf muscles.

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1295 MOTOR CORTICAL UNIT ACTIVITY RELATED TO SEVERAL CONDITIONED HINDLIMB FORCES IN THE MONKEY. S.A. Sahrmann, M.H. Clare, and W.M. Landau. Dept. Neurol., Sch. Med., Washington U., St. Louis, Mo. 63110

Rhesus monkeys were conditioned with light signals to perform four randomly selected tasks with the hindfeet. Force was exerted bilaterally on fixed bars with attached strain gauges. The tasks were: (1) to exert a strong plantarflexion force, hold it a random time, on cue exert a strong, brief, rapid dorsiflexion force and return to rest (rapid-relax); (2) to do the converse; (3) to plantarflex into a small force window, holding until signalled to return to rest; and (4) to perform the converse of (3).

Preliminary analysis of 86 cortical cells is presented. Based on data for 200 msec. before (pre-Cn) and after (post-Cn) the achievement of force criterion, six types of cortical neurons have been identified: Plantarflexor cells (1) maximal activity occurred preceding the achievement of the required force (phasic cells, 27%), and (2) those with strong activity continuing during the hold period (tonic cells, 20%); (3) dorsiflexor phasic cells, 22%; (4) dorsiflexor tonic cells, 20%; (5) cells whose major activity related to the time of initiation of and maintenance of small plantarflexion hold, 8%; and (6) cells whose greatest firing occurred during the return to rest at the end of each task, 3%.

Average resting discharge frequency (ADF) was calculated for the 500 msec. preceding the presentation of the first signal of each task. Among plantarflexor cells, most phasic cells had low ADFs (56% less than 13/sec.) while only a few of the tonic cells had low ADFs (21%). There were higher ADFs for the majority of dorsiflexor cells (64% tonic, 73% phasic).

Phasic cells (69% plantarflexor and 87% dorsiflexor) fired faster in the initiation of the rapid-relax action than in the initiation of the hold action. During the 200 msec. post-Cn discharges continued at a faster rate (greater than 10% difference) after rapid-relax action than with hold action (64% of all cells). Less than 50% of the cells showed greater activity with a strong hold force than with a small hold force.

1296 COMPARISON OF MORPHOLOGICAL FEATURES OF FAST AND SLOW PT CELLS REVEALED BY INTRACELLULAR PRESSURE INJECTION OF HRP. H. Sakai* and C.D. Woody. (SPON: E. Eldred) UCLA Medical Center, Los Angeles, CA. 90024.

Recordings were obtained from the motor cortex of 14 awake cats using 4% HRP-1M KCl filled electrodes. Morphological features of 19 PT cells were analyzed. Eight of the cells were also injected with 1mM cGMP. Intracellular injections were made with a pressure of 60-90 psi applied for 0.5-2 sec following assessment of the response of each cell to antidromic stimulation. The cells were recovered by core biopsy. The pyramidal tract was stimulated ipsilaterally at the level of the facial nucleus (P. 8.0; L. 1.3; V. -10) using concentric, bipolar electrodes (two, 300 Hz pulses of 0.03 msec duration delivered every sec). Distance from the recording site in the coronal-pericruciate cortex to the locus of antidromic stimulation was between 4 and 5 cm. Cells which were activated antidromically with latencies shorter than 2 msec were classified as fast PT cells (N=11) with conduction velocities of at least 20m/sec. Cells activated with latencies longer than 2.5 msec were classified as slow PT cells (N=6). Two cells with latencies between 2.0 and 2.5 msec could not be classified into either group.

The following observations were made:

1) The diameters of axons measured at a level 10-20μm beyond the axon hillock were larger in the fast conducting PT cells (correlation coefficient, r=0.738 between axon diameter and conduction velocity).
2) All PT cells with somas recovered (N=17) were pyramidal cells located in layer V. Fast PT cells had larger somas (30-70μm) than did slow PT cells (18-30μm). This confirms the result of Naito et al. (Brain Res. 1969) obtained by methyl blue staining.
3) The lateral extent of the dendritic field within layer V was greater in fast PT cells than in slow PT cells.
4) Slow PT cells had high densities of spines on dendrites in layer III in agreement with Labelle and Deschenes (Brain Res. 1979), but counterstaining revealed that one-third of the fast PT cells injected with HRP without cGMP did also. Thus the density of spines in layer III was not as reliable an index for separating fast and slow PT cells as soma size and axon diameter.
5) Electron microscopic examination done collaboratively with C. Ribak, U.C. Irvine, showed dense amounts of HRP reaction product within neurons without spread of HRP into the extracellular space. Although the internal structures were obscured by reaction product, the membranes and synaptic junctions were well preserved. (Supp. by HD 05958 & E. Gruen)

1297 VOLUNTARY MOVEMENT AND EXCITABILITY OF HUMAN EYEBLINK REFLEXES. Jerome N. Sanes* and James R. Ison. Dept. Psychology, University of Rochester, Rochester, NY 14627.

This study reports on changes in eyeblink reflexes when humans perform voluntary phasic and sustained movements with either the reflex agonist or remote muscle groups. In response to a reaction stimulus subjects either briefly blinked or contracted forearm muscles or began sustained contractions of orbicularis oculi or forearm muscles. Eyeblink reflexes were elicited before and after movement. Surface electromyographic activity (EMG) was recorded from orbicularis oculi muscle and eyeblink reflexes were elicited by percutaneous shocks to the supraorbital branch of the trigeminal nerve. The reflex consists of a 12 msec latency ipsilateral response (R1) and a 30-50 msec latency consensual response (R2). A disynaptic trigemino-facial path mediates R1, a polysynaptic path through ponto-medullary reticular formation mediates R2.

Prior to both movements, at the onset of reaction stimuli R1 increased, then increased further beginning 75 msec before the reaction, peaking at 25-10 msec before voluntary EMG onset. During this period R2 was moderately suppressed. Nearly immediately following onset of phasic voluntary movement R1 was maximally enhanced and then declined almost to normal after about 400 msec. R2 was further suppressed, with a trough at 50-100 msec, and recovered by about 400 msec after movement onset. Tonic movements produced the initial R1 enhancement and also a final but considerably reduced stable facilitation; the R2 function nearly approximated that found with brisk movements. However, for tonic eyelid activity only, R2 showed a secondary potentiation peaking about 1500 msec following EMG onset.

These findings (1) partially substantiate the claim that reflex excitability is a sensitive and specific measure of pyramidal tract and other supraspinal influences on motoneuronal pools before and after voluntary movement; (2) further elucidate characteristics of reflex recovery after onset of movement; and (3) reveal differential influences of voluntary action on separable neuronal pathways. Enhancement of R1 in relation to agonist muscle activity is understandable in light of findings of pyramidal tract neuronal activity, presumed to influence the final common motoneurons, before and during voluntary movement. Changes accompanying remote muscle activity possibly reflect activity in collateral pathways or action of a more generalized motor readiness system. Reflex suppression was the predominant effect on R2, even though its final common path was potentiated, as shown by increased R1 strength. The locus of this net inhibition must lie in the longer associative pathways for R2 elaboration. Thus these data illustrate net facilitative and inhibitory effects by the same behavior on reflexes with identical afferent and efferent limbs but distinctive central pathways.

1298 FACILITATION AND SUPPRESSION OF FORELIMB MUSCLE ACTIVITY FROM SINGLE INTRACORTICAL MICROSTIMULI IN BEHAVING MONKEYS. S. Sawyer*, P.D. Cheney, R.F. Martin and E.E. Fetz, Dept. of Physiol. and Reg. Primate Res. Ctr., Univ. of Wash., Seattle, WA 98195

The effects of single precentral microstimuli on activity of 12 identified forearm muscles were documented in monkeys making active wrist movements. Stimulus-triggered averages (StTA's) of rectified EMG activity detected subthreshold facilitation and suppression of muscle activity produced by intracortical stimuli (.2 ms, biphasic), using stimulus intensities too weak to evoke overt responses (5-10 μa) and repetition rates too low to involve temporal summation (≤15/s). When applied at cortical sites of cells producing post-spike facilitation in spike-triggered averages of EMG activity, stimulus pulses evoked a pattern of post-stimulus facilitation (PStF) identical to, but more intense than the pattern of post-spike facilitation.[1] As stimulus intensities increased from lowest effective levels (ca. 1 μa) up to 10 μa the magnitudes of post-stimulus effects increased, generally preserving the relative amplitude in each muscle. Above 15 μa stimuli often affected additional muscles and evoked more complex effects. (In comparison, trains of repetitive microstimuli (400 pps; 50ms) under the same conditions evoked stronger and more widespread effects. With the monkey at rest, the threshold response to repetitive microstimuli was usually activation of the muscle which had shown the greatest PStF in StTA's.) The PStF usually appeared in several synergist muscles, even for minimal stimulus intensities. Post-stimulus suppression (PStS) was sometimes obtained in antagonists of the facilitated muscles and occasionally also in synergists. From certain cortical sites suppression was obtained without evidence of facilitation in any muscles, in both stimulus- and spike-triggered averages. The mean latency of strong PStF was 7.6±0.8 ms (mean±SD), and of strong PStS was 8.2±1.3 ms. In mapping studies 10 μa stimuli were delivered at successive points along histologically reconstructed electrode penetrations into the precentral bank. Corticospinal cells were identified by retrograde transport of HRP from cervical cord. As a function of cortical depth, the amplitudes of post-stimulus effects in different muscles typically increased and decreased together, being strongest from layer 5. In tracks tangential to layer 5 the amplitude of PStF in individual muscles exceeded half-maximal values over distances of 850±180 μm; PStS was evoked from wider zones, with a half-width of 1600±480 μm. A given muscle was often facilitated from multiple sites, usually in combination with different sets of synergists. These results suggest that output effects elicited from specific precentral sites typically affect multiple muscles and may include inhibition.[1,2]

1. Cheney & Fetz, Neurosc. Abst. 3:269; 2. Jankowska, Padel & Tanaka, J. Physiol. 249:637 & 258: 467.

1299 CNS SIGNAL TO MUSCLE: PARAMETERS OF MOVEMENT REPRESENTED IN EMG DURING RAMPS AND HOLDS. Marc H. Schieber* (SPON:M.H.Clare).Dept. Anat. & Neurobiol., Wash. Univ. Sch. Med., St. Louis, MO 63110.

Two Rhesus monkeys were trained to perform wrist movements guided by a continuous pursuit tracking display. The target, and therefore the wrist, moved in a hold/ramp/hold track. Parameters of the target's motion were selected from preset values, directing the monkey to make a specific wrist movement for a fruit juice reward. Maintained torque load, initial holding position, ramp velocity, and final holding position were individually varied between blocks of trials while all other parameters remained constant. (Ranges: load, 0.00 to 0.24 Nt-m in either direction; position, 60° flexion to 30° extension; velocity, 8 to 29°/sec in either direction.) Bipolar electromyograms (EMGs) were full-wave rectified, digitized and integrated for analysis.

Extensor digitorum communis (EDC) was studied in greatest detail since it consistently gave the largest EMGs. Its pattern of activity was similar to that of all forearm muscles studied. During extension ramps with no imposed load, EDC showed a progressive increase in discharge as the wrist was progressively extended. This changing activity was correlated with instantaneous wrist position. Similarly, discharge during the holding periods before and after the ramp correlated with the maintained position of the wrist. EMG amplitude did not change with ramp velocity over the range tested, except when the muscle shortened against a large load. During the hold prior to flexion ramps EDC's activity was again correlated with wrist position. But during flexion ramps with no imposed load, EDC was silenced as the ramp began and remained silent until the self-paced return extension. When the monkey performed extension or flexion ramps under maintained torque loads, EDC's activity was the sum of two components: that activity present under no load; plus a maintained change in the level of activity, proportional to the load.

The representation of wrist position in EDC's activity during holds and extension ramps may be due both to the muscle working against elastic restoring forces exerted by the soft tissues of the forearm and wrist, and to the decreasing ability of the muscle to generate tension as it approaches its minimum length (length-tension relationship). The absence of a velocity representation in the EMG (except when shortening against load) implies that both the effective viscous load, and the motoneuron signal controlling velocity per se, are relatively small. No co-contraction of opposing muscles was detected in the present experiment. Nor was there any difference in muscle activity during the holding periods, dependent on the direction of the impending or completed ramp.

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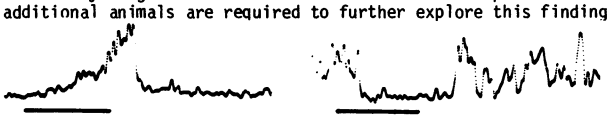
1300 EXCITATION AND INHIBITION OF FOREARM MUSCLES EXPLORED WITH MICROSTIMULATION OF PRIMATE MOTOR CORTEX DURING A TRAINED TASK. E.M. Schmidt and J.S. McIntosh. Lab. of Neural Control, NINCDS, NIH, Bethesda, MD 20205.

Monkeys were trained to make alternate wrist flexion and extension movements against different loads at up to 6 different wrist positions. Conventional microelectrodes were used to explore the forearm area of the precentral cortex for cells that were temporally correlated to the task. The cell's sensory field, response to torque pulses, and whether or not the cell projected into the pyramidal tract was determined prior to intracortical microstimulation (ICMS) through the recording electrode. Trains of 17 stimuli (.2 ms biphasic pulses at 400 Hz and current levels to 20 μa) were delivered at specific times during the task when the motoneuron pools to the forearm muscles were involved in different phases of the movement. By applying the ICMS under the same load and movement conditions repeatable EMG responses in forearm flexors and extensors were obtained. ICMS produced a larger excitation when a movement was being initiated against a constant load than when the muscle had been opposing the same load for approximately 1 sec. The time delay between the start of the ICMS train and excitation or inhibition of forearm muscles was as short as 11 ms. Quite often when excitation was produced, a ramp increase in EMG activity was recorded as shown in Fig. 1A. This was not due to cortical reverberating circuits because the EMG activity would end abruptly 11 ms after the last stimulus pulse of the train. Areas of inhibition of a given muscle have been observed as close as 100 μm to areas of excitation of the same muscle. EMG patterns produced by ICMS have ranged from inhibition of a single muscle (Fig. 1B), inhibition and excitation of synergists, inhibition and excitation of antagonist, to excitation of single muscles. There is some indication that excitatory regions to a specific muscle are bordered by inhibitory regions to the same muscle. Experiments on additional animals are required to further explore this finding.



A B

Fig. 1. Rectified and averaged (32 trials) EMG responses in flexor carpi radialis (FCR) to ICMS in precentral cortex. The calibration bars below the records (40 ms) indicate the time and duration of stimulation. (A) Ramp increase in EMG activity of FCR during movement. (B) Inhibition of FCR at a site 4 mm from A.

1301 PROCESSING OF CENTRALLY PROGRAMMED MOTOR COMMANDS. B.T. Shahani and R.R. Young. Clinical Neurophysiology Laboratories, Neurology Department, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts 02114

Central programs subserving the execution of stereotyped fast elbow flexion movements during a visual matching task produce a triphasic EMG pattern: an initial burst in the agonist biceps (Ag1) followed by activity in the antagonist triceps (An1) during which there is electrical silence in the agonist (Ag1-Ag2 interval) and finally a second burst in agonist (Ag2) which brings the arm to the target. The present experiment was designed to identify mechanisms at the segmental and/or suprasegmental level which could modify this "centrally programmed" EMG activity. Unloading of muscle spindles in the agonist at random intervals resulted in prolongation of the duration of Ag1 and An1 and decrease in the Ag1-Ag2 interval when a 2 kg weight was electromagnetically released 20-60 msec prior to initiation of Ag1 in seven normal subjects. With training, four subjects could overcome changes in these parameters and produce normal triphasic pattern during unloading. The timing (20-60 msec) of these changes and subjects ability to overcome them as well as their similarity to changes noted in patients with cerebellar deficits, suggests a suprasegmental site (perhaps cerebellum) for modulation of centrally programmed EMG activity.

1302 A MAPPING OF BRAINSTEM NEURONS PROJECTING TO THE SPINAL CORD IN THE GOLDEN HAMSTER. Randall C. Shults and James D. Rose. Dept. Psychol., Univ. of Wyoming, Laramie, WY 82071.

As part of an investigation into the brainstem control of estrous reflexes in the female hamster, brainstem neurons projecting to the spinal cord were identified with the horseradish peroxidase (HRP) technique. A 50% solution of HRP was pressure injected into one side of the cord at the upper cervical, lower cervical, or lumbar levels. After a three day survival period, the brains were processed by the technique of Mesulam (J. Histochem. Cytochem., 1978). Injections at all cord levels yielded labeled cells predominantly in the following regions: (1) medulla; caudal lateral tegmental field, nucleus reticularis gigantocellularis, and Deiter's nucleus, all principally ipsilaterally, as well as nucleus raphe' pallidus; (2) pons; nucleus reticularis pontis caudalis, ipsilaterally, nucleus reticularis pontis oralis, bilaterally, and a ventrolateral reticular cell group in the contralateral anterior tegmentum; (3) midbrain; contralateral red nucleus, and a few cells in the reticular formation, bilaterally. The number of cells labeled in these regions diminished progressively as a function of more caudal HRP injection placement. Relatively more neurons, especially ipsilaterally, were labeled in the medial reticular formation of the anterior medulla and caudal pons with more anterior injections. The density of contralaterally labeled cells, however, was more constant with all levels of HRP injection. In addition to labeling many more cells, upper cervical injections also resulted in labeling of some contralateral deep tectal neurons. Some neurons in the midbrain central gray and Edinger-Westphal nucleus had projections to the cervical cord.

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1303 BEHAVIORAL RELATIONS OF MEDULLARY RETICULAR FORMATION CELLS. Jerome M. Siegel and Rebecca L. Wheeler*. Sepulveda VA Med. Ctr. and Dept Psychiatry, Sch. Med., UCLA, Los Angeles, CA 90024.

Behavioral and photographic techniques were used to determine the movement correlates of discharge in medullary RF units recorded in unrestrained cats. These cells could be divided into two major classes on the basis of the behavioral correlates of their discharge; cells with laterally asymmetrical movement relations, and cells with laterally symmetrical movement relations. Fifty-four percent of encountered cells (n=21) had laterally asymmetrical movement relations while 38% (n=15) had laterally symmetrical movement relations. The remaining cells (n=3, 8% of total) did not relate to any specific motor behavior.

The largest subgroup (n=16) of cells with laterally asymmetrical movement relations was comprised of cells related to movements of the head or vertebral column in the horizontal plane. Of these, 12 responded when the head was moved passively. In all of these cells, increased discharge was related to passive head movement to the contralateral side. None responded to passive movement to the ipsilateral side. This asymmetry is highly significant ($p<.0005$). Cells responding to passive head movement to the contralateral side did not discharge when the same movement was made spontaneously by the cat. Instead these cells discharged only when the cat turned to the ipsilateral side.

Cells without lateralized behavioral relations (n=15) could be divided into those related to head or spinal movements (n=12) and those related to other movements. Of those related to spinal movements, six discharged in relation to active forward movements of the head when it was in a lowered position, a seventh cell discharged only during active ventral movements of the lumbar spine, and an eighth discharged during fixation of the lumbar region. Four responded during spontaneous upward movements of the neck. Three of the four cells that responded to passive head movement responded only when the head was moved in a direction opposite to the optimal spontaneous movement.

The behavioral relations shown by these cells indicate that many may have a pattern of synaptic relationships that coordinates excitation and inhibition of several motoneuron pools involved in producing ipsilateral movement. The present results may reveal the cellular mechanism underlying the classic "tegmental response" of ipsilateral curvature of the spinal column after electrical stimulation of the RF.

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1304 MOTOR CAPABILITIES OF THE CHRONIC SPINAL CAT: RECRUITMENT OF SLOW AND FAST EXTENSORS OF THE ANKLE. J.L. Smith, L.A. Smith*, and K.L. Dahms*. Dept. Kinesiology and Brain Research Institute, UCLA, Los Angeles, CA 90024.

Myopotentials from two ankle extensors, the soleus (SOL) and the lateral gastrocnemius (LG) were recorded from chronic spinal cats transected at T_{13} either at 2 or 12 weeks (W) of age. Four cats in each group were exercised daily on a motorized treadmill for 10-20 min, and testing was completed 4-6 mo post-surgery. With the forelimbs and trunk suspended above the treadmill, all cats fully supported the weight of their hindquarters during stepping. Most cats periodically used their hindlimbs unassisted during quadrupedal standing and overground locomotion. Muscles were implanted with electrode wires under sterile operating conditions as described by Betts, et al (Brain Res. 117:529, 1976). The electromyography (EMG) was synchronized with the cat's movement by a video system and recorded on FM tape for subsequent integration and period analyses by minicomputer (Smith, et al, J. Neurophysiol. 40:503, 1977).

During treadmill locomotion, the SOL was recruited at all speeds (0.13 to 0.89 m/s), which elicited the entire range of gaits from a slow walk to a slow gallop. The peak amplitude of the rectified-averaged (RA) EMG, which in normal adult cats is correlated with peak tendon forces measured in vivo (Walmsley, et al, J. Neurophysiol. 41:1203, 1978), increased about 20% for SOL and about 300% for LG over the range of speeds tested. During unassisted standing and stepping overground, the SOL was recruited alone, and the RA-EMG was similar to that recorded during slow treadmill stepping.

When the cats were held vertically with hind legs pendant, air stepping was recorded at 3-5 c/s, and the SOL was active generally without the LG. Clonus, elicited by tendon taps or during slow treadmill stepping and standing, ranged from 10-15 c/s and was predominant in the SOL.

During rapid and alternate ankle movements of 10-14 c/s, produced by sticking tape to the plantar pads, the LG alone was active, as is the pattern for normal adult cats (Smith, et al, Neurosc. Abst., 1976), only in those cats in which the contraction time (CT) of the SOL, measured at sacrifice, was >50 ms. However, in those cats in which the SOL had a CT of < 50 ms, both LG and SOL were recruited simultaneously. All weight-bearing 2W cats had "fast" (21-40 ms) SOL, while all but one of the weight-bearing 12W cats had a "slow" (50-58 ms) SOL (Edgerton, et al, Neurosc. Abst., 1979). Recruitment of a "fast" SOL would not slow the rhythm of rapid paw shaking, and the variation in segmental input to motoneurons may reflect this fact.

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1305 USE OF THE (^{14}C)2-DEOXYGLUCOSE TECHNIQUE TO STUDY OCULOMOTOR FUNCTION: METHODS AND PRELIMINARY RESULTS. David Sparks, Lawrence Mays and T. L. Hickey. Univ. of Alabama in Birmingham, Birmingham, AL 35294.

Macaque monkeys, trained to make repetitive saccades, were used to examine alterations in the metabolic activity of neurons in brain regions known to be involved in the generation of eye movements. Monkeys were taught to follow a saccade target presented on a large screen oscilloscope. After acquisition of the target and a variable fixation interval, the target was moved instantaneously to a new position, requiring another acquisition saccade and fixation interval. Since trials were presented without an intertrial interval and water reinforcement was delivered during the fixation period, the number of spontaneous saccades was minimized. For example, one monkey made over 2000 5^{0} rightward saccades and over 700 15^{0} leftward saccades during a 45 minute period. The number of saccades with other vectors occurring during this period was negligible.

After extensive training, the unanesthetized monkey (with indwelling intravenous catheter) was placed in the experimental chamber, the head restrained, and the tracking task begun. Horizontal and vertical eye position signals (Fuchs and Robinson, 1966) were sampled at a rate of 500/sec throughout the 45 minute session and stored on a 9-track digital magnetic tape. Thus, it was possible to reconstruct the vector of each eye movement that occurred during the experimental session. If satisfactory performance continued after an initial injection of physiological saline, a rapid injection of (^{14}C)2-deoxyglucose, 75 μCi/kg in 1.5 ml of saline was given. At the end of the 45 minute tracking period, the animal was anesthetized with Nembutal and perfused intracardially with 3.3% phosphate buffered formalin at pH 7.4. The brain was removed, blocked, and slowly submerged in Freon 22, chilled by an acetone/dry ice mixture.

Autoradiographs revealed increased levels of metabolic activity in expected oculomotor areas of the brain stem: the oculomotor, trochlear and abducens nuclei; pontine oculomotor regions; and the cerebellum. Sections from other oculomotor areas are currently being processed. Selective labelling was particularly noticeable in the cerebellum, with specific regions of discrete folia showing increases in activity. The midline vermis region showed generalized increases in label, somewhat denser in the nodulus and the deeper folia of lobes, V, VI, and VIIIB. Laterally, discrete patches of label were observed in lobules IVC and VIIIA of the vermis, in the simple and paramedian lobules and in Crus IIa. All folia of the flocculus examined showed dramatic, but uniform, increases in metabolic activity.

In combination with appropriate behavioral procedures, the (^{14}C)2-deoxyglucose technique (Sokoloff et al., 1977) appears to be suitable for investigations of the oculomotor system.

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1306 TOPOGRAPHICAL ORGANIZATION OF ASCENDING CEREBELLAR PROJECTIONS FROM THE DENTATE AND INTERPOSED NUCLEI IN MACACA MULATTA. Gregory Stanton. Dept. of Anat., Coll. of Med., Howard Univ., Washington, D.C. 20059.

Discrete elcrolytic lesions were placed in the dentate and interpositus cerebellar nuclei and the resulting anterograde degeneration was stained with the Wiitanen technique. The dentate and interpositus anterior (IA) nuclei project topographically onto the somatotopically organized divisions of the red nucleus and the ventral lateral-ventral intermediate nuclei of the thalamus (VL-Vim). Caudal parts of the dentate project to face areas in medial parts of the parvocellular red nucleus (RNpc) and VL-Vim, rostrolateral parts to central arm areas in these nuclei. There is a small projection from the rostroventral dentate to lateral hindlimb areas in RNpc and VL. Dorsal parts of the dentate project to ventral parts of RNpc and medial VLc-Vim whereas ventral parts of the dentate project to dorsal parts of RNpc (with some overlap ventrally) and dorsomedial parts of VLc. The dorsoventral topography of dentate terminals in VL-Vim may be related to proximal-distal musculature representation in these thalamic nuclei. Dentate projections to the nucleus of Darkschewitsch and the thalamic nuclei VA, area "X", and VLps also appear to be topographically organized. These nuclei are related by afferent and/or efferent connections with the premotor cortex.

Caudal and rostral parts of IA project to medial (forelimb) and lateral (hindlimb) areas in the magnocellular red nucleus (RNmc) and VLc-Vim. Projections from IA are greatest to distal musculature areas in VL-Vim. No projections from the interpositus posterior nucleus (IP) to the red nucleus could be identified but projections to the nucleus of Darkschewitsch and dorsal parts of VLc were found suggesting a functional relationship of IP to the proximal limb musculature and premotor areas of the cerebral cortex.

1307 CENTRAL AND PERIPHERAL CONTROLS OF SWIMMING IN ANURAN LARVAE. Donald J. Stehouwer* and Paul B. Farel. Dept. Physiol., Sch. Med., Univ. N. Carolina, Chapel Hill, NC 27514.

The isolated nervous system of the bullfrog tadpole spontaneously exhibits episodes of patterned motoneuronal bursting at intervals ranging from about 30 to 180 sec. Each episode is a few seconds to 60 sec in duration and consists of alternating bursts between each segmental pair of ventral roots in the thoracic, lumbar, and tail regions of the spinal cord. This patterned activity is found only in the medial division of the ventral roots, whose axons terminate in the axial musculature used for swimming. The lateral division of the eighth, ninth, and tenth ventral roots innervates the limb musculature and shows only a continuous burst beginning with the onset of each episode. It is believed that the alternate bursting of the medial rootlets reflects motoneuronal activity associated with swimming and that the discharge from the lateral rootlets is associated with the hindlimb extension that is normally observed during swimming.

The period of the medial rootlet bursts within each episode varied, but was usually about 100 to 500 msec. On each side of the cord, bursts were synchronous the entire length of the cord. Midline sections of the cord at various levels and of varying length suggested that the mechanisms generating the alternate bursting were present throughout the spinal cord. However, it was also found that patterned bursting was maintained across a divided section of the cord if, and only if, a more rostral section of the cord was intact. This suggests that patterned activity arising in rostral segments can be conducted caudally down each side of the sectioned cord, but that patterned activity generated caudally is not conducted rostrally.

Electromyograms obtained from intact, freely-swimming tadpoles were virtually identical to the root recordings obtained in vitro with respect to the rate of alternation. Unlike the in vitro preparation, these EMG's revealed a rostro-caudal phase lag. Neither peripheral destruction of the lateral line organs nor section of lateral line nerves eliminated the phase lag. Complete spinal deafferentation, leaving the lateral line system intact, was effective in eliminating the phase lag. It appears that the basic program for swimming is generated entirely within the spinal cord at the segmental level, that coordination between segments is accomplished rostrocaudally, and is modified by dorsal root input.

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1308 TIMING BETWEEN HAND TREMOR OSCILLATION AND EXTENSOR EMG MODULATION AT THE TREMOR FREQUENCY. Robert N. Stiles. Dept. Physiol. and Biophysics, Univ. Tenn. Center. Health Sciences, Memphis, TN 38163.

Merton, in his classic work of 1953 on servo-control of movement (The Spinal Cord, pp. 247-255. Churchill.), proposed that the position of a limb may be controlled by a negative feedback, servo-loop mechanism (a length servo) involving primarily the stretch reflex. He also noted that, for certain limbs, the long time lag around the servo loop would have to be compensated for by derivative, or rate, feedback if oscillations of these limbs were not to occur. Merton further noted that the muscle spindles are well adapted to provide that compensation because of their very marked response to the time rate of change of muscle stretch. The question of whether the rate feedback normally does compensate for the time delay in the servo loop has been considered since by many different workers. However, it appears that the question is still unanswered.

A measure of the time lag in the servo loop(s) can be obtained for postural hand tremor by performing cross-covariance analysis of the demodulated extensor EMG and the hand motion. Using this procedure, the delay (in msec) between when the peak upward position of the oscillating hand occurred and the time later that the maximum (peak) of the amplitude modulation of the extensor EMG occurred was calculated. This time difference (designated Δt) was determined for nine normal human subjects. For seven of the nine subjects, the Δt increased from control values of between 30 and 40 msec for control tremors with root-mean-square (rms) displacements of about 50 micra, to values as large as 70 to 80 msec for hand tremors with rms displacements of between 10,000 to 30,000 micra. One subject's tremor increased very little in displacement amplitude over two different 60-min periods that the subject maintained the unsupported hand in a horizontal position. For the 56 16-sec records analyzed for this subject, the Δt values increased very little above control levels of about 30 msec. For the remaining subject, the Δt values changed very little as the subject's tremor amplitude increased from control levels of about 40 micra to its largest value of 6,000 micra. For eight of the nine subjects, the displacement amplitude of postural hand tremor appears to depend in part upon the time lag in the neural feedback pathway(s). (Supported in part by USPHS Grant NS 14730.)

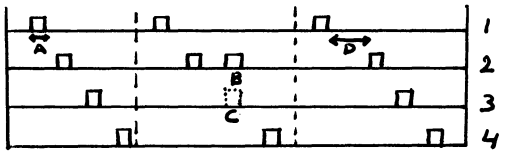
1309 **AUTOMATED AND QUANTITATIVE ASSESSMENT OF SOFT NEUROLOGICAL SIGNS.** **Cornelis L.J. Stokman. Dept. Child Psychiatry, N.Y.S. Psychiatric Institute, New York, N.Y. 10032.**

Soft neurological signs, i.e. signs without clear neuropathological focus, are associated with various forms of neuropsychiatric dysfunction. Certain soft signs show an excess prevalence in children with learning disabilities and conduct disorders as well as in schizophrenic adults. However, the high number of false positives and false negatives severely limits the value of soft signs for diagnostic or screening purposes. Operational definitions, explicit scoring criteria, normative data, etc., which are largely lacking, are essential to improve the diagnostic and predictive value of such signs.

This report illustrates the use of automated response devices, developed by the author, and response recording to operationally define and measure selected clinically important motor signs (thumb-finger apposition, hand pronation-supination, heel-toe tapping, hopping) and relevant response characteristics such as speed and frequency, errors of omission and commission, intensity or duration, rhythmicity, and asymmetry.

Data are presented on inter- and intrarater reliability in normal adults and adolescent psychiatric in-patients. For a pilot group of nine adolescent psychiatric in-patients rank order correlation coefficients for total score were significant between raters both within test ($r = 0.77$; $p < 0.01$) and retest ($r = 0.93$; $p < 0.01$) sessions, and within rater for test-retest (rater 1: $r = 0.92$; $p < 0.01$, and rater 2: $r = 0.77$; $p < 0.01$).

Figure 1 presents a drawing of the response measures for the thumb-finger apposition test, with various response characteristics such as duration (A), errors of commission (B) and omission (C), and arhythmicity (D, variable interresponse time).



1310 VISUAL ASPECTS OF POST-DEAFFERENTATION ATAXIA AND DYSMETRIA. D.E. Teodoru,* T.A. Tran,* J. Herskovic,* and A.J. Berman. Department of Neurosurgery, V.A. Hospital, Bronx, N.Y. 10468

Five preoperatively trained dorsal rhizotomized (DR) monkeys performed a visually cued reaching task within two months of surgery (D. Berman, et.al., 1978). Testing was performed in the dark and the visual cue was brief so as not to permit visual guidance of the limb. We now report that these same monkeys, in a lighted room, did not reach and grasp a food pellet from a 1.0 cm diameter platform until the fourth post-operative month. At that time, the movements used for food retrieval were severely ataxic and dysmetric. The response pattern soon changed, however, to a rapid, ballistic movement. The arm was "thrown at the target," as described by Gilman et.al. (1976) The trajectory of the movement became smooth and accurate as dysmetria and ataxia diminished. In a further test of motor function, the DR animals were required to reach horizontally into a 7.0 cm diameter cylinder as it was moved to a stop position. Ataxia and dysmetria persisted on this test longer than on the platform test. Severe oscillation of the DR limb made entry of the hand into the cylinder difficult. If, however, the cylinder was kept in a fixed position and exposed to the animal by lifting a screen, ataxia was noticeably reduced, reach trajectory improved, and reach time dramatically shortened.

When a target is immobile, reefference associated with the oculomotor command producing "foveal capture" of the target may be used to pre-program the reaching movement (Paillard, 1978). Proprioception is, therefore, not necessary. If the target is in motion, on the other hand, oculomotor reefference must constantly be updated. The movement, therefore, becomes dependent on visual feedback. In the visually cued task, without the possibility of visual guidance, animals used a visually triggered response. In the platform test, they may have initially attempted to use a visually guided response but later switched to a visually triggered one. In the cylinder test, animals may have used a visually guided response when the cylinder was in motion but a visually triggered response when it was stationary. We propose that the observed oscillations in reach of the DR limb result from the absence of velocity-related muscle afferents which normally counteract the instability of the visuomotor servo-loop (Murphy, et.al., 1975). This instability is a function of the inherent processing time of visual feedback, which has been shown to be far slower than that of somatosensory feedback to the motor cortex (Evarts, 1974) or cerebellum (Buchtel, et.al., 1972).

1311 2-DEOXY-(^{14}C) GLUCOSE AS A MARKER FOR IDENTIFYING ACUTELY ACTIVE SKELETAL MUSCLE FIBERS IN INDIVIDUAL MOTOR UNITS. J. Toop*, R.E. Burke, R.P. Dum, M.J. O'Donovan* and C.B. Smith*. (Spon. F.T. Hambrecht) Lab. of Neural Control, NINCDS, NIH and Lab. of Cerebral Metabolism, NIMH, Bethesda, MD 20205.

The injection of trace amounts of (^{14}C)-deoxyglucose (DG) permits autoradiographic evaluation of rates of glucose metabolism in structures in the central nervous system (Sokoloff et al., J. Neurochem. 28:897, 1977). The uptake of DG has also been studied in rat muscle using this technique, where it was found that electrical stimulation greatly increases the uptake of tracer (Rapoport et al., Exp. Neurol. 60:168, 1978). Our present aim has been to evaluate DG autoradiography as a label for muscle fibers belonging to an individual motor unit (i.e., those innervated by a single alpha motoneuron), which has previously been done by depleting intrafiber glycogen stores during prolonged stimulation (Edstrom & Kugelberg, J. Neurol., Neurosurg. & Psychiat. 31:424,1968). Glycogen depletion provides an independent "marker" for acutely active muscle fibers. In each of 5 adult cat flexor digitorum longus (FDL) or soleus (SOL) muscles (normal or self-reinnervated), a physiologically identified motor unit was stimulated repetitively while monitoring muscle unit force output (13 - 18 pulses in 40 Hz trains delivered once per sec. through a micropipette in the innervating motoneurons; see Burke et al., J. Physiol. 234:723, 1973). Immediately after the onset of stimulation, DG was injected intravenously (approx. 40 µC/kg in saline) and stimulation was continued for 16 to 75 minutes. Between 20 and 72 minutes was allowed to elapse between DG injection and removal of the test muscle, which was then quickly frozen whole in isopentane at -180° C and stored in a liquid nitrogen freezer. Frozen sections were cut at 10 µm in a cryostat at -20° C and quickly air-dried. Autoradiograms were made in two ways: 1.) rapidly dried frozen sections on glass slides were placed in contact with X-ray or Kodak Plus-X film in dry cassettes; or 2.) sections were picked up in the cryostat under safelight directly onto slides covered with AR.10 stripping film. In the latter case, the film-section sandwich was developed and fixed after suitable exposure and then stained by the PAS reaction for glycogen. In 2 self-reinnervated FDL muscles, there was good correspondence between groups of depleted fibers and areas of increased silver grains and in one of these, grain densities in some cases corresponded to individual glycogen-depleted fibers. In the other 3 muscles, there was much less correspondence between depleted fibers and grain densities. The results suggest that active muscle fibers take up DG, but that the use of the method to map single muscle fibers belonging to individual motor units requires refinement of present methods.

1312 EYE MOVEMENT CONTROL: THE EFFECTS OF JOINT SUPERIOR COLLICULUS AND FRONTAL EYE FIELD STIMULATION. Sean True*, Peter H. Schiller* and Janet Conway* (SPON: R. Held) Dept. Psyc., MIT, Cambridge, MA 02139.

Electrical stimulation of both the frontal eye fields (FEF) and the superior colliculus (SC) produces saccadic eye movements whose directions and amplitudes depend on the site of stimulation. Simultaneous stimulation at two loci within each of these structures produces a single saccade whose size and direction is a weighted function of the saccades produced singly from each site (Robinson, D.A. Vision Res. 12: 1795, 1972. Robinson, D.A. and Fuchs, A.F. J. Neurophysiol. 32: 637, 1969). This averaging could be produced either within these structures or at a locus to which both project.

The aim of this study was to determine what the interactions are between the FEF and the SC when they are jointly stimulated, either simultaneously or in succession. Stimulation of these structures was carried out in the alert monkey using implanted search coils to monitor eye movements. The animal's head was restrained during the experiment. Stimulation was between 10-600 μA, typically at 300Hz, 70-300 msec train durations with 0.5 msec pulse widths. For the most part microelectrodes were used for this purpose which permitted localization of each structure by recording prior to stimulation.

Our results showed that paired stimulations produce results similar to those obtained when two sites are activated within each structure: for simultaneous stimulation the resultant saccade was the weighted function of individually elicited saccades, where the relative intensity of stimulation is the weighting factor. With contralateral stimulation this can result in nulling of the saccadic response, provided the two sites stimulated produce saccades in opposite directions. Sequential stimulation showed a refractory period in which the second stimulation has no apparent effect on eye movement. This refractory period corresponds to that seen with paired stimulation in a single structure. We did not see any case of interruption of a saccade by a following saccade under sequential stimulation.

These findings suggest that the FEF and the SC converge on a site in the brainstem where the averaging function observed is performed. Since we have previously shown that ablation of the SC does not alter stimulation elicited saccades from the FEF (Schiller, P.H. Brain Res. 122: 154, 1977.), it is unlikely that the effects reported here could be attributed to the FEF → SC pathway. (Supported by NSF grant #BNS 76-82543 and NIH grants 5 R01 EY00676 and #1 T31 GM07484)

1313 UNIDIRECTIONALLY PROPAGATED ACTION POTENTIAL GENERATION IN PERIPHERAL NERVE BY SHORT STIMULI. Christopher van den Honert*, and J. Thomas Mortimer. Dept. Biomed. Eng., Case Western Reserve University, Cleveland, Ohio 44106.

Electrical stimulation of peripheral nerve ordinarily elicits two action potentials propagated in opposite directions. A stimulation technique has been developed which generates a single, unidirectionally propagated impulse. Propagation in the opposite direction is inhibited by imposition of a longitudinal potential gradient in the extracellular medium which opposes the flow of excitatory action currents. The time course of that potential is roughly equivalent to the driving force behind the action currents, i.e. the action potential itself. Both stimulation and block are effected through an asymmetrical tripolar cuff electrode using a single central cathode. The total stimulating current is divided unequally between the anodes.

The method has been successfully demonstrated at rates up to 50 Hz in motor axons of cats using EMG measurements from medial gastrocnemius during sciatic nerve stimulation. The compound sciatic neurogram was recorded proximal to the electrodes. Stimuli consisted of regulated current pulses (1 - 10 mA, 0 - 8 ms) with a linear or exponential falling phase (0 - 10 ms).

This technique may be used as an investigative tool, or to effect a clinical motor nerve block by introduction of antidromic impulses on the peripheral nerve. The block arises from the head on collision (and subsequent mutual anihilation) of the natural efferent activity and the artificially generated antidromic impulses. Such a collision block could provide control of spasticity, particularly in the urinary sphincter muscle of spinal cord injury patients. Short term sphincter block would facilitate bladder voiding by electrical stimulation of the detrusor musculature.

This research was supported by NIH Grant No. NINCDS NO1-NS-2-2314 and GM 01090-16 Training Grant.

1314 THE UNUSUAL STRUCTURE OF TRAPEZIUS MOTONEURONS IN THE ADULT CAT. S. J. Vanner* and P. K. Rose. Dept. of Physiology, Queen's University, Kingston, Ontario, Canada K7L 3N6.

Anatomical studies of the spinal cord using Golgi stains have established that dendrites of spinal motoneurons have an organization dominated by rostrally and caudally directed dendrites usually 1000 μm in length. However, the consistency of this structural framework has not been systematically studied. We now report that the trapezius motoneurons have structural characteristics which are strikingly different from other spinal motoneurons. These motoneurons are located in the cervical cord and are involved in head and forelimb movements.

Using chloralose anesthetized cats, 21 trapezius motoneurons were antidromically identified and injected intracellularly with horseradish peroxidase. Subsequent histological processing revealed that the trapezius motoneuron pool formed a column of neurons extending from the caudal C2 segment to the rostral C6 segment. The unusual feature of these motoneurons was their remarkably long rostrally and caudally directed dendrites. Occasionally these dendrites measured over 2000 μm from the soma and most exceeded 1000 μm in length. The total extent of the dendritic tree measured 3000 μm to 4000 μm. These rostral and caudal dendrites formed a distinct bundle which was usually confined to the trapezius motoneuron nucleus. Ventromedial and dorsally projecting dendrites also measured over 1000 μm in length but lacked the distinct grouping of the rostrally and caudally directed dendrites. Although few in number, lateral dendrites projected into the lateral funiculus occasionally reaching the edge of the spinal cord.

The functional significance of these long dendrites will depend on their membrane characteristics. If the membrane resistivity of the trapezius motoneurons matches that reported for hindlimb motoneurons it is possible that synapses located on the distal dendrites will have little effect on the membrane potential of the soma. Alternatively, if the membrane resistivity is substantially larger, trapezius motoneurons may have electrotonic lengths similar to hindlimb motoneurons.

(Supported by the Canadian Medical Research Council.)

1315 UNIT ACTIVITY IN THE MESENCEPHALIC RETICULAR FORMATION (MRF) ASSOCIATED WITH SACCADES AND POSITIONS OF FIXATION DURING A VISUAL ATTENTION TASK.
David M. Waitzman and Bernard Cohen, Department of Neurology, Mount Sinai School of Medicine, New York 10029

The MRF projects to the superior colliculus as well as to the ipsi and contralateral pontine reticular formation where rapid eye movements are generated. Electric stimulation of the MRF causes contralateral saccades, and MRF lesions cause ipsilateral gaze preference. We are studying whether MRF neurons participate in the production of visually guided saccadic eye movements and positions of fixation. Unit activity is monitored while monkeys fix a small spot of light on a TV screen in the Wurtz paradigm. When the spot dims, they release a bar for a water reward. Eye movements are recorded with EOG. The spot is under computer control and can jump to new locations during the task. Animals sometimes break fixation to execute off-target saccades. Their eyes usually return to the spot with an on-target saccade after several hundred milliseconds.

To date several types of task-related neurons have been found in the MRF. They were located in the region of nucleus cuneiformis, lateral to the oculomotor nucleus. The first group fires 50 to 200 msec before the onset of on-target contralateral saccades. Frequencies can exceed 600 Hz, and in some units are dependent upon the difficulty of the visual task: the smaller the contrast between spot brightness and ambient light, the higher the firing rate. A second group of neurons fires with a burst of spikes just before the animal makes small contralateral on-target saccades of less than 1^{o} to 2^{o}. These cells are silent during larger on-target saccades or spontaneous eye movements. A third set of MRF neurons fires only when the animal maintains fixation. They stop firing 100-200 msec before saccades that take the eyes off-target and begin to fire with the occurrence of the next on-target saccade. Their firing rates are also higher when the visual attention task is made more difficult. Other MRF neurons are not task-related. They fire about 50 msec after the onset of all rapid eye movements and blinks. The lesion, stimulation and unit data support the postulate that important visual-oculomotor processing occurs in the MRF. Activity of the task-related neurons could be used to generate contralateral horizontal saccades and periods of fixation during visual attention. Activity of MRF cells that follows eye movement could be used to signal the occurrence of rapid eye movements to cerebral structures.

Supported by NEI Grant EY 00296

1316 REPRESENTATION OF THE JAW MUSCLES IN THE FACIAL AND TRIGEMINAL NUCLEI OF THE PIGEON (COLUMBA LIVIA). J.M.Wild and H.P.Zeigler. Department of Animal Behavior, American Museum of Natural History, New York, N.Y. 10024.

As part of a series of anatomical and physiological studies of effector mechanisms controlling components of the feeding behavior of the pigeon, we have mapped the somatotopy of the jaw muscles in the Vth and VIIth cranial nerve nuclei using horseradish peroxidase histochemistry.

In the case of the protractor of the lower jaw, M. depressor mandibulae, the only jaw muscle innervated by the facial nerve, the labeled neurons were located totally and discretely within the medial facial nucleus lying in the center of the caudal pontine reticular formation. Labeled axons could be observed to leave the nucleus in dorsal and dorsomedial directions before turning laterally, caudally and then laterally again to pass through the brainstem immediately ventral to the rostral portions of the lateral vestibular nucleus. The axons then pierce the descending trigeminal tract and exit the lateral brainstem caudal to their nucleus of origin.

In the case of the six jaw muscles within the orbit, all but the protractor of the upper jaw, M. protractor quadrati et pterygoidei, were represented within the largest or chief motor trigeminal nucleus located lateral and rostral to the medial facial nucleus. The adductor complex was represented ventrolaterally, M. pseudotemporalis superficialis ventrally, M. pseudo temporalis profundus dorsolaterally, and the two pterygoid muscles medially, centrally and rostrally. M. protractor quadrati et pterygoidei was represented in a more scattered group of neurons predominantly located between the medial facial nucleus and the caudal pole of the chief motor trigeminal nucleus, forming, as it were, a V-VII motor column.

Many labeled axons could be seen to exit the brainstem directly from the lateral and ventrolateral aspects of the chief motor trigeminal nucleus. However, many others ascend dorsally before turning laterally to join the discrete arching bundle of axons leaving the dorsomedial facial nucleus of Karten and Hodos, (1967). The fibers from both these nuclei then descend together ventrolaterally to exit the brainstem on the ventral aspect of the entering sensory root of the trigeminal nerve.

The data suggest a somatotopic organization of the trigeminal motor complex but, just as there is considerable overlap in the position of the muscles with respect to each other within the orbit, so there is considerable overlap in their representation within the nucleus. In addition some facial and trigeminal neurons innervating the two jaw opening muscles lie in close proximity. (Supported by NIMH Grant MH 08366 and a CUNY FRAP award to H.P.Z.)

1317 POSTROTATORY NYSTAGMUS, OPTOKINETIC AFTERNYSTAGMUS AND THE VESTIBULO-OCULAR RESPONSE IN DUTCH-BELTED RABBITS. Barbara J. Winterson, Han Collewijn* and Hans van der Steen*. Dept. Physiol. Med. Fac., Erasmus U., Rotterdam, The Netherlands.

Measurements of the decay of the velocity of the slow components of postrotatory nystagmus (PRN) in rabbits gave estimates of time constants (9-15 sec) for the vestibulo-ocular response (VOR) which were inconsistent with time constants estimated from phase relations obtained during sinusoidal rotations (2-4 sec). This difference led us to examine in the same animals the following: PRN after constant velocity rotations (30, 60, & 150°/sec); VOR over a range of frequencies (0.03-2.0 Hz) and amplitudes (5-20°); optokinetic afternystagmus (OKAN) after constant velocity drum rotations (30 & 60°/sec); and the response to constant velocity rotations (15, 30, & 60°/sec) in the light.

We found: 1) The velocity of slow components of both PRN and OKAN decayed at a constant rate (0.5-4°/sec per sec) rather than the exponentially declining rate which has been reported and modelled in monkey. 2) This constant rate of decay was largely independent of initial eye velocities or whether prior stimulation was rotational or optokinetic. 3) The total compensatory response was maintained at constant gain when rotations were 30°/sec or less. 4) In response to sinusoidal rotations a small effect of amplitude on phase was found. Lower amplitudes were associated with phase advances. 5) Time constants estimated from phase relations ranged from 2-3 sec.

These results indicate the source of the inconsistency in estimates of time constants from different methods. PRN with a constant rate of decay has durations that are determined by the initial velocity of the eye and therefore can never be described by a single time constant. The higher the initial eye velocity, the longer the duration of PRN; the lower the initial velocity, the shorter the duration and thus the apparent time constant. During sinusoidal rotation of modest amplitude, velocities and durations of eye movement are not of such magnitude to appreciably affect the mechanism underlying PRN.

The similarity between the time courses of decay of PRN and OKAN suggest shared neural circuity. Fortunately, this similarity insures maintenance of constant gain during moderate rotational velocities in the light, because reductions in VOR gain are compensated by comparable growth in gain of the optokinetic response.

1318 MOTOR AND SOMATIC SENSORY CORTEX SINGLE UNIT BEHAVIOR WITH PASSIVE AND ACTIVE LIMB MOVEMENTS AND POSITION MAINTENANCE IN PRIMATES. Jonathan R. Wolpaw. Neurobiology Dept., Armed Forces Radiobiology Research Institute, Bethesda, MD 20014.

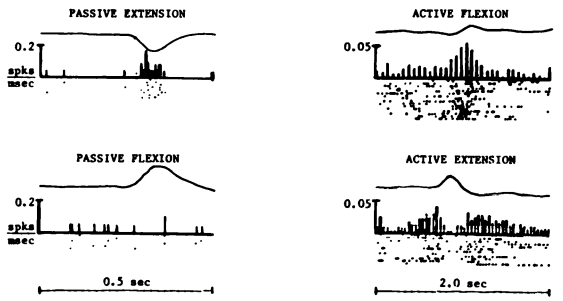
Four monkeys maintained hand position against a range of steady background forces. 50-ms force pulses, causing abrupt passive wrist flexion or extension, were superimposed. Pulse responses, background activity, and activity during active movements were recorded from 630 units in areas 4, 3, 1, 2, 5, and 7, which responded in 60 ms.

Three-quarters of task-related units in areas 4 and 2 behaved similarly with a pulse in one direction, background force in the same direction, or active movement in the opposite direction. In contrast, closely task-related units in areas 5 and 7 behaved similarly with passive or active movement in the same direction. In areas 3 and 1, few force pulse responsive units were related to background force or active movement.

Postcentral areas were similar in average response latency(27-30 ms). Area 4 was significantly later(32.5 ms). In areas 4 and 2, flexion pulses caused most excitation and extension pulses caused most inhibition.

The results resolve the discrepancy between Porter and Rack (Br. Res. 103: 201, 1976), and Conrad et al. (Br. Res. 71: 507, 1974) and Evarts and Fromm (Prog. Clin. Neurophysiol. Vol. 4, Karger, 1978, p 56) in regard to area 4 unit behavior during active vs passive movements.

The particular correlation in areas 4 and 2 between unit behavior during passive movement, active movement, and position maintenance, combined with the shorter latency of area 2, is consistent with the position that short latency sensory input, largely from muscle stretch receptors, had significant control over area 4 throughout performance, and that much of this input reached area 4 through area 2.



Area 2 unit, typical of 4 and 2, excited with passive (force pulse-induced) movement in one direction (extension) or active movement in the other (flexion). Each histogram sums the trials in the raster. Traces are hand position; up is wrist flexion, down is extension.

1319 INTERACTIONS BETWEEN THE ACHILLES TENDON REFLEX AND THE FUNCTIONAL STRETCH RESPONSE IN NORMAL SUBJECTS AND PATIENTS WITH MULTIPLE SCLEROSIS. Marjorie H. Woollacott. Neurological Sciences Institute, Good Samaritan Hospital & Medical Center, Portland, OR 97209.

The EMG activity of four leg muscles [gastrocnemius (G), tibialis anterior (TA), hamstrings (H) and quadriceps (Q)] was monitored while freely standing normal and spastic humans were subjected to unexpected movements of a platform capable of six independent degrees of rotation (horizontal, vertical and rotational displacements of each foot). At different latencies during the platform perturbations an electromechanical hammer was used to elicit the Achilles tendon reflex, in order to determine if any changes in the state of the myotatic stretch reflex occurred during unexpected postural perturbations. In 50% of the subjects tested the tap to the Achilles tendon also elicited a smaller muscle response in TA and in occasional subjects in H and Q muscles. This reflex response organization to the tendon tap also occurred in quietly standing subjects and was not correlated with functional stretch response (FSR--latency 110-120 mm) organization to platform movements.

In the majority of normal subjects results indicate that the moytatic stretch reflex in the gastrocnemius muscle is inhibited during the FSR of its antagonist muscle, the tibialis anterior. The inhibition occurred during all platform perturbations used (synchronous translateral, rotational and vertical motion in addition to reciprocal vertical motions). The duration of the inhibition was correlated with the duration of the antagonist's FSR and was usually within the range of 100-300 msec.

Previous reports by Nashner and Woollacott show that certain platform movements (vertical lifts and drops and direct ankle rotations) cause FSR's which are inappropriate for postural stability and are subsequently adapted to very low levels within 3 to 5 trials. In the present experiments the Achilles tendon reflex was still inhibited even in those instances in which the TA responses had adapted to nearly zero.

In patients with multiple sclerosis a delayed inhibition of the Achilles tendon reflex in the G was observed concomitant with a delay in the tibialis anterior FSR.

1320 ℓ-BACLOFEN PREFERENTIALLY DEPRESSES EXTENSOR VS FLEXOR VENTRAL ROOT REFLEXES IN THE UNANAESTHETIZED SPINAL CAT. Kiran Yashpal*, Steven Backman*and James L. Henry (SPON: R.B. Malmo). Dept. of Research in Anaesthesia, McGill Univ., Montréal, Québec,H3G 1Y6.

Recently we found that the ℓ-isomer of baclofen was 10-100 x more potent than the d-isomer in depressing the mono- and polysynaptic L_7 ventral root reflexes (Fed. Proc. 38:900, 1979). Because baclofen's clinical use is as an antispastic agent it was determined whether it has a preferential effect on extensor or on flexor ventral root reflexes. L_7 ventral root reflexes were elicited by electrical stimulation of the medial or lateral gastrocnemius (extensor) nerve and of the anterior tibial (flexor) nerve in the unanaesthetized, decerebrated cat spinalized at L_1, paralysed and ventilated mechanically. ℓ-baclofen was administered into the jugular vein and the effects noted on the amplitudes of the evoked reflexes. ℓ-baclofen (0.1-1.0 mg/kg) had a dose-dependent depressant effect on all evoked responses: the time course of the onset of depression was faster for the extensor nerve evoked reflexes, and at any one dose the degree of the depression was greater on the extensor nerve evoked reflexes. For both extensor and flexor nerve evoked responses, depression of the monosynaptic reflex was more rapid and greater in magnitude than the polysynaptic reflex. Low doses of strychnine sulphate (0.05-0.2 mg/kg) reversed depression induced by low doses of ℓ-baclofen (0.1-0.3 mg/kg), but higher doses of ℓ-baclofen (0.5-1.0 mg/kg) overcame this reversal. Bicuculline methiodide (1-10 mg/kg i.v.) failed to reverse the depression induced by ℓ-baclofen. The preferential effect of ℓ-baclofen on extensor nerve reflexes is consistent with its clinical use as an antispastic agent, especially in cases of increased extensor tone. Its antagonism by strychnine suggests a glycinergic mechanism is involved in the expression of its effects in the ventral horn.

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1321 MOTOR PROGRAMMING PRECEDING SPEECH. Donald H. York, Tom W. Jensen, W. Casey Lenox* and John G. Rosenfeld*, Dept. of Physiology and Communications Disorder Unit. School of Medicine, University of Missouri, Columbia, MO 65212.

The question of whether there are identifiable event-related potentials preceding speech is quite controversial. The present study was designed to answer this question. In all previous studies examining averaged EEG preceding voicing, the high frequency response of the EEG amplifier was seldom set beyond 50Hz. In the present study an electrode was placed on Cz and linked ear lobes served as reference. A ground was attached to the right forearm. The electrodes were connected to a differential amplifier with bandwidth 1Hz - 1000Hz and recorded on magnetic tape. A second channel of the tape was used to record a square wave trigger pulse obtained from the voice onset phonogram. Ten right handed female subjects were studied. Each subject produced 100 utterances of the words ATE and YES. A control consisted of random sampling of the EEG while the subject was still seated in a reclined chair, in a semi-darkened room with eyes fixed on a spot 30 inches distant and no voice production. Electromyograms from obicularis oris, obicularis oculi and masseter muscles were recorded on a third channel on tape. Analysis of the data involved backward averaging of at least 100 repetitions of the utterance using the voice onset to trigger the averager. Sample periods of 128 msec were analyzed up to 668 msec preceding voicing. Averaged data were entered into a microprocessor for peak identification and latency assignment to all positive and negative going waves. A subsequent program then compared across individuals examining for identical peaks ±1 msec over the range 0 - 668 msec. The results showed that in the time period, from 216-344 msec preceding speech, in at least 6 out of 9 subjects, there were 6 - YES peaks, 8 - ATE peaks and 2 - CONTROL peaks which were positive going and did not overlap. In this period 2 - YES peaks, 5 - ATE peaks and 3 - CONTROL peaks were negative going, with only one overlap of a control peak. No averaged electromyogram activity could be found in the time period 216-344 msec. These results suggest that distinct temporal patterns of activity exist preceding the production of an utterance and furthermore that these patterns are utterance specific across subjects. The precise role and origin of this averaged EEG activity is presently being investigated.

1322 MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS OF BURST INHIBITORY NEURONS IN THE ALERT CAT. K. Yoshida*, R.A. McCrea, A. Berthoz* & P. Vidal*. Lab. de Physiol. du Travail, 41 rue Gay Lussac, 75005, Paris, France; Dept. Physiol. & Biophys., New York Univ. Med. Ctr., New York, N.Y. 10016

The location and inhibitory synaptic effects of medullary burst inhibitory neurons (BIN) on abducens (Abd) motoneurons has been described by Hikosaka and Kawakami (1978) in the decerebrate cat. The purpose of the present study was to quantitatively describe the activity of BINs in the alert cat and to determine their location, somadendritic morphology, axonal trajectory and termination sites. Neurons that exhibited a burst of activity prior to ipsilateral saccades were found in the medulla 0.7-1.0 mm from the midline at the level of the caudal Abd nucleus to 3.0 mm posterior to the Abd and 0.8-3.5 mm below the surface of the IVth ventricle. BINs discharged prior to and during ipsilateral voluntary saccades as well as the quick phase of vestibular and optokinetic nystagmus. The duration of the activity was related to saccade duration and the number of spikes was related to the saccade amplitude. BINs exhibited no activity during fixation, but occasionally responded weakly during contralateral saccades. The axons of four BINs which demonstrated all the above characteristics were intracellularly injected with HRP in the alert cat. The somata of the injected neurons were located in the region described above and gave rise to 4-7 proximal dendrites. In one injected neuron, the dendritic tree extended over 0.6 mm rostrocaudally, 1 mm mediolaterally and 1.3 mm dorsoventrally. Each of the injected BIN axons terminated extensively throughout most of the contralateral Abd, although some areas were free of termination in each case. The terminal arborization usually arose as a series of branches from the main axon as it coursed rostrolaterally beneath the nucleus. The synaptic boutons of BIN axons ranged in size from 0.5 μ to 3 μ and often occurred in close clusters. BIN axons also terminated profusely in the contralateral medullary reticular formation, particularly in the area where BINs are located. The main axon of a BIN accompanied by one or more branches with terminal arborization in the Abd nucleus entered and terminated in the medial vestibular nucleus. Terminal collaterals were also given off to the contralateral rostral prepositus nucleus. No BIN collaterals were seen to ascend rostral to the Abd. In conclusion, we have demonstrated in the alert cat that BINs which discharge prior to and during ipsilateral saccades, terminate in the contralateral Abd nucleus, reticular formation, medial vestibular nucleus and prepositus nucleus. These morphological data suggest that BINs may also in part be responsible for the pause observed in some secondary vestibular and prepositus neurons during contralateral saccades. Supported by CNRS, CNAM and NS-13742.

1323 A PHYSIOLOGICAL CLASSIFICATION OF MOTOR UNITS IN HUMAN FIRST DORSAL INTEROSSEOUS. Joseph L. Young and Richard F. Mayer. Dept. Neurol., Sch. Med., U. of Md., Baltimore, Md. 21201.

Isometric twitch characteristics of 122 human First Dorsal Interosseous (FDI) motor units (MUs) have been studied using the electronic averaging, microstimulation technique. Of these, 40 units were further classified according to their twitch contraction times (Tc) and fatigabilities following repetitive trains of stimuli. MUs were considered fast (F) or slow (S) by their Tc$\leq$ 75 msec or Tc$\leq$75 msec respectively. 75 msec was chosen on the basis that:-

1. all units that fatigued have Tc$\leq$ 75 msec, and
2. all units that "sag" also have Tc$\leq$ 75 msec.

"Sag" was used in the usual sense to mean any fall in the steady-state peak tension in response to an unfused tetani. "Sag" was tested systematically in this study but was not used in classifying MUs because of the base-line often fluctuates. This inherent technical difficulty made a decision about "sag" in many cases difficult. Fast units were further divided into fatigable (FF) or fatigue resistant (FR) by comparing the initial twitch to the second twitch following 2 minutes of repetitive trains. Each train has a stimulus frequency of 30 Hz lasting 1/3 of a second. This train of stimuli was repeated once every second. In general, large MUs are fast contracting in agreement with other studies of MUs in cat and man. But there are many fast units in the FDI that produce small twitch tensions. MUs in human FDI is composed of physiologically broader groups of MUs as compared to the cat medical gastrocnemius muscle. It is concluded that the criteria set forth here is suitable for separating MUs physiologically into three groups.

1324 TETANIC TENSION APPEARS TO BE A PERFECT PREDICTOR FOR RECRUITMENT OF PLANTARIS (PL) MOTOR UNITS IN THE CAT. Felix E. Zajac and Joel S. Faden. Univ. of Maryland, College Park, MD 20742.

Various studies on the whole population of motor units in a given muscle show that recruitment is statistically ordered either according to properties which reflect the "size" of the motoneuron or to properties which indicate the contractile strength of the motor unit. These findings are consistent with the fact that for the full range of motor units there are correlations between motoneuron size, conduction velocity (CV) of the motor axon and tetanic tension(TT) of the motor unit. However, such correlations between CV and TT are not high in mixed hindlimb muscles of the cat and are non-existent in the 61% subpopulation of fast-twitch(type F) motor units. This study (for methods see Neurosci. Abstr. 3:271,1977) emphasized the recruitment order of type F units to distinguish whether the order is more related to axonal CV or to TT of the unit. Small intact filaments, each containing one PL axon, were dissected free from the L7 ventral root in decerebrate cats and axonal CV, TT and type(S,FR,F-int, FF) of each motor unit isolated was determined. Each filament was cut distally and discharges from its PL axon were recorded from its proximal end during monosynaptic reflexes. Discharge from one PL axon was compared with the presence or absence of discharge from another PL axon in another filament. Recruitment order of each pair of PL axons was compared with their motor unit properties. For each pair of units(both type S, or both type F, or of mixed type) the one with the smallest TT was recruited first(Table 1, col B). In contrast the unit with the slowest CV was recruited first in only 47% of type F pairs and in 73% of all pairs studied(Table 1, col C). These results are consistent with the probability that there is a monotonic trend between CV and TT(compare col C with D, Table 1). The probabilities were calculated on data from 134 PL motor units. TT is thus a better predictor for recruitment order in this motor pool than CV.
(supported by NIH grant NS 11518)

TABLE 1

A	B	C	D
Motor Unit Type In Each Pair	Smallest Tension Producing Unit Recruited First	Slowest Conducting Motor Axon Recruited First	Monotonic Trend Between CV and TT
Both type S	100%(9/9)	88%(8/9)	p=0.95
Both type F	100%(20/20)	47%(9/19)	p=0.47
Type S/Type F	100%(13/13)	100%(13/13)	p=0.88
All pairs	100%(42/42)	73%(30/41)	p=0.74

NEUROCHEMISTRY

1325 INHIBITION OF PYRIDOXAL KINASE BY GAMMA-AMINOBUTYRIC ACID. D. M. Abercrombie* and D. L. Martin* (SPON: A.T. Campagnoni). Dept. of Chemistry, University of Maryland, College Park, MD. 20742.

Pyridoxal kinase is inhibited by gamma-aminobutyric acid (GABA), a neurotransmitter in the vertebrate central nervous system. Because GABA is synthesized by a pyridoxal-P requiring enzyme, this inhibition raised the possibility of a metabolic feedback loop for control of GABA synthesis. The results show that there was little inhibition of pyridoxal kinase by GABA at low concentrations of pyridoxal, but that the inhibition became stronger as the concentration of pyridoxal was raised. Similar results were obtained when β-alanine and δ-aminovaleric acid were substituted for GABA. Conventional models of enzyme inhibition did not fit the inhibition data and GABA did not inhibit when pyridoxamine was the substrate suggesting that GABA did not inhibit by interacting directly with the enzyme. Substrate depletion and direct inhibition of pyridoxal kinase by the pyridoxal-GABA imine were considered as alternative mechanisms. To distinguish between the two mechanisms, the equilibrium constants for pyridoxal-GABA imine formation were determined at pH 6.2,7.3, and 8.0 (μ=0.2 M, 37°C). The values were 0.23 M^{-1}, 3.4 M^{-1}, and 14.0 M^{-1}, respectively. The association constant for the pyridoxal-glycine imine was 1.0 M^{-1} (pH 6.2). These values were used to calculate the concentrations of free pyridoxal and pyridoxal-GABA imine under assay conditions. The results showed that the pyridoxal concentration was not appreciably reduced by GABA indicating that inhibition was not the result of substrate depletion. This conclusion was supported by the failure of glycine to inhibit the enzyme. The calculated pyridoxal-GABA concentrations were used to analyze the inhibition data. The results showed that the pyridoxal-GABA imine behaved as a simple noncompetitive inhibitor with respect to pyridoxal thereby supporting the hypothesis that pyridoxal kinase was inhibited by the pyridoxal-GABA imine. In view of this indirect mechanism and the high concentration of GABA needed for inhibition, it seems unlikely that GABA inhibition of pyridoxal kinase represents a specific control point for GABA synthesis.

1326 BRAIN REGIONS AND ACTIVATIONAL STATE ALTER THE INFLUENCE OF pH ON TYROSINE HYDROXYLASE ACTIVITY. Ann Acheson*, Linda Kennedy*, Gregory Kapatos*, and Michael Zigmond. Departments of Biological Sciences and Psychology, and Department of Pharmacology, School of Pharmacy, University of Pittsburgh, Pittsburgh, PA. 15260.

Tissues from various rat brain regions were homogenized in Tris-HCl buffer, pH 6.0, and the high-speed supernatent was assayed for tyrosine hydroxylase (TH) activity in the presence of 75 μM L-(1-^{14}C) tyrosine, 6MPH$_4$, catalase, and a dihydropteridine reductase regenerating system. We found that the influence of pH on the TH activity of these crude enzyme preparations varied with brain region. Striatum, a brain region rich in dopamine (DA)-containing nerve terminals, had a pH optima of 5.65. However hippocampus and cerebellum,regions rich in norepinephrine (NE)-containing terminals, had a pH optima in the range of 6.1 - 6.2. Brain areas with mixed DA and NE innervation had intermediate pH optima(frontal cortex, 5.8), or displayed biphasic pH curves with one peak between 5.7 and 5.9 and a second peak at 6.1 (olfactory tubercle and hypothalamus). TH from areas containing cell bodies displayed less sensitivity to pH than TH from terminal regions. Activity from cell body areas was maximal over a broad range of pH values, which encompassed the individual pH optima of the terminal regions to which these cell bodies project (substantia nigra, 5.65 - 5.9; locus coeruleus, 5.95 - 6.3).

We also found that activation of TH by phosphorylating conditions (0.2mM cAMP, 1.0 mM $MgCl_2$, 0.5 mM ATP, and 2.0 mM theophylline) produced a decrease in the sensitivity of the enzyme to pH. Activated enzyme from striatum or hippocampus showed little decrease in maximal activity when assayed at a sub-optimal pH, thus increasing the effect of phosphorylation relative to basal activity. For example, the activity of TH incubated under phosphorylating conditions was 4 times that of control activity at pH 6.5, whereas at pH 6.2, the activity was not significantly different from control.

These data support previous suggestions that TH exists in different forms in DA- and NE-rich brain regions. They also suggest the importance of assay conditions in regional comparisons of TH activity and in studies of TH activation.

(Supported in part by USPHS grants MH-29670 and 00058)

1327 USE OF AN ANTICHOLINESTERASE AS PROBE IN THE STUDY OF MICROWAVE-INDUCED BLOOD BRAIN BARRIER CHANGES. Y. Ashani*, F. H. Henry* and G. N. Catravas. Biochemistry Department, Armed Forces Radiobiology Research Institute, Bethesda, MD 20014.

Groups of rats were administered an anticholinesterase agent (phospholine iodide) and exposed to pulsed microwave radiation at a frequency of 2.45 GHz and average power density of 10 mW/cm^2. The pulse width was 2 μsec with a pulse repetition frequency of 500 Hz. Irradiations were conducted for a period of 10 min in an anechoic chamber under near field conditions as described by Thomas, et al. (Science 203:1357-1358, 1979). A statistically significant decrease in body temperature, compared to controls, was observed in rats that were given the drug and 10 min later were exposed to microwaves. When irradiation preceded drug administration these changes could not be observed. The enhancement of the anticholinesterase effects on body temperature in the presence of low levels microwave irradiation may be used as a potential simple rapid tool in the study of effects of low power density microwaves on biological systems such as blood brain barrier.

1328 IMMUNOCHEMICAL STUDIES OF MAMMALIAN NEUROFILAMENTS. L. Autilio-Gambetti*, M.E. Velasco*, P. Gambetti, J.M. Sipple*. Division of Neuropathology, Institute of Pathology, Case Western Reserve University, Cleveland, Ohio 44106.

Mammalian neurofilaments (NF) have recently been shown to be made of three polypeptides: 200K, 145K and 68K daltons. These three polypeptides from NF-rich fractions of rat spinal cord (Shecket and Lasek, Trans Am Soc Neurochem 10, 1979) were separated in SDS slab gels; antisera to each of the NF polypeptides were raised in rabbits by injecting the corresponding bands from these gels. By indirect immunofluorescence and peroxidase antiperoxidase (PAP) methods, antisera were seen to stain central and peripheral axons from human and rat. Electron microscopy of PAP preparations of peripheral nerve showed that the immunostain reaction takes place at the NF.

The PAP method was adapted for immunostaining of antigenic peptides in SDS slab gels. Each of the antisera stained strongly their corresponding polypeptide and less itensely the other two NF polypeptides; in addition, a band migrating with tubulin was also stained. The 50K dalton polypeptide obtained from preparations of bovine brain filaments by the axonal floatation method (Liem et al, J Cell Biol 79:637, 1978) was not stained by either antisera. These results indicate cross reactivity between the three NF polypeptides and antigenic identity between central and peripheral NF from different species.

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1329 Ca^{++}-ACTIVATED NEUTRAL PROTEINASE IN NORMAL AND TRAUMATIZED SPINAL CORD. Naren L. Banik*, James M. Powers*, Karen Smith* and Edward L. Hogan (SPON: W. Boggan). Departments of Neurology, Biochemistry and Neuropathology, Medical University of South Carolina, Charleston, S. C. 29403.

Experimental spinal cord trauma affects myelinated axons and causes paralysis in animals. Myelin proteins are progressively degraded, and ultrastructurally myelin is disrupted with vesiculation of myelin lamellae and phagocytosis. The degradation of myelin proteins suggests a role for proteolytic enzymes and has prompted study of the nature and levels of proteinases in intact and traumatized spinal cord. Levels of these enzymes in extracts from animals following cord injury have previously been reported (Trans. ASN 10:153). Since calcium accumulates in myelinated axons in experimental trauma, some of these proteinases may be activated by Ca^{++}. Experimental spinal cord trauma was produced in rats by dropping 10 g from 30 cm upon exposed, dura-invested spinal cord. Extracts in distilled water, pH 5.8, were prepared from homogenates of normal and lesion regions of traumatized rats. The homogenate was centrifuged at 100,000 g (90 min.) and the supernatant freeze-dried and assayed for enzyme activity. The activity of neutral proteinase was assayed in 0.025M phosphate buffer, pH 7.6, at 37°C (60 min.). One microgram of protein extract was incubated with 30 µg of purified myelin basic protein in a final volume of 0.08 ml. The incubation was carried out with and without $CaCl_2$ (5 mM), EDTA (5 mM) and leupeptin (25 µg). The degradation of basic protein at pH 7.6 was greatly stimulated by Ca^{++} (5 mM) and a 69% loss of protein was observed. The addition of EDTA (5 mM) prevented the breakdown of basic protein. This Ca^{++}-activated neutral proteinase is inhibited by leupeptin (25 µg/0.025 ml). These results suggest that like muscle there is a Ca^{++}-activated neutral proteinase in spinal cord which is sensitive to leupeptin - and may play an important role in the breakdown of myelin in spinal cord trauma. Thus the use of leupeptin may be useful in preventing the myelinolysis in spinal cord injury.

Supported by PHS Grant No. NS11066.

1330 OVEREXPRESSION OF MONOAMINE OXIDASE IN NEUROBLASTOMA X FIBROBLAST HYBRIDS. James Barbosa*, John Pintar*, Uta Francke*, Morris Hawkins Jr. and Xandra Breakefield. Dept. Human Genetics, Yale Univ. Sch. Med., New Haven, CT 06510.

The effect of different chromosomal constitutions on expression of enzyme activity can be assessed using somatic cell hybrids. Cell fusions were performed using a mouse neuroblastoma clone, originally derived from the C1300 tumor, and a normal human fibroblast line, grown from a skin biopsy. The neuroblastoma parent, N1E-115TG2, is heteroploid and has no monoamine oxidase (MAO) or hypoxanthine phosphoribosyltransferase (HPRT) activity. This clone was derived from line N1E-115 which expresses MAO activity at a level of 100 pmol/min/mg protein. The fibroblast parent, GM316, has a normal diploid male complement of 46 chromosomes, and has both HPRT and MAO activity. Monoamine oxidase activity in this line is expressed at a level of 16 pmol/min/mg protein. Following hybridization, parental cells were selected against by growth in the presence of hypoxanthine, aminopterin, thymidine (HAT) and ouabain. Hybrid cells were cloned in HAT medium to select for retention of the human X chromosome which contains the gene coding for HPRT. Ten hybrid clones retained the human X and all mouse chromosomes, but only a few human autosomes. These clones expressed varying levels of MAO activity from 0 to 100 pmol/min/mg protein. This finding suggests that if there is only one gene determining expression of this enzyme, it is probably not on the human X chromosome. Hybrid clones with high MAO activity were subcloned under non-selective conditions allowing random loss of the human X chromosome. Several subclones had MAO activities of >1000 pmol/min/mg protein, over 100-fold higher than either parent. The MAO activity in these hybrids could result from a turn on of the mouse gene(s) and/or retention of the human gene(s) coding for this enzyme. The overexpression of activity reflects the interaction a few specific human chromosomes with a predominantly mouse neuroblastoma genotype. Studies are underway to identify which human chromosomes are retained and whether any alterations in the mouse chromosomal complement occur in subclones with high MAO activity. Electrophoretic techniques are being used to distinguish the species of origin of the MAO.

1331 THE EFFECTS OF SEVERAL NEUTRAL AMINO ACIDS ON d-AMPHETAMINE AND APOMORPHINE INDUCED CIRCLING IN RATS WITH LESIONS IN THE SUBSTANTIA NIGRA. John M. Beaton and Issam H. Humaideh*, Neurosciences Program, University of Alabama in Birmingham, B'ham, AL 35294.

Following unilateral destruction of the nigrostriatal dopamine system little behavioral change may be noticed. However, compounds which either directly stimulate post-synaptic dopamine receptors (e.g. apomorphine) or increase synaptic dopamine content (e.g. amphetamine) produce unilateral turning. The directionality of the turning is dependent upon whether the test compound acts pre- or post-synaptically. Amphetamine acts pre-synaptically and produces turning in the direction ipsilateral to the lesion. Apomorphine which acts post-synaptically induces contralateral turning. There is a large body of evidence which indicates that the brain levels and synthesis of the putative neurotransmitters, serotonin, dopamine and norepinephrine, can be modified by acute or chronic alterations of the peripheral levels of the large neutral amino acids (e.g. leucine, valine). The present study was carried out to study the effects of pretreatment with L-valine, D-valine or L-leucine (250 mg/kg) on rats with substantia nigra lesions, treated with various doses of d-amphetamine (2,4,6 or 8 mg/kg) or apomorphine (0.25, 0.5 or 1.0 mg/kg). The rats were lesioned electrolytically in the right substantia nigra. At least ten days were allowed between the surgery and drug testing. The various amino acids tested were all injected subcutaneously in the back 30 minutes prior to the intraperitoneal injection of either amphetamine or apomorphine. After injection of the drug the animals were placed individually in the middle of a circular open field and turns to the left or right were counted for 1 minute at 0,15,30,45 or 60 minutes after the administration of the drug. L-valine pretreatment at 250 mg/kg resulted in a marked decrease in amphetamine-induced circling in rats with unilateral substantia nigra lesions but had no effect upon apomorphine-induced circling. It is suggested that this suppression of the amphetamine effect is due to the lowering of brain tyrosine levels by the administration of the L-valine. L-leucine induced similar effects. D-valine at a similar dosage was inactive in suppressing either the amphetamine or the apomorphine-induced circling. This latter finding is not surprising since the carrier system for the entry of these amino acids into brain is thought to be stereospecific.

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1332 UPTAKE OF ^{3}H-CHOLINE AND SYNTHESIS OF ^{3}H-ACh BY THE RABBIT CORNEA. C. Belmonte*, C. Gonzalez* and S. Fidone (SPON: B.R. Kripke). Dept. Physiol., Facultad Med., Valladolid, Spain, and Dept. Physiol., Univ. Utah Col. Med., Salt Lake City, UT 84108.

The presence of large amounts of acetylcholine (ACh) and choline acetyltransferase (CAT) in the corneal epithelium of different species and their persistence after sensory denervation of the cornea is well established.

The uptake of choline in the cornea has been studied in several laboratories but there has been considerable controversy regarding the presence in the cornea of a high affinity system for choline uptake. In this study, we examine the uptake of choline in normal and denervated corneas, and further, we extend the investigation to a consideration of ACh synthesis in this structure.

Corneas of anesthetized white albino rabbits were visualized with the aid of a dissecting microscope and denervated by making a circular incision through the epithelial and stromal layers with a fine scalpel. Twelve days later the success of the denervation procedure was assessed by the absence of a blink reflex in response to stimulation of the central part of the cornea. The corneas from normal and denervated rabbits were removed and cut into pieces weighing 5-10 mgs. (wet weight). After establishing the period of linear active uptake (total-passive), the kinetics of the uptake process were determined by incubating corneas with ^{3}H-choline in concentrations ranging between 0.5 - 70 µM.

The incorporation of ^{3}H-choline could be resolved into two components, one with a Km = 6.1 µM and Vmax = 7.9 pmols/mg and the other with a Km = 42.0 µM and Vmax = 36.1 pmols/mg. Both components persisted after denervation with minor modifications of the kinetic parameters. In other experiments, the incorporation of ^{3}H-choline (10 µM) into corneal phospholipids and ACh together with the levels of free ^{3}H-choline in the tissue were determined. We found that the synthesis of ACh reached a plateau after a 1 hr. incubation but the incorporation of ^{3}H-choline into phospholipids continued to increase for up to 4 hrs. These values were unchanged following denervation of the cornea.

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1333 ^{125}I-RADIOLABELED α-BUNGAROTOXIN DERIVATIVES: KINETIC PROPERTIES OF THEIR INTERACTION WITH NICOTINIC ACETYLCHOLINE RECEPTORS.
Edward L. Bennett, Hiromi Morimoto*, Michael R. Hanley and Ronald J. Lukas, Chemical Biodynamics Division, Lawrence Berkeley Lab., University of California, Berkeley, CA 94720.

Radiolabeled derivatives of post-synaptic neurotoxins have been extensively used in characterization of nicotinic acetylcholine receptors from central, autonomic and peripheral nervous tissues. Nevertheless, there is considerable variation in reported kinetic properties of receptor-toxin interactions, which may be attributable, at least in part, to use of different chemical modification procedures in preparation of radiolabeled toxins. One popular radiolabeled toxin derivative is ^{125}I-labeled α-bungarotoxin, prepared by chloramine T., lactoperoxidase or iodine monochloride procedures. We have earlier reported (Biochem. 17, 2308, 1978) that iodination of α-bungarotoxin (α-Bgt) leads to perturbations in its physical structure, as detected by circular dichroism spectra, and causes diminution of its affinity for toxin receptors in rat brain, relative to native or tritium-labeled α-Bgt. Using detailed kinetic studies, we have found evidence that alteration of toxin biological activity on iodination is more pronounced than indicated from earlier results. For example, the dissociation (following removal of free toxin and 50-fold dilution) of radiolabeled toxin from complexes of toxin and rat brain receptor is characterized by a half-time of ∿100 hr for tritiated α-Bgt, ∿60 hr for mono-^{125}I-labeled α-Bgt and ∿10 hr for di-^{125}I-labeled α-Bgt. If experiments are performed by addition of a large excess of native toxin to radiolabeled toxin-receptor complexes, dissociation of iodinated toxins appears to be further accelerated. In general, the same phenomena is characteristic of dissociation of radiolabeled toxins from sites on Torpedo californica electroplax nicotinic receptors, and peculiarities of toxin-receptor association kinetics are observed for iodinated toxin species. Thus, the possibility exists that reports of anomolous kinetics of toxin-receptor interaction may reflect the nature of the chemical modification used to introduce radiolabel, rather than intrinsic complexity of native toxin-membrane bound receptor interactions.

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1334 RECENT EVIDENCE FOR MULTIPLE BENZODIAZEPINE RECEPTOR COMPLEXES WITH INTIMATE ASSOCIATION BETWEEN ɣ-AMINOBUTYRIC ACID (GABA) RECOGNITION SITES AND IONOPHORES.
Donald I. Benson*, Richard F. Squires and Claire A. Klepner*, Medical Research Division, American Cyanamid Co., Pearl River, NY 10965.

The existence of multiple benzodiazepine (BDZ) receptor complexes is strongly supported by several independent lines of evidence including selective protection of one class of ^{3}H-flunitrazepam binding sites against heat inactivation by phosphate ion, flat dose response curves for several triazolopyridazines exhibiting high affinity for the BDZ receptor with Hill coefficients near 0.6, and multiphasic dissociation of receptor bound ^{3}H-diazepam.

ɣ-Aminobutric acid (GABA) and several other GABA-mimetic substances have been found to significantly increase brain specific ^{3}H-BDZ binding. In addition, we have found that GABA, muscimol and β-guanidino proprionic acid (β-GPA) can protect the BDZ receptors against thermal inactivation at 60° C in 25 mM Tris·HCl buffer, pH 7.5. Heat inactivation time courses revealed three populations of BDZ receptors, two of which appear to be protected by GABA or muscimol. Dose response curves for GABA, muscimol and β-GPA revealed that both high and low affinity GABA binding sites are involved in the protective effects.

In contrast to GABA and muscimol, two GABA-mimetics, tetrahydroisoxazolopyridinol (THIP) and isoguvacine, inhibited BDZ binding in a highly selective manner. Dose response curves revealed a "plateau" effect at concentrations of 200 μM and above, whereby a maximum of 60% or 40% of the receptors were inhibited by THIP or isoguvacine, respectively. Interestingly, addition of 1 mM GABA fully reversed inhibition of binding produced by these substances. Also, neither THIP nor isoguvacine was found to protect against thermal inactivation at any concentration tested.

In addition to the phosphate ion, several other anions (chloride, citrate, malonate, sulfate, etc.) can protect certain BDZ receptor complexes. Furthermore, it has been found that both ^{3}H-flunitrazepam binding and the relative inhibition of binding produced in the presence of THIP is highly dependent upon anion concentration.

These results further support the contention of multiple BDZ receptor complexes consisting of a single BDZ recognition site, one or two GABA receptors and one or several ionophores.

1335 MUSCARINIC CHOLINERGIC STIMULATION INCREASES CYCLIC GMP LEVELS IN RAT HIPPOCAMPUS.
Asa C. Black, Jr., Dean Sandquist*, James R. West, James K. Wamsley*, and Terence H. Williams. Dept. Anatomy, Univ. Iowa Coll. Med., Iowa City, IA 52242, and Dept. Pharmacol. and Exptl. Therap., Johns Hopkins Sch. Med., Baltimore, MD 21205.

The rat hippocampus receives a cholinergic innervation which is derived from cell bodies located in the medial septal nucleus, the nucleus of the diagonal band, and possibly the intermediolateral regions of the septum (Brain, 90:521; Brain Res., 119:1). Considerable evidence has been produced to support the view that cyclic GMP is involved in muscarinic cholinergic neurotransmission.

Whole hippocampi from decapitated adult Sprague-Dawley rats were pre-incubated for 30 min at 37 C in Eagle's Medium containing 5 mM theophylline, and then incubated in the same solution containing 2.2 mM $CaCl_2$ and bethanechol. Samples were analyzed for cyclic GMP and protein (J. Neurochem., 32:1033).

INCUBATION CONDITIONS	CYCLIC GMP pm/mg. protein ± S.E.M. (No. samples)
0.25 Min., 500 μM Bethanechol	0.33 ± 0.01 (4)*
0.50 Min., 500 μM Bethanechol	0.30 ± 0.02 (5)*
0.75 Min., 500 μM Bethanechol	0.38 ± 0.05 (8)*
1.0 Min., 500 μM Bethanechol	0.39 ± 0.04 (5)*
2.5 Min., 500 μM Bethanechol	0.53 ± 0.03 (4)*
5.0 Min., 500 μM Bethanechol	0.44 ± 0.05 (5)*
Controls	0.091 ± 0.02 (7)
2.5 Min., 100 μM Bethanechol	0.21 ± 0.01 (6)*
2.5 Min., 250 μM Bethanechol	0.37 ± 0.03 (4)*
2.5 Min., 500 μM Bethanechol	0.53 ± 0.03 (4)*
2.5 Min., 1000 μM Bethanechol	0.48 ± 0.07 (6)*

*P<0.001 (Student's "t" test).

The cyclic GMP increases were calcium-dependent and were blocked by atropine sulfate. Other studies suggest that the septum gives rise to a muscarinic cholinergic projection to the basal and apical dendrites of hippocampal pyramidal cells. Acetylcholine and cyclic GMP each have excitatory effects on rabbit hippocampal pyramidal cell responses to electrophysiological stimulation of the pathway from the medial septal region to hippocampal field CA1 (Fed. Proc., 37:524). It thus seems reasonable to infer that cyclic GMP is generated by activity in the muscarinic cholinergic projection from the septum to the hippocampus in the rat (Supported by NS 11650 to THW).

1336 IN VIVO INCREASE IN HYPOTHALAMIC CYCLIC AMP FOLLOWING ACTIVATION OF SEROTONERGIC RECEPTORS IN THE RAT.
Marsha C. Bundman and Ronald A. Browning. Southern Illinois University, School of Medicine, Carbondale, IL 62901.

Recently we reported that 5-hydroxytryptophan (5-HTP) resulted in a significant increase in hypothalamic cyclic-AMP levels when administered (100mg/kg i.p.) to serotonin (5-HT) depleted rats (pre-treated with 5,7-dihydroxytryptamine, 150μg i. vent.). We postulated that this rise in c-AMP was coupled to 5-HT receptor activation (Bundman and Browning, Soc. Neurosci. Absts. 4:578, 1978). It is known, however, particularly in the absence of significant numbers of 5-HT nerve endings, that 5-HTP can be taken up by and converted to 5-HT in catacholamine neurons. In order to evaluate the specificity of the 5-HTP induced increase in c-AMP, we have now studied the effects of tryptophan on c-AMP levels and have examined the ability of serotonin receptor blockers to prevent this change.

Trytophan (100mg/kg i.p.), which is converted to 5-HT exclusively in serotonergic neurons, plus the MAO inhibitor, tranylcypromine (TCP) (20mg/kg i.p.) were administered to rats 10 days following 5,7-dihydroxytryptamine (50μg i. vent.). This resulted in a 46% increase in c-AMP levels ($p < 0.05$) as compared with animals receiving TCP alone.

The 5-HT receptor blocker, metergoline, in a dose of 5.0mg/kg i.p. given 90 min prior to challange with 5-HTP (100mg/kg i.p.) resulted in a 53% inhibition of the c-AMP increase ($p < 0.05$). However, methysergide (40mg/kg i.p., 30 min prior to 5-HTP or 10mg/kg i.p., 10 min prior) had no significant effect on the c-AMP rise, although at these doses it was successful in blocking the behavioral syndrome characteristic of 5-HT receptor activation. This suggests the possibility of two different 5-HT receptors, only one of which is coupled to changes in c-AMP.

The present findings support the hypothesis that the 5-HTP induced increase in hypothalamic c-AMP is specifically a serotonin receptor mediated phenomenon.

1337 ROLE OF GLUTAMIC ACID IN THE EARLY NEUROTOXIC EFFECTS OF 3-ACETYL PYRIDINE IN RATS. Roger F. Butterworth, François Jolicoeur*, Daniel Rondeau*, Edith Hamel* and André Barbeau. Dept. Neurobiol., Clin. Res. Inst. of Mtl., Montreal, Quebec, Canada.

Treatment of rats with a single dose of 3-acetyl pyridine ((3AP), 75 mg per kg, i.p., LD_{50}) results in severe neurological impairment in surviving animals. Histological studies have shown that 3AP produces partial degeneration of facial, hypoglossal and ambiguous nuclei and complete destruction of the inferior olive nucleus with resulting degeneration of cerebellar climbing fibres. These lesions were detectable as early as 7 hr after a single injection of 3AP and 48 hr after injection, few, if any intact climbing fibres remained (Desclin and Escubi, Brain Res., 77, 349 (1974)).

Rats showed decreased locomotor activity, catalepsy as well as a distinctive muscle rigidity 6 hr after the administration of 3AP. Abnormalities of gait (ataxia) were apparent 12 hr post treatment and by 24 hr all rats showed loss of righting reflex.

Measurement of amino acids in several discrete regions of brains of affected animals 72 hr after 3AP treatment revealed the following:-

(a) A decreased taurine concentration in cerebellum and medulla oblongata.

(b) Decreased glutamic acid concentration in cerebellum, medulla oblongata, cerebral cortex, striatum, hippocampus and olfactory bulbs.

(c) No changes in concentrations of GABA, glycine or aspartic acid in any region of brain studied.

These findings are consistent with the hypothesis that glutamic acid may be the excitatory neurotransmitter of cerebellar climbing fibres. The importance of the amino acid changes in the development of catalepsy and rigidity produced by 3AP will be discussed.

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1338 SIMILARITIES AND DIFFERENCES IN THE STRUCTURE OF A AND B FORMS OF MONOAMINE OXIDASE. Richard M. Cawthon*, Maria R. C. Costa* and Xandra O. Breakefield. Dept. Human Genetics, Yale Univ. Sch. Med., New Haven, CT 06510

Three methods of polyacrylamide gel electrophoresis were used to analyze the structure of ^{3}H-pargyline-labelled monoamine oxidase (MAO) from cells with A and B activity. These two forms of the enzyme are known to differ in their substrate selectivity, drug sensitivity, and tissue distribution. It has not been clear whether these differences result from intrinsic variation in the structure of MAO or from extrinsic modulation by its membrane microenvironment. Here the irreversible inhibitor ^{3}H-pargyline was bound to crude mitochondrial fractions from rat hepatoma line MH_1C_1, with A and B activity, and rat glioma line C6, with A activity. Specific labelling of only the A or B forms of hepatoma cells was controlled by pre-incubation with selective A and B inhibitors. Electrophoresis in sodium dodecyl sulfate (SDS)-polyacrylamide gels revealed a single protein band of MW 57,000 for both forms. Electrophoresis in non-equilibrium pH gradient gels again showed a single labelled band for both forms, indicating they also have a similar charge; however, binding of ^{3}H-pargyline to the A form was more labile than to the B form under these conditions. To further examine the structure of the A and B forms of MAO, the labelled protein bands obtained by SDS-polyacrylamide gel electrophoresis were subjected to limited proteolysis and electrophoresis in SDS-polyacrylamide gels to identify peptide fragments. Site-specific proteases can be used against fully solubilized and denatured MAO to identify discrete differences in amino acid composition or modification, independent of associated lipids. Analysis of fragments of MW >10,000 generated by Staphylococcus aureus V8 protease revealed a unique fragment present only when the B form of the enzyme was labelled. The other three fragments observed were common to both A and B forms.

In conclusion, the catalytic polypeptides responsible for A and B types of activity are similar in MW and charge, but differ in their characteristics of ^{3}H-pargyline binding and in their covalent molecular structure.

1339 MODULATED RNA RELEASE FROM ISOLATED BRAIN NUCLEI IN A CELL-FREE SYSTEM. Ming J.W. Chang, Thomas E. Webb* and Adalbert Koestner. Dept. Vet. Pathobiol. and Dept. Physiol. Chem., OSU, Columbus, OH 43210.

A reconstituted cell-free system is described which supports the release (processing/transport) of rapidly labeled, putative messenger RNA from rat brain nuclei. The nuclei, prelabeled for 30 min. in vivo with [3H] uridine, are incubated in surrogate cytoplasm consisting of dialyzed cytosol, spermidine, dithiothreitol, buffer, salts, low molecular weight RNA as RNase inhibitor, ATP and an energy-regenerating system. The nuclei were completely stable in this medium. The RNA release was dependent on (a) energy (ATP), (b) non-dialyzable factors in the cytosol and (c) to some extent the age and sex of the cytosol donor. As predicted for messenger RNA, a maximum of approximately 6-8% of the nuclear counts were released in RNA over a 30 min. incubation period at 30°C and a significant fraction of the RNA was polyadenylated. Contrary to non-neural tissues this energy-dependence of RNA release is not lost during carcinogenesis (neuro-oncogenesis) nor in the transformed cell line, T22. It is suggested that this system will be useful in analyzing nuclear RNA processing and transport in normal and neoplastic brain cells.

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1340 ISOLATION AND CHARACTERIZATION OF A PROTEOLIPID ASSOCIATED WITH (^{3}H) SPIROPERIDOL BINDING ACTIVITY. Yvonne C. Clement-Cormier and Barbara Boyan-Salyers. Depts. of Pharmacology and Neurobiology, Univ. Tex. Sch. Med. and Dept. of Microbiology, Univ. Tex. Dent. Branch, Houston, TX 77025.

A binding protein has been extracted with potassium chloride from the microsomal fraction of the calf striatum. The extract binds (^{3}H) spiroperidol with high affinity and exhibits stereoselectivity for the (+) and(-) isomers of butaclamol. The results of stability studies indicate that the solubilized component is stable to storage at -20°C for several weeks and at 4°C for one week. The IC 50 values for displacement of (^{3}H) spiroperidol binding by dopamine and other neuroleptic agents was determined to be similar to that of the native membrane binding component. Gel filtration chromatography of the soluble membrane preparation on Sephadex 100 revealed several major peaks of stereoselective binding activity for (^{3}H) spiroperidol. When the soluble microsomal fraction was extracted with chloroform methanol, stereoselective binding was recovered in both the aqueous and organic solvent phases. Fractionation of the chloroform methanol extract into the neutral and crude phospholipid (CPL) phases revealed that the highest specific binding activity for (^{3}H) spiroperidol was associated with the CPL which is enriched in hydrophobic protein. CPL binding of (^{3}H) spiroperidol represented a 1000 fold purification over that observed in the microsomal extract. Chromatography of the soluble chloroform methanol extract on Sephadex LH-20 resulted in the elution of a single peak of specific binding activity which contained both protein and lipid. Analysis of the phospholipids associated with the binding site revealed a high content of phosphotidyl inositol and triphosphotidyl inositol. Electron micrographs of the KCl striatal extract revealed the presence of electron dense particles, some of which aggregated while drying on a formvar film to form microlamellar patterns similar to that observed with highly purified proteolipids and the soluble acetylcholine receptor. Overall, these data suggest that at least one of the binding sites for the dopamine receptor in the CNS may be a proteolipid.

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1341 DIHYDROERGOPEPTINE COMPOUNDS DISPLAY HIGH AFFINITY TO VARIOUS NEUROTRANSMITTER RECEPTORS IN THE BRAIN. A. Closse*, D. Hauser* and M. Seiler* (SPON: S. H. Snyder).Preclinical Research, SANDOZ Ltd., Basel, Switzerland.

A variety of labelled ligands have been used to identify α-adrenergic, serotoninergic and dopaminergic receptors in the brain. The specific binding of these ligands is inhibited by low concentrations of dihydroergopeptines, such as dihydroergotamine, dihydroergotoxine and its components.

We have now performed comparative binding studies with ^{3}H-dihydroergotamine, ^{3}H-dihydro-α-ergocryptine, ^{3}H-dihydroergocristine and ^{3}H-dihydroergocornine. All these ligands bind in vitro with high affinity to rat brain membranes, displaying dissociation constants between 10^{-9} and 10^{-10} M. α-Adrenergic, serotoninergic and dopaminergic agonists and antagonists compete variably for these ergot binding sites.

The present studies confirm the notion that dihydroergopeptin derivatives show a high affinity to various neurotransmitter receptor sites, although in different proportions. Whereas dihydroergotamine seems to prefer serotonin receptors, dihydro-α-ergocryptine binds predominantly to α-adrenergic sites. Dihydroergocristine and dihydroergocornine show about equal affinity to all three of the neurotransmitter receptors mentioned above.

1342 AN IMPROVED METHOD FOR CALCULATION OF HIGH AFFINITY CHOLINE UPTAKE: THE USE OF CHOLINE ACETYLTRANSFERASE AS A CHOLINERGIC MARKER David O. Cooper* and Dennis E. Schmidt, Vanderbilt University, Nashville, Tennessee, 37232

In current methods, the rate of Na^{+}-dependent high affinity choline uptake (HACU) is expressed in terms of total synaptosomal protein (TSP). Tissue protein is not, however, a specific index of cholinergic synaptosomal presence, and reflects primarily non-cholinergic synaptosomes, glial elements, and other subcellular fractions. In recent studies utilizing choline acetyltransferase (CAT) as a specific marker for the presence of cholinergic synaptosomes, it was found that within individual experiments there was a variable degree of correlation between TSP and CAT activity (r value range .46 - .93). When these experiments are pooled, all correlation disappears (r = .18) suggesting that TSP is a poor indicator for calculation of HACU. Furthermore, when HACU in different brain regions is calculated on the basis of TSP, the values reflect primarily the degree of cholinergic innervation. HACU rates based on CAT activity, however, are similar in different brain regions (Table 1) indicating that a relative measure of HACU per cholinergic synaptosome is being determined.

TABLE 1

	pmol choline/ug protein/min	fmol choline/pmol CAT/min
Striatum	0.161 ± 0.017 (24)	0.639 ± 0.059 (24)
Hippocampus	0.056 ± 0.008 (80)	0.695 ± 0.045 (80)
Cortex	0.057 ± 0.006 (24)	0.970 ± 0.070 (24)

Finally, HACU values based on CAT activity were consistently less variable and statistically more significant than comparable values based on TSP. It is, therefore, suggested that under conditions where CAT activity is not altered by experimental manipulations, HACU based on CAT activity represents a better and more conceptually sound method for determination of cholinergic function.

This work was supported by USPHS grants MH 29182, DA 02050 and the Tennessee Dept. of Mental Health and Mental Retardation, the United States Brewer's Association, Inc. Dr. Cooper is a recipient of a post-doctoral fellowship award PHS grant # MH 15432-01.

1343 CHEMICAL COMPOSITION OF NORMAL AND MULTIPLE SCLEROSIS BRAINS. W. Craelius, D. Rosenheck† D.Schaefer† and A. Shaer* Dept. Biol.,Lafayette Coll., Easton, Pa. 18042.

The lipid and elemental composition of ten control and eleven multiple sclerosis (M.S.) brain samples were determined using sensitive analytical techniques. Semi-quantitative lipid analyses were done using thin-layer chromatography. No gross abnormalities in the lipid composition of normally appearing M.S. white matter were observed. However, in white matter containing plaques,a dramatic rise in free fatty acids was noted in eight M.S. brains. Sulfatides appeared to be decreased in plaque-enriched white matter, and cholesterol esters were usually elevated.

Ten elements, Ca, Cu,Fe, K, Mg, Mn, Na, Ni, P, and Zn, were within the analytical range of detection, and accurate values for these were obtained. Twenty other elements were below detectable limits. White matter from controls was compared with normally appearing white matter from M.S. brains, and marked differences were noted in some elements, as listed below:

Element	Control White Matter (10 samples) (In ug/g(wet wt.))	M.S. White Matter (11 samples)	Significance
Ca	52.0	65.8	$p < .05$
Fe	44.1	57.6	$p < .01$
P	4212.0	3725.1	$p < .02$
Zn	5.0	10.6	$p < .001$

The significance of these elemental alterations is not known. The possibility that demyelination could be related to the differences is supported by data obtained on a M.S. plaque, whose Ca, Fe, P, and Zn levels were: 91.9, 205, 3071, and 15.3 ug/g respectively. There appeared to be no substantial elemental differences between white and grey matter.

1344 PRODUCTION OF NEUROFILAMENT ANTISERA USING AXONAL PREPARATIONS ISOLATED FROM BOVINE WHITE MATTER AS THE ANTIGEN. Doris Dahl and Amico Bignami. Spinal Cord Injury Research, West Roxbury Veterans Administration Medical Center and Department of Neuropathology, Harvard Medical School, Boston, Ma. 02132.

In a recent communication from this laboratory (Trans. Am. Soc. Neurochem. 10, 141, 1979) it was shown that the polypeptides of the neurofilament "triplet" in axonal preparations isolated from bovine white matter according to Norton's procedure have different cyanogen bromide peptide maps. It was also shown that the neurofilament antisera raised against urea-soluble chicken antigen (J. Comp. Neurol. 176, 645, 1977) allow the isolation of the ∿ 70K component of the triplet by immunoaffinity chromatography. We now report experiments aimed at producing neurofilament antisera against urea extracts of the axonal preparation. All antigens were denatured in 1% SDS and injected with an equal volume of Freund's complete adjuvant. Most rabbit produced GFA antisera due to the high antigenicity of the SDS-denatured gliofilament protein constituting one third of the axonal preparation and the major fraction in the 50K mol. wt. range (BBA, in press). However, one rabbit responded with the production of an antiserum which stained both glio- and neurofilaments by immunofluorescence, only the gliofilament activity being absorbed with GFA protein. The neurofilament activity was absorbed by the axonal preparation but not by the chicken brain antigen used to produce the antiserum against the ∿ 70K component of the neurofilament triplet. The possibility of raising antisera directed against different components of the neurofilament triplet was further investigated. The ∿ 150K band in axonal preparations was cut from non-stained SDS-polyacrylamide gels and the eluted protein injected into 2 rabbits. The immunohistological localization of the 2 antisera (anti-70K and anti-150K) was identical in sections of the rat cerebellum. Experiments aimed at determining whether both polypeptides are contained in experimentally produced neurofibrillary tangles will be presented. Supported by USPHS grant NS 13034 and by the Veterans Administration.

1345 GLUCOSE AND PROTEIN METABOLISM IN THE MONOCULARLY DEPRIVED SPLIT-BRAIN PIGEON. Dale G. Deutsch and Anton Reiner, Depts. of Biochem. and Psych. SUNY at Stony Brook, Stony Brook, New York 11794

Transection of the dorsal supraoptic decussation disrupts interhemispheric transfer of monocularly learned visual discrimination tasks in birds. In the present study, brain glucose and protein metabolism were studied in such "split-brain" pigeons after monocular visual deprivation. Split-brain pigeons were prepared (R.E. Meier, Psychol. Forsch., 34, 220, 1971), fitted with aluminum goggles, and one eye was occluded for seven days by insertion of an opaque disc into the goggle.

To monitor glucose metabolism the autoradiographic 2-[^{14}C] deoxyglucose (2-DG) technique (L. Sokoloff et. al., J. Neurochem. 28, 897, 1977) was employed. Following monocular visual deprivation there were notable differences in glucose utilization between the forebrain and midbrain visual structures of the deprived and nondeprived (control) sides of the brain. Within the telencephalon, the deprived ectostriatum and visual Wulst showed marked decreases in glucose utilization relative to the control side and in the optic lobe, the tectum of the deprived side showed a marked decrease compared to the control tectum. Non-visual structures, such as those of the auditory pathway, were unaffected by the visual deprivation.

To analyze protein levels and synthesis the telencephalic hemispheres and optic lobes were dissected from the deprived and control sides of the brain. Tissue slices were prepared and incubated in Elliot's media with [^{3}H]L-leucine for one hour at 37°C. Membrane and soluble fractions were prepared and fractionated on one- and, in some cases, two-dimensional electrophoresis. Commassie blue staining and fluorography were employed for detection of protein levels and radioactive label, respectively, and the resulting patterns were analyzed on a scanning microdensitometer. No significant differences were detected between the protein patterns from the deprived and control telencephalic hemispheres or between the two optic lobes.

These results suggest that functional alterations in brain activity, as evidenced by the 2-DG technique, may not necessarily be accompanied by changes in protein levels or synthesis. However, it is possible that protein changes occur within the ectostriatum or visual Wulst, for example, and that these changes were masked by analyzing the entire telencephalic hemisphere. We thank Harvey J. Karten and Melvin V. Simpson for their help and support during these studies. Supported by USPHS 1 F 32 NS 05682001 (A.R.) and New York State Health Research Council #855 (D.G.D.)

1346 MEASUREMENT BY HPLC WITH ELECTROCHEMICAL DETECTION OF 3,4-DIHYDROXYPHENYLACETIC ACID (DOPAC), HOMOVANILLIC ACID (HVA), AND 5-HYDROXYINDOLE-3-ACETIC ACID (5-HIAA) IN RAT STRIATUM: ALTERATIONS INDUCED BY STIMULATION OF THE SUBSTANTIA NIGRA OR DRUG TREATMENTS. D.D. Dietz*, C.D. Kilts*, R.B. Mailman, R.A. Mueller and G.R. Breese. Departments of Psychiatry, Pharmacology and Anesthesiology, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27514.

A high performance liquid chromatographic (HPLC) method has been developed for the simultaneous measurement of DOPAC, HVA and 5-HIAA in rat brain homogenates. Tissues were homogenized in 0.1 N HCl and extracted with anhydrous ether. After back extraction into 0.25 M phosphate buffer (pH 6.5), aliquots were injected into an HPLC equipped with an anion exchange column (Whatman Partisil-10 SAX). A mobile phase of 0.05 M sodium acetate (pH 4.0) was pumped at 0.9 ml/min, and quantitation was by electrochemical detection (E = 0.80 v). To evoke release of dopamine, unilateral electrical stimulation of the ventral tegmentum was used (5-40 Hz; 1.5 msec duration; 20-250 μA intensity; and 15 or 30 min stimulation time). Frequency, intensity and time dependent increases in DOPAC were observed, while HVA concentrations were less markedly changed. For example, stimulation at 250 μA, 25 Hz for 15 min increased DOPAC and HVA levels 193 and 136%, whereas 30 min of stimulation increased DOPAC and HVA levels 253 and 176%, respectively. Striatal 5-HIAA content was unaffected by electrical stimulation of this site. Probenecid (250 mg/kg) and haloperidol (0.3 mg/kg) given 1 hr prior to sacrifice increased concentrations of all metabolites. (Supported by USPHS Grants HD-10570, HD-03110, ES-01104 and MH-00013.)

1347 THE USE OF HYDROPHOBIC CHROMATOGRAPHY IN THE PURIFICATION OF RAT BRAIN CHOLINE ACETYLTRANSFERASE. George W. Dietz, Jr* (SPON: G. Stone). Division of Neurosciences, City of Hope National Medical Center, Duarte, CA 91010.

Choline acetyltransferase (ChAT) has proven difficult to purify by conventional techniques, and we have sought new methods for its purification. We noted several suggestions in the literature that affinity chromatography of ChAT, for example on styrlpyridinium sepharose, reflects nonspecific interactions rather than specific interactions of a substrate-enzyme type (H.G. Mautner (1977) C.R.C.Crit. Rev. in Biochem. Nov. 341-370). However, the conditions used were not appropriate for the demonstration of hydrophobic bonding, and more critical experiments were called for. We have studied a variety of hydrophobic matrices for their ability to bind rat brain ChAT. In particular n-octyl sepharose and phenyl sepharose have been found to bind ChAT tightly. While it has not been possible to recover ChAT from the n-octyl sepharose, we were able to recover ChAT activity from the phenyl sepharose, and a chromatographic step has been developed using a gradient of ethylene glycol to elute the enzyme. The binding to phenyl sepharose seems to be unrelated to the catalytic site, since all attempts to elute ChAT with the substrate, acetyl CoA have been unsuccessful. For our final purification step we have employed an affinity chromatography on blue dextran sepharose, and have obtained highly purified preparations with specific activities in the range of 50-100 umoles of acetylcholine formed/mg/min. Our most highly purified fraction gave, upon SDS electrophoresis, three closely spaced bands with apparent molecular weights in the range of 68,000 daltons, corresponding to the reported molecular weight for the rat brain ChAT. We have not yet been able to identify the enzyme band(s) since we cannot recover activity from the gel. However, we are intrigued by the possibility that these bands represent the three charge isozymes of rat brain ChAT previously noted. (Supported by NIH grant NS 12116).

1348 FORMATION OF THE FREE RADICAL, SEMIDEHYDROASCORBATE, DURING DOPAMINE-β-HYDROXYLATION: COUPLING OF DOPAMINE-β-HYDROXYLASE TO SEMIDEHYDROASCORBATE REDUCTASE. Emanuel Diliberto, Jr.* and Pamela Allen* (Spon: James L. Howard) Dept. of Medicinal Biochemistry, Wellcome Res. Lab., Research Tri. Park, NC 27709.

Dopamine-β-hydroxylase (DBH), a mixed function oxidase, catalyzes the ascorbate-dependent oxidation of dopamine to norepinephrine. Currently, β-hydroxylation is thought to occur by the transfer of two electrons from one molecule of ascorbate to DBH with the release of dehydroascorbate; the reduced enzyme is then reoxidized by molecular oxygen and substrate with the formation of the β-hydroxylated product and water [Friedman and Kaufman, JBC, 240: 4763 (1965)]. No neural enzyme system catalyzing the reduction of dehydroascorbate has been found. Recently, Staudinger, et al. [Ann. N.Y. Acad. Sci., 92: 195 (1961)] discovered a rat liver enzyme, semidehydroascorbate reductase (SDR) which catalyzes the NADH-dependent reduction of semidehydroascorbate to ascorbate. Studies on the mechanism of catechol oxidation by DBH suggested the involvement of partially reduced DBH in the catalytic oxidation of catechol [Diliberto and Kaufman, Fed. Proc. 37: 835 (1978)]. For these reasons, the possibility of generation of the free radical, semidehydroascorbate, during β-hydroxylation and the recycling of ascorbate by SDR in the adrenal medulla was investigated. SDR was found in all membrane fractions of the bovine adrenal medulla, including the chromaffin vesicle membrane, but not in the soluble fractions. Since DBH is a chromaffin vesicle enzyme, this localization suggests a role for SDR in dopamine-β-hydroxylation. Using SDR from rat liver microsomes, the formation of semidehydroascorbate during β-hydroxylation was examined. Indeed, a tyramine-dependent oxidation of NADH was observed when SDR was present during β-hydroxylation. Further experiments confirming the generation of the free radical, semidehydroascorbate, during β-hydroxylation will be presented. In the presence of catalytic amounts of ascorbate, a coupling of SDR to DBH has been demonstrated: regeneration of active cofactor is observed by the catalytic oxidation of NADH and formation of β-hydroxylated product; delayed addition of SDR results in a decrease in the rate of NADH oxidation; and, with further delay, no oxidation of NADH was observed, indicating the lability of the free radical intermediate. At low rates of hydroxylation, a stoichiometric oxidation of NADH was obtained in the presence of SDR during the formation of octopamine from tyramine. Tyramine dependent oxidation of NADH, inhibited by fusaric acid and stimulated by fumaric acid, can be obtained with purified chromaffin vesicle membranes, suggesting a coupling of membrane bound DBH and SDR. These results are consistent with a role for SDR in β-hydroxylation through regeneration of the active cofactor, ascorbate.

1349 LOCALIZATION OF DOPAMINERGIC RECEPTORS IN RABBIT CAROTID BODY. B. Dinger*, C. Gonzalez*, K. Yoshizaki* and S. Fidone (SPON: J.W. Woodbury). Dept. Physiol., Univ. Utah Col. Med., Salt Lake City, UT 84108.

The precise role of the Type I cells and the afferent nerve terminals in the sensory transduction process of the carotid body has not been experimentally clarified. Ultrastructural studies have repeatedly demonstrated abundant dense-cored vesicles in the Type I cells, characteristic of catecholamine storage. These studies have also identified synaptic-like contacts between the Type I cells and the afferent nerve terminals. Recent biochemical studies have characterized the synthesis and release of dopamine from the carotid body, the most abundant catecholamine in this organ. Pharmacologically, it appears that exogenous dopamine is excitatory in the rabbit and inhibitory in the cat with respect to its effect on afferent fiber discharge. In order to gain further insight concerning the site of action of dopamine, we have attempted in this study to detect and characterize dopaminergic receptors in the rabbit carotid body.

Pairs of rabbit carotid bodies were rapidly removed from pentobarbital-anesthetized animals, cleaned of surrounding connective tissue, and incubated in a waterbath-shaker at 37°C for 20 min. in a modified Tyrode's solution containing different concentrations of ^{3}H-spiroperidol, with and without 0.02 µM (+)-butaclamol. Following incubation the tissue was washed for 6 min. in ice cold Ringer's solution. The weight of each carotid body was determined to the nearest tenth of a microgram. Levels of tissue and incubation media radioactivity were obtained using liquid scintillation spectrometry. Specific binding was defined as the amount of spiroperidol bound in the absence of (+)-butaclamol less the amount bound in the presence of (+)-butaclamol. The data were analyzed on a Scatchard plot. The K_A of binding was calculated to be 0.38 nM, and 4.17 pmoles of receptors are found per gram of tissue. These values compare favorably with those for high affinity dopaminergic receptors in central and peripheral nervous tissue.

In addition, we have begun a study of receptor binding in carotid bodies denervated by removal of the carotid sinus nerve 14 days previously. Preliminary results indicate that denervation does not change either the K_A or the total number of receptors. The available data is suggestive of an autoreceptor system on Type I cells similar to that of dopaminergic neurons in the corpus striatum.

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1350 ABSORPTION AND RETENTION OF HALOTHANE IN FETAL BRAIN. P. Divakaran*, B.M. Rigor*, R.C. Wiggins (Spon H. Kaufman, M.D.). Depts. Neurobiology & Anatomy and Anesthesiology, Univ. Texas Medical School at Houston, Houston, TX 77025.

Because of current interest in the effects of chronic prenatal exposure to inhalation anesthetics, we compared the tissue concentrations of halothane in fetal and adult (maternal) tissues following exposure of pregnant rats to 0.5% halothane. Following various exposure regimes, rats were killed, tissues were collected, frozen in liquid nitrogen, and eventually extracted with 1,2-dichloroethane for analysis by gas chromatography using a flame ionization detector. Our minimum instrument sensitivity was 2 ng; our lower limit of detection in tissue was estimated at 1 µg of halothane per tissue sample. Adult rats were exposed to 0.5% halothane and the concentration in brain and liver was determined at various times between 1 and 30 minutes after start of exposure. Accumulation was initially rapid and within three minutes, adult brain values were 30-40 µg of halothane per gram. After thirty minutes brain values slightly increased to 40-50 µg/g. Adult liver increased from 14 µg/g at three minutes to 30 µg/g at thirty minutes. After exposing 18 day pregnant rats, fetal brain and whole body (minus the head) were analyzed for halothane. After an initial delay of about two minutes, during which time little halothane appeared in the fetus, the halothane concentration in fetal brain increased rapidly from about 8 µg/g at three minutes to about 25 µg/g at six minutes, and then slowly to 25-30 µg/g at thirty minutes. Thus the placental separation of adult and fetal incubation caused only an initial delay in the appearance of halothane in fetal brain. Tissue levels of fetus and mother were comparable after several minutes of exposure. Retention of halothane was determined by exposing groups of pregnant rats to 0.5% halothane for three hours and determining the percentage of halothane remaining at various times after termination of the exposure. After three hours of exposure, tissue concentrations of halothane in these rats were 29 ± 7 µg/g in maternal liver and brain, 26 ± 7 µg/g in fetal brain, and 24 ± 6 µg/g in fetal body. Within three hours after termination of exposure, all tissue values had decreased by about half. Within 24 hours, values were below our detection capability. From these experiments we conclude that interposition of a placenta causes only a brief delay in the entry of halothane into fetal tissue and after thirty minutes, or more, of exposure fetal and maternal tissues contain essentially identical concentrations of halothane. The half life of tissue halothane is about three hours, although we can not rule out long term residual trace binding below our detection limits. This work was supported by Public Health Service Grant NS-14355.

1351 CHRONIC MATERNAL ETHANOL CONSUMPTION: EFFECT ON CNS SYNAPTIC PLASMA MEMBRANE PROTEINS AND GLYCOPROTEINS IN OFFSPRING. M.J. Druse-Manteuffel and A.B. Noronha*, Dept. of Biochemistry, Loyola Univ. Med. Ctr., Maywood, IL 60153.

Female Sprague-Dawley rats were pair-fed using either the control or ethanol liquid diets of Lieber & DeCarli (BioServ) for 2 months prior to conception through parturition. At 9, 16 and 23 days of age, control and ethanol offspring were injected intraventricularly with either ^{3}H- or ^{14}C-fucose. Eighteen hours later rats were sacrificed and synaptic plasma membranes (SPM) were isolated (BBA 249, 380; 1971). ^{3}H-labeled SPM from a control animal were combined with ^{14}C-labeled SPM from an ethanol pup prior to the extraction and separation of proteins by SDS-polyacrylamide gel electrophoresis (Brain Res. 76, 423; 1974). The assignment of isotopes was also reversed. Gels were stained with Fast Green, scanned photometrically and sliced for radioactivity.

The mixture of SPM from control and ethanol pups had numerous proteins. The three major proteins had molecular weights of approximately: 1) 95-100,000; 2)55-60,000 and 3) 45-50,000. The intensity of staining of bands 2 and 3 were nearly equal in 22-day-old control rats. However band 2 stained much more intensely than band 3 in the mixture of SPM proteins from 10-, 17- and 24-day-old control and ethanol pups. This difference was greatest at 10 days. Analysis of the distribution of ^{3}H and ^{14}C radioactivity on gels demonstrated that the ethanol pups incorporated more radioactive fucose into protein 2 than controls. In addition the ethanol pups consistently incorporated more radioactivity into high molecular weight glycoproteins than control rats. These results suggest that the protein and glycoprotein composition of SPM from ethanol pups are abnormal. The observed SPM abnormalities may result in altered synaptic connectivity and function.

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1352 THE INFLUENCE OF SODIUM CHLORIDE AND GUANYL-5'-YL IMIDODIPHOSPHATE ON MUSCARINIC RECEPTOR BINDING. Frederick Ehlert*, Lois Rosenberger*, William Roeske*, and Henry Yamamura. Dept. of Pharmacology, University of Arizona Health Sciences Center, Tucson AZ 85724

The effect of sodium chloride and guanyl-5'-yl imidodiphosphate (Gpp(NH)p) on muscarinic receptor binding in the rat brain and longitudinal muscle of the rat ileum was investigated with the specific muscarinic receptor affinity label [^{3}H](-)quinuclidinyl benzilate ([^{3}H](-)QNB). The concentrations of sodium chloride and Gpp(NH)p employed during binding experiments were 200mM and 30µM respectively. All binding assays were run using 50mM TRIS-HCL buffer (pH 7.4) as the incubation medium. When measured by competitive displacement of ileal [^{3}H](-)QNB binding, the IC_{50} of oxotremorine increased 3 to 10 fold in the presence of sodium chloride. A similar increase in the IC_{50} of oxotremorine was observed when ileal binding measurements were made in the presence of Gpp(NH)p. In contrast, sodium chloride and Gpp(NH)p only caused small increases in the IC_{50} of oxotremorine as determined in whole brain binding experiments. These results illustrate some qualitative differences between central and peripheral muscarinic receptors. The influence of sodium chloride and Gpp(NH)p on the binding of muscarinic antagonists was investigated by competitive displacement of [^{3}H](-)QNB binding by atropine and by direct saturation studies with [^{3}H](-)QNB. In both the ileum and the brain, sodium chloride and Gpp(NH)p only produced small effects on antagonist binding. Thus, sodium chloride and Gpp(NH)p appear to have a preferential effect on agonist binding to the muscarinic receptor. Supported in part by USPHS grants, Huntington's Diseased and Hereditary Disease Foundations.

1353 THE EFFECTS OF ORALLY ADMINISTERED U-0521 (COMT), AN INHIBITOR OF CATECHOL-O-METHYLTRANSFERASE. Stanley Fahn and A.L.N. Prasad*
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U-0521 given intraperitoneally to rats was an effective inhibitor of COMT, but was toxic in high dosages when administered with levodopa. In this present study, U-0521 was administered p.o. to rats. To effectively inhibit methylation of orally-administered levodopa, U-0521 had to be administered 30-60 min prior to levodopa. U-0521 blocked methylation periphally and in brain for up to 4 hrs following levodopa administration. A dose-response curve revealed that 400 mg/kg of U-0521 produced maximum inhibition of methylation of levodopa. Lowering the dose of levodopa did not increase the percent of inhibition of methylation, indicating that the active sites of COMT were not saturated at a dose of 250 mg/kg or 400 mg/kg of U-0521. Of potential therapeutic importance was the observation that doses of up to 500 mg/kg of U-0521 were not toxic to rats.

1354 CYCLIC NUCLEOTIDES IN THE CONE-DOMINANT RETINA OF HIBERNATING GROUND SQUIRREL. Debora B. Farber, David Chase*, Dennis Souza* and Richard N. Lolley. Jules Stein Eye Institute, UCLA School of Medicine, Los Angeles, CA 90024, and V.A. Medical Center, Sepulveda, CA 91343.

The retina of the 13-line ground squirrel contains almost exclusively cone photoreceptors. Upon entering or emerging from hibernation, cone photoreceptors of the ground squirrel undergo major changes in morphology, some of which have been reported by Reme and Young (Invest. Ophthalmol. 16, 815, 1977) and by Kuwabara (Invest. Ophthalmol. 14, 457, 1975). In addition to a reduction in the length of cone outer segments, synaptic ribbons are decreased in size and number during the early stages of hibernation and are re-formed upon emergence from hibernation. Changes in synaptic ribbon morphology are specific to visual cells because synaptic ribbons of the bipolar terminals are unchanged during hibernation. Cyclic AMP levels of non-hibernating, dark-adapted ground squirrels exceed those of cyclic GMP by about 8-fold; white light causes a significant reduction in cAMP levels exclusively. During hibernation at 6°C, cAMP levels are modified but cGMP content is not affected. During the first days of hibernation, retinal cAMP levels rise above the values measured in non-hibernating squirrels in a dark-room environment. Following one and up to three months of hibernation, cAMP levels are stabilized at a value which is similar to that of non-hibernating animals in the light. Within three days after arousal from hibernation, cAMP levels return to pre-hibernation levels, and cone morphology re-forms simultaneously. These observations suggest that hibernation alters cone cell morphology and cAMP metabolism both. These data also support the proposition that cone photoreceptors contain higher levels of cAMP than cGMP. (Supported by NIH Grant EY02651, RCDA 1 K04 EY00144 (to DBF) and the Medical Research Service of the Veterans Administration.)

1355 SYNTHESIS AND RELEASE OF CATECHOLAMINES BY THE CAT CAROTID BODY: EFFECTS OF HYPOXIC STIMULATION. S. Fidone, C. Gonzalez* and K. Yoshizaki*. Dept. Physiol., Univ. Utah Col. Med., SLC, UT 84108.

The presence of large amounts of catecholamines (CA), mainly dopamine (DA), in cat carotid body is well established. The inhibitory effect of DA on the chemosensory activity in this animal is apparently different from its action in the rabbit, rat and perhaps dog, where its effects are reportedly mainly excitatory. In previous studies, we have characterized in detail the process of biosynthesis and release of DA from the rabbit carotid body. Because of the difference in DA actions in rabbit vs. cat, it was of interest to compare certain aspects of DA metabolism in these two species.

In experiments dealing with the synthesis of CA by cat carotid body, hypoxic stimulation was effected by exposure of the animals for a 3 hr. period in an atmosphere of 10% O_2 in N_2 immediately prior to removal of the carotid bodies for incubation in modified Tyrode's solution containing ^{3}H-tyrosine or ^{3}H-dopa. In the release experiments, the carotid bodies were first pre-loaded with labelled DA synthesized from ^{3}H-tyrosine, and then mounted in a superfusion system that permitted simultaneous collection of the superfusates and monitoring of the chemosensory discharge from the carotid sinus nerve. Hypoxic stimulation involved superfusion with media equilibrated with different O_2 tensions.

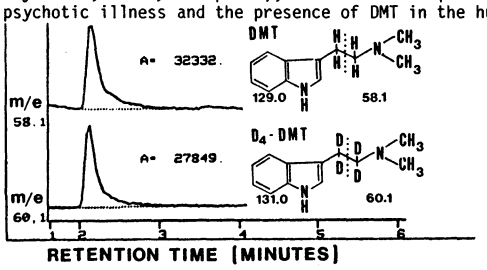
Our findings can be summarized as follows: 1) The kinetic characteristics of the process of synthesis of CA in the cat carotid body is similar to that observed in the rabbit, and both are comparable to that described for other catecholaminergic structures. 2) As in the rabbit carotid body, hypoxic stimulation in the cat does not modify the rate of synthesis when ^{3}H-dopa is used as precursor but with ^{3}H-tyrosine the synthesis is increased by more than 80% above control value. 3) There is spontaneous release of ^{3}H-DA from cat carotid body as well as a stimulus-related increase in ^{3}H-DA release which is proportional to the intensity of the hypoxic stimulation. The increase in chemoreceptor discharge from the carotid sinus nerve parallels the increase in ^{3}H-DA release. These findings are similar to our previous observations with rabbit carotid body. Thus, although other studies in our laboratory have shown clear differences between rabbit and cat with regard to the long-term effects of hypoxia on tyrosine hydroxylase activity in the carotid body, the present study fails to uncover any striking differences in the short-term synthesis of DA or its pattern of release in these two animals. Supported by USPHS grants NS07938 and NS12636.

1356 BIOCHEMICAL DEMONSTRATION OF THE MYELIN-ASSOCIATED GLYCOPROTEIN IN PERIPHERAL NERVE. D. A. Figlewicz*, R. H. Quarles*, N. H. Sternberger*, and G .R. Barbarash* (SPON, W. D. Lust). NINCDS, NIH, Bethesda, MD 20205

Recent immunocytochemical studies with antisera prepared to the CNS myelin-associated glycoprotein (MAG) revealed staining in both Schwann cells and the periaxonal portions of myelin sheaths in the rat trigeminal ganglion (Sternberger et al, PNAS 76: 1510, 1979). However, previous biochemical studies had not demonstrated MAG in the PNS. A combination of biochemical and immunological procedures has now been used to show that rat sciatic nerve myelin contains a high mol. wt. glycoprotein with properties very similar to MAG of the CNS. Sciatic nerves of adult rats were injected with 25 to 50 μcuries of (^{3}H)fucose, and myelin was isolated from the nerves 18 h later. In addition, the myelin-related "W" fraction released by osmotic shock was isolated (McIntyre et al J. Neurochem. 30: 991, 1978). Approximately, 4.4 mg myelin protein and 1.7 mg "W" fraction protein were isolated per g sciatic nerve tissue. Electrophoresis of myelin and "W" fraction proteins on SDS 10% polyacrylamide gels revealed that a large percentage of (^{3}H)-fucose was incorporated into the P_O protein, with smaller proportions into two lower mol. wt. proteins. Also, a small amount of radioactivity could be detected in several high mol. wt. proteins. The "W" fraction had consistently higher levels of incorporation into these high mol. wt. glycoproteins than the PNS myelin fraction. Some of these glycoproteins could be separated from P_O and the smaller glycoproteins by application of the lithium diiodosalicylate(LIS)-phenol procedure previously shown to selectively extract MAG from CNS myelin (Quarles and Pasnak, Biochem. J. 163: 635, 1977). Electrophoresis of the LIS extracts from the PNS myelin and "W" fractions, together with an internal standard of (^{14}C)fucose-labeled CNS myelin, demonstrated a prominent ^{3}H peak that had a small second peak or shoulder on its trailing edge which appeared to co-electrophorese with the CNS MAG. The LIS extracts of PNS myelin and "W" fractions were incubated with a specific rabbit antiserum to rat brain MAG. Addition of goat anti-rabbit IgG resulted in the precipitation of a small percentage of the (^{3}H)fucose-labeled glycoproteins. Electrophoresis of this immune precipitate revealed a single ^{3}H peak that migrated almost identically with (^{14}C)MAG from CNS myelin. Thus sciatic nerve has a minor glycoprotein with immunological and biochemical properties similar to MAG of the CNS. It seems likely that this periaxonal glycoprotein could function similarly in glial-axonal interactions in the PNS and CNS.

1357 DETERMINATION OF N,N-DIMETHYLTRYPTAMINE IN HUMAN CEREBROSPINAL FLUID (CSF), INTRAVENTRICULAR FLUID (IVF) AND RAT BRAIN USING GAS CHROMATOGRAPHY-MASS SPECTROMETRY. Joachim P. Gardemann, John M. Beaton, George B.Brown and John R. Smythies, Neurosciences Program, University of Alabama in Birmingham, B'ham, AL 35294.

The hallucinogen N,N-dimethyltryptamine (DMT), which may be produced by aberrant N-methylation of tryptamine, has been postulated as a psychotoxin responsible for the symptoms of schizophrenia. The studies to be presented here were carried out to determine (1) if exogenously administered DMT is taken up into the synaptic regions of rat brain and (2) if DMT is an endogenous substance in human CSF or IVF. Adult male Long-Evans rats were injected IP with 5, 10 or 20 mg/kg α, α, β, β-tetradeuterated DMT (D_4-DMT) 30 min. prior to sacrifice. Purified synaptic vesicles were then prepared from the brains of groups of three rats. The vesicles were extracted with methylene chloride and the extract derivatized with heptafluorobutyryl imidazole (HFBI). Samples of the derivatized extract were then analyzed on a GC/MS. The electron impact mass spectrum of the DMT as HFBI - derivative exhibits m/e 58.1 as the base peak due to alpha - cleavage of the tertiary amino group. Aliquots of 1 to 3 µl were chromatographed over a four-foot glass column, 2mm i.d., of 2% SP-2250 at 150°C for 2 min., followed by a programmed temperature increase of 10°/min. to 250°C. Under these conditions, DMT-HFB and D_4-DMT-HFB eluted at 2.2 min. Identification of the two compounds was based upon retention time and selected ion monitoring of the mass fragments 58.1 and 129.0 for DMT-HFB and 60.1 and 131.0 for D_4-DMT-HFB. The exogenously administered D_4-DMT was found to be present in the rat brain synaptosomes after intraperitoneal injection of 5, 10 or 20 mg/kg. Quantitative analyses of human CSF and IVF were achieved by using D_4-DMT as an internal standard. The occurrence of endogenous DMT could be shown in some human samples. The data below indicate approximately 100 ng DMT per 1 ml, occurring in a sample of human IVF. In extending similar studies from our laboratory (Biol. Psychiat., 1979, in press), no relationship was found between psychotic illness and the presence of DMT in the human CSF or IVF.



1358 BRAIN ALKALINE PHOSPHATASE IN MAMMALIAN SPECIES. David J. Goldstein and Harry Harris*. Dept. Human Genetics, School Medicine, University of Pennsylvania, Phila., Pa. 19104

At least three gene loci are involved in the expression of the various forms of human alkaline phosphatase (ALP); one coding for the placental form of the enzyme; at least one coding for the intestinal form; and at least one coding for the liver, bone and kidney forms. These three classes of human alkaline phosphatase can be discriminated one from another by their behavior with certain inhibitors, by thermostability, by electrophoresis and by immunologic characteristics.

We studied the alkaline phosphatase in brains and livers from several species including rodents (mouse, rat, guinea pig, hamster), a carnivore (cat), ruminants (cow, sheep) and man. By thermostabilities and degree of inhibition with L-phenylalanine, L-homoarginine and L-phenylalanylglycylglycine, the brain ALP in all these species, including man, closely resemble the liver/bone/kidney ALP.

When comparing the activities of brain ALP of certain adult and of fetal animals, a large developmental difference was discovered. Fetal guinea pig brain contained nearly twenty-fold greater activity than an adult. Newborn lamb brain contained about five-fold greater activity than adult in the cortex and cerebellum and about a ten-fold greater difference in the mid and hind brain. In addition, fetal rat brain contains six-fold greater activity than adult rat brain.

These findings raise interesting questions about the role of alkaline phosphatase in the brain.

1359 EFFECTS OF LOW Ca^{++}, HIGH Mg^{++} ON CHEMOSENSORY DISCHARGE AND RELEASE OF DOPAMINE FROM RABBIT CAROTID BODY. C. Gonzalez*, K. Yoshizaki* and S. Fidone (SPON: P.R. Burgess). Dept. Physiol., Univ. Utah Col. Med., Salt Lake City, UT 84108.

The presence of a synapse between the Type I cells of the carotid body and their sensory nerve terminals is a striking ultrastructural feature of this chemoreceptor organ. A high concentration of dopamine (DA) and numerous dense-cored vesicles in the Type I cell cytoplasm are also characteristic of carotid body ultrastructure, and it has been suggested that DA may play a key role in the mechanisms of chemoreception.

In previous studies we established that with varying levels of hypoxia the release of DA from rabbit carotid body increased with increases in chemosensory discharge. In the experiments described here, we investigated the effects of removal of Ca^{++} from the superfusion medium and its substitution by Mg^{++}. Carotid bodies were pre-loaded with ^{3}H-DA synthesized from ^{3}H-tyrosine and were then mounted in a superfusion system that allowed collection of the superfusates together with the simultaneous recording of the sensory discharges from the carotid sinus nerve. A single experiment consisted of 4 superfusion cycles, each of 45 min. duration subdivided into pre-stimulus (control), stimulus and post-stimulus collection periods. The stimulus period consisted of 5 min. of superfusion with 20% O_2 in N_2-equilibrated media. During the remaining periods, the carotid body was superfused with 100% O_2-equilibrated media. In the first and last cycles, superfusions were performed with media of normal ionic constitution, while in the middle two cycles the media contained either 0 Ca^{++} or 0 Ca^{++} plus 2.1 mM Mg^{++}. We found that during superfusion with normal-ionic media, the stimulus-related release of ^{3}H-DA was 10-13 times greater than basal release levels and the chemoreceptor discharges were 6-9 times basal levels. Neither the basal discharge or the basal release of ^{3}H-DA were affected by the 0 Ca^{++}, high Mg^{++} media, but the stimulus-related release of ^{3}H-DA was increased only 1.3-1.5 times control values and the sensory discharges were increased only 2.6-4.9 times control levels. These data suggest that the hypoxia-induced release of DA from rabbit carotid body is Ca^{++}-dependent, and that DA release may be important for the full expression of chemosensitivity in this organ.

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1360 CHARACTERIZATION OF ENKEPHALINASE. Charles Gorenstein and Solomon H. Snyder. Dept. Pharmacol., Sch. Med., Johns Hopkins Univ., Baltimore, MD 21205

Enkephalinase, a membrane bound dipeptidyl-carboxypeptidase, can degrade enkephalin in a highly specific fashion. Its high affinity for enkephalin, regional variation paralleling the opiate receptor and changes during addiction suggest that the enzyme is responsible for the inactivation of the neurotransmitter (Malfroy et al., Nature 276:523, 1978; Swerts et al., Eur. J. Pharmacol. 53: 209, 1979). We have developed a binding assay using a ^{3}H-peptide ligand displaying uniquely high affinity for the enzyme. Peptide specificity for the binding site is identical to that of enkephalinase activity assayed by measuring enkephalin degradation. Binding and catalytic activity of the enzyme in mouse and rat brain have been explored in the soluble and purified state. Alterations in binding and catalytic activity have been evaluated as a function of changed sensitivity to opiates, angiotensin and Bradykinin also substrates for enkephalinase. (Supported by USPHS grant DA-00266)

1361 DISTRIBUTION OF THE OLFACTORY MARKER PROTEIN IN THE OLFACTORY MUCOSA OF PRE- AND POST-NATAL MICE. P. P. C. Graziadei, G. A. Monti Graziadei* and R. S. Stanley* (SPON: J. S. Elam). Dept. Biol. Sc., F.S.U., Tallahassee, FLA 32306.

With immunohistochemical techniques it has been shown that the olfactory marker protein (OMP) is localized in the mature olfactory neurons of adult mice. The immature stem elements of the neurons and the supporting cells do not stain with the peroxidase-antiperoxidase method, and are presumed not to contain the protein (G. A. Monti Graziadei et al., J. Histochem. Cytochem. 25, 1977). The peroxidase-antiperoxidase method can consequently be used to determine the proportion of mature versus immature elements in embryos as well as in animals of different age. Differentiated olfactory neurons, with an apycal dentrite and an axon, are present at ten days gestation in the deepest part of the olfactory pit. At 14 days the olfactory epithelium contains a continuous layer of differentiated elements. The OMP begins to appear at this time, when it is present in few scattered neurons. The number of neurons containing the OMP rapidly increases. At 17 days of gestation positive neurons are found all through the sensory area, however some regions of the neuroepithelium are devoided of marker. In neonatal mice the common histological stains evidentiate an epithelium which is not substantially different from the one of the adult animals. However, the mature neurons stained by antiserum to OMP are restricted to a small band which occupies the distal portion of the neuronal layer. The number of stained neurons increases till 30 days postnatally, when an equilibrium is reachedbetween mature and immature elements. However, the proportion mature/immature neural elements is not constant all through the sensory sheath. Even in the adult animals there are zones where the immature elements prevail over the mature ones. These data are in agreement with previous morphological and autoradiographic observations (Graziadei and Monti Graziadei, J. Neurocytol. 8, 1979) which have shown in the neuroepithelium zones where the neurogenetic process is more/less vigorous. These zones have been termed accordingly: active and quiescent zones.

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1362 MOUSE PNS MYELIN: AN ANALYSIS OF PROTEIN COMPOSITION FOR YOUNG AND ADULT QUAKING AND NORMAL MICE. S. Greenfield*, M. J. Weise*, H. Sarvas*, S. W. Brostoff* and E. L. Hogan. Departments of Neurology, Biochemistry and Immunology, Medical University of South Carolina, Charleston, S. C. 29403.

In order to determine whether the abnormalities in ultrastructure and lipid content of Quaking (Qk) mouse PNS are accompanied by alterations in the protein composition of PNS myelin, mouse sciatic nerve myelin was isolated from 21-25 day old and from adult normal (N) and Qk mice. The yield of myelin isolated from adult Qk mouse sciatic nerve was two-thirds of control. In contrast to the initial characterization of the protein compositions of Qk and N adult mouse PNS myelin (Greenfield et al., Trans. Am. Soc. Neurochem. 55, 1978), a recent report (Matthieu, Biochem. J. 173, 989, 1978) has shown a drastic decrease in basic protein content of Qk compared to N sciatic nerve myelin. In our current studies, as before, we find that most differences are limited to 30% or less. Exceptions to this are 1) a 42% decrease in P_m (the small basic protein analogous to B_S of CNS myelin - Milek et al., Trans. Int. Soc. Neurochem., 1979) of Qk vs. N at 21-25 days, and 2) a two-fold increase in "10.5K" (Singh et al., Brain Res. 144, 303-311, 1978) in Qk vs. N adults. Analysis of myelin protein preparations in a basic protein RIA shows only 20-30% less basic protein for Qk vs. N mouse.

Our analysis of myelin from young and adult mice also suggests the occurrence of an age-related shift towards an increase in the ratio of the small to large PNS basic proteins (P_m and P1, respectively) similar to that observed for B_S and B_L of CNS myelin. This shift during maturation may not be as pronounced in Qk mice. Thus, although the P_m and P1 proteins have been shown by SDS gel and RIA analysis to be analogous to B_S and B_L (Milek et al.), the pathology in the Qk mouse appears to have a lesser effect on the protein composition of PNS compared to CNS myelin.

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1363 BENZODIAZEPINES AND PURINERGIC DEPRESSION OF CENTRAL NEURONS. Leif Hertz, Peter H. Wu* and John W. Phillis. Depts. Anat. and Physiol., Sch. Med., Univ. Sask., Saskatoon, Canada S7N OWO.

Adenosine and adenine nucleotides depress the spontaneous firing of rat cerebral cortical neurons (Can. J. Physiol. Pharmacol. 52, 1226, 1974). Inosine, adenine and 2'-deoxyadenosine have a similar but weaker action. Hypoxanthine and guanosine are virtually devoid of depressant activity. The benzodiazepines, diazepam and flurazepam, also depress the firing of cerebral cortical neurons and in sub-threshold amounts potentiate the depressant actions of adenosine and its nucleotides. Theophylline antagonizes the depressant effects of both the purines and flurazepam. Inosine, inosine 5'-monophosphate, adenine, 2'deoxyadenosine and guanosine did not antagonize adenosine-evoked depressions.

We have previously shown that adenosine is taken up into astrocytes in primary cultures with a Km of 3.4 µM and a Vmax of 0.36 n mol/min per mg protein (J. Neurochem. 31, 55, 1978). In the present work the effects of diazepam, 2'deoxyadenosine, inosine, hypoxanthine and guanosine on adenosine uptake by cultured astrocytes were studied at an adenosine concentration of 5 µM. Uptake of adenosine was greatly inhibited by diazepam (IC_{50}=20 µM) and 2'deoxyadenosine (IC_{50} < 0.25 mM), less efficiently inhibited by inosine and guanosine (IC_{50}=0.5 and 1.0 mM respectively) and only little, if at all, by hypoxanthine (IC_{50} >2.5 mM). These findings are consistent with the hypothesis that diazepam may elicit its depressant actions by enhancing the levels of extracellular endogenously released adenosine and further suggest that compounds such as inosine and 2'deoxyadenosine exert their depressant actions, at least in part, by a similar mechanism, namely by inhibiting the uptake of adenosine into brain cells. The failure of iontophoretically applied weak agonists or inactive substances such as inosine, guanosine, adenine and 2'deoxyadenosine, which like adenosine displace diazepam from its binding site in brain tissue (Life Sci. 24, 851, 1979), to antagonize adenosine-elicited depression is a further indication that the diazepam binding site in brain tissue cannot be identical with the adenosine receptor. The diazepam binding site may therefore be the adenosine uptake site.

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1364 EXPERIMENTAL ALLERGIC NEURITIS IN THE RAT - STRAIN DIFFERENCES IN THE RESPONSE TO BOVINE P2 PROTEIN. P. M. Hoffman*, J. M. Powers* and S. W. Brostoff* (SPON: G. F. Young). V.A. Medical Center, Charleston, S.C. and Departments of Neurology and Pathology (Neuropathology), Medical University of South Carolina, Charleston, S.C. 29403.

Bovine peripheral nerve myelin antigens were tested for their ability to produce experimental allergic neuritis (EAN) in three inbred and one outbred strains of rat. Each antigen was emulsified with complete Freund's adjuvant (CFA) (Difco H37ra) and injected intradermally into both hind footpads in a total volume of 0.2ml/animal. Clinical disease and histologic lesions characteristic of EAN were prominent in Lewis rats but not in Sprague-Dawley, Bufflo or Wistar rats, when bovine P2 or whole PNS myelin was used. Lewis rats responded with the most severe lesions and clinical signs when challenged with bovine PNS myelin (2 mg/animal) which contains P2 protein in its native configuration. Isolated P2 protein (200 ug/animal) produced definite but less severe disease. Reduction of disulfide bonds by treatment with mercaptoethanol enhanced the neuritogenicity of P2 protein. When rats were challenged with bovine galactocerebroside in CFA in the absence of bovine P2, no clinical or histologic evidence of EAN was present in any of the strains tested. These studies confirm the marked susceptibility of Lewis rats to EAN induction with isolated, intact bovine P2 protein (Nature 277, 140, 1979) in contrast to rabbit, guinea pig and monkey, which show no such susceptibility. As we earlier noted in the rabbit (Nature 268, 752, 1977), the conformation of the P2 protein may be important in determining its ability to induce EAN.

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1365 PHOSPHORYLATION OF RIBOSOME-ASSOCIATED PROTEINS IN CEREBRAL CORTEX. Larry A. Holbrook and Sidney Roberts. Dept. Biol. Chem. Sch. Med., and Brain Res. Inst., UCLA, Los Angeles, CA 90024.

Several investigators have shown that intraperitoneal administration of a loading dose of phenylalanine to infant rats results in polyribosome disaggregation and alterations in ribosomal function in the brain. Current investigations in our laboratory indicate that protein phosphorylation mechanisms at the ribosome level are involved in these phenomena. The present studies were designed to investigate the possibility that phenylalanine treatment may alter brain protein synthesis by changing the phosphorylation state of initiation factors and other ribosome-associated proteins whose activity is known to be affected by this process. Infant (7-day old rats) were given [^{32}P]orthophosphate intracisternally and shortly thereafter, phenylalanine (1 mg/g body wt) or an equivalent volume of 0.9% NaCl was injected intraperitoneally. The animals were killed 1 h later. Purified polysomes were prepared from the cerebri and incubated in buffer containing 500 mM KCl to release ribosome-associated proteins. After repelleting the ribosomes, the supernatant was made to 70% $(NH_4)_2SO_4$. The resultant protein pellet was analyzed on sodium dodecyl sulfate slab gels. Five major phosphoproteins were consistently found in both saline and phenylalanine-treated rats with estimated molecular weights around 110,000, 72,000, 60,000, 36,000, and 31,000. A second method of isolating initiation factors after ^{32}P-labelling in vivo was also used which involved binding of proteins present in the cerebral cytosol to a heparin-Sepharose affinity column. A wide spectrum of bound proteins distinguishable by staining and ^{32}P-labelling eluted with a 70-500 mM KCl gradient. A fraction eluting around 350 mM KCl stimulated reinitiation in a cell-free protein-synthesizing system. This fraction had major phosphoproteins of approximate MW 125,000, 112,000, 49,000, 37,000, and 17,000. The high-salt wash preparation contained only ribosome-associated proteins while the heparin-bound presumably had both free and bound translational factors. The heparin-bound initiation-stimulating fraction also had protein kinase activity which selectively phosphorylated a 38,000 MW protein in the presence of cyclic AMP. Overall phosphorylation of the proteins in these preparations appeared to be increased in phenylalanine-treated animals, but the specific factors responsible for this increase have not yet been identified. These results suggest that variations in the phosphorylation of translational factors may be involved in alterations in brain protein synthesis in experimental hyperphenylalaninemia. [Supported by a Canadian MRC Fellowship and research grants NS-13295 from the National Institutes of Health and R-259 from the United Cerebral Palsy Research and Educational Foundation.]

1366 REGIONAL DISTRIBUTION OF ACETYL-COENZYME A HYDROLASE IN RAT BRAIN. L.L. Hsu*, S.-C. Liang*, P. Hicks* and J. Claghorn (SPON: L.-P. Chao). Texas Research Institute of Mental Sciences, 1300 Moursund Avenue, Houston, TX 77030.

Acetyl-coenzyme A hydrolase (EC 3.1.2.1, AcCoA H) has been described in brain, pineal gland and blood. The distribution of this enzyme in brain, however, has not been reported. Since AcCoA H may play an important role in regulating the endogenous acetyl-coenzyme A levels in brain, and thus trigger acetylcholine synthesis, we investigated its distribution in several brain regions of the rat, for comparison with CAT activities. Five adult male Sprague-Dawley rats were sacrificed by decapitation and selected brain regions were dissected and frozen at -20°C until analysis. AcCoA H and CAT activities were determined in corpus striatum, hypothalamus, hippocampus, diencephalon, midbrain and septum. Our results showed that septum had highest AcCoA H activity (10.29 ± 0.85) followed in descending order by corpus striatum, midbrain, diencephalon and hippocampus. Hypothalamus had the lowest activity (0.66 ± 0.022 nmol AcCoA hydrolyzed/mg protein/20 min.). Similarly, septum also had the highest CAT activity (17.49 ± 1.44 nmol acetylcholine formed/mg protein/15 min.), followed in descending order by corpus striatum, (14.17 ± 0.59), hippocampus, diencephalon and midbrain. Hypothalamus again showed the lowest CAT activity (4.71 ± 0.22). In conclusion, the regional distribution of AcCoA H in brain correlates ($r = 0.82$, $p<0.05$) well with that of CAT and these observations further support the notion that AcCoA H may influence the biosynthesis of ACh in brain by regulating the acetyl-donor levels.

1367 EFFECTS OF SODIUM FLOURIDE, A PHOSPHATASE INHIBITOR, ON TYROSINE HYDROXYLASE ACTIVITY IN VITRO. T.H. Joh, M.E. Ross*, M.J. Brodsky*, D.H. Park and D.J. Reis. Lab. of Neurobiol., Dept. of Neurol., Cornell Univ. Med. College, New York, NY 10021.

We have proposed that native tyrosine hydroxylase (TH) from the caudate nucleus (CN) of rat brain is composed of a mixture of active and inactive forms, and that phosphorylation of the enzyme by cAMP-dependent protein kinase and ATP converts an inactive (-P) to an active (+P) form (PNAS, 75:4744, 1978). This implies that an active form (+P) can be converted to an inactive form (-P) by protein dephosphorylation which may be mediated by an endogenous protein phosphatase in crude enzyme extracts. If so, addition of NaF, a commonly used phosphatase inhibitor, to the TH assay mixture may block dephosphorylation, thereby increasing the TH activity. We have studied the effects of NaF on TH activity in crude tissue preparations (39,000g supernatant) in which protein phosphatase may be present. In order to examine whether the effect of NaF is tissue-specific, various tissues containing TH were used, including substantia nigra (SN) and CN (dopaminergic neurons), locus ceruleus (noradrenergic neurons) (LC) and adrenal medulla. Addition of NaF to the assay mixture of dopaminergic neurons significantly ($P<.001$) increased TH activity when using $(MePH_4)=1x10^{-4}$M: in SN it increased from 836±132 (pmol/mg/20min±SEM) to 1531±328 and in CN from 1886±292 to 3446±540. In noradrenergic neurons, TH activity in the LC was increased by only 10% (378±56 to 432 ±58) while no increase was observed in adrenal. The increase in TH activity was more apparent in measurements of initial (5 min) velocity: SN to 195%, CN to 220%, LC to 130% without change in adrenal. When $(MePH_4)=1x10^{-3}$M was used, the initial velocity of only dopaminergic neurons was increased by 30%: in SN (1108±194 to 1435±256) and CN (2716±302 to 3543±350). This NaF effect was found to be concentration-dependent with the maximum increase in TH activity occurring at (NaF)=50mM. These results indicate that dephosphorylation of TH may occur in vivo and that dephosphorylation by phosphatase decreases TH activity, especially in dopaminergic neurons. The smaller effect of NaF observed at higher concentrations of $MePH_4$ can be explained by the finding that high $MePH_4$ concentrations produce a cooperative activation of TH in crude tissue extracts, regardless of whether TH is in the (+P) or (-P) form (Trans. Am. Soc. Neurochem., 10:164, 1979). These findings confirm our hypothesis that TH, especially in dopaminergic systems, consists of (+P) and (-P) forms which coexist in a state of equilibrium. The regulation of TH activity may depend on interconversion of (-P)⇌(+P) by protein kinase (⟶) and phosphatase (⟵).

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1368 ADENOSINE REGULATES CYCLIC AMP FORMATION IN SPINAL CORD THROUGH ALPHA-ADRENERGIC RECEPTORS. David J. Jones and Laurie F. McKenna*. Depts. Anesth. and Pharmacol., Univ. Tex. Hlth. Sci. Ctr., San Antonio, Tex. 78284

Previous studies from our laboratory have demonstrated norepinephrine-stimulated formation of cyclic AMP in rat spinal cord tissue slices to be mediated by both alpha and beta-adrenergic receptors. The regulation of the alpha component of this response by adenosine was investigated in the present studies using incubated rat spinal cord tissue slices. 150-175 Gm ARS/Sprague Dawley rats were decapitated and cervical to sacral spinal cord removed. The cord was then sliced bi-directionally using a McIlwain tissue chopper set at 300 μm and slices preincubated for 40 min in oxygenated Krebs-Ringer bicarbonate buffer. Aliquots of tissue were subsequently distributed into incubation flasks with fresh buffer for an additional 15 min, at which point NE, phenylephrine (PE) or isoproterenol (ISO) were added. Antagonists phenoxybenzamine (PBA) or propranolol (PPL) and/or adenosine were added 10 min prior to NE, PE or ISO addition. Following addition of agonists the incubations were carried out for 10 additional min at the end of which tissue slice protein was acid-denatured and cyclic AMP measured by radioimmunoassay. All units are pmoles cyclic AMP/mg protein.

The addition of 10^{-4} M NE stimulated cyclic AMP accumulation from 7.3 ± 0.7 to 41.0 ± 3.2. The prior addition of 10^{-4} M adenosine potentiated the response with NE to 60.5 ± 5.3 ($p<.01$ vs NE). Adenosine alone produced an insignificant increase in cyclic AMP accumulation (10.5 ± 1.8). The alpha-adrenergic receptor stimulant PE at 10^{-4} M produced only a slight increase in cyclic AMP (10.9 ± 0.9, NS). However, when added in presence of adenosine, a 4-fold increase occurred (45.4 ± 6.3). ISO at 10^{-4} M stimulated cyclic AMP formation (16.7 ± 0.9, $p < .025$) which was not altered in the presence of 10^{-4} M adenosine. 10^{-5} M PBA blocked completely the increase in cyclic AMP due to adenosine with either NE or PE, whereas PPL did not. In addition the adenosine receptor blocking agent theophylline at $5x10^{-4}$ M also blocked the enhanced response due to adenosine with either NE or PE. It is evident from this data that adenosine modulates the alpha receptor response for cyclic AMP accumulation in spinal cord tissue slices.

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1369 SYNAPTOSOMAL DOPA DECARBOXYLASE (DDC) AND TYROSINE HYDROXYLASE (TH) ACTIVITIES ARE SENSITIVE TO CHANGES IN AMINO ACID TRANSPORT. Ira R. Katz* (SPON: Nansie S. Sharpless). Dept. of Psychiatry, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

Previous studies of the extent to which amino acid transport limits catecholamine formation have compared the rates of transport into heterogeneous synaptosome preparations with synthetic rates. For greater specificity, I studied the effects on DDC and TH activity of amino acids expected to interact with the substrate carrier, using rat striatal P_2 fractions. DDC and dopamine formation from tyrosine (requiring both TH and DDC) were assayed by release of $^{14}CO_2$ from carboxy labelled DOPA and tyrosine; TH, by release of ^3H from 3,5-$^3H_2$-tyrosine. In intact synaptosomes, DDC is inhibited by amino acids of the "L" transport system. Lysis, osmotically or by detergent, gives variable effects on DDC activity; the remaining activity is consistently insensitive to amino acid inhibition. The requirement for synaptosome integrity implies interaction at the level of transport. At 2uM DOPA, various amino acids at 100uM gave inhibition as follows: leucine, 58%; methionine, 32%; valine, 11%; phenylalanine, 60%; p-chlorophenylalanine 58%; and aminobicycloheptanecarboxylic acid, 50%. Leucine was chosen for further study. With 2uM DOPA, 12,38, & 50% inhibitions were seen at 10,25, & 50uM Leucine. Though inhibition was not strictly competitive, it decreased with increasing DOPA. In studies of the interaction between leucine and 3-hydroxybenzylhydrazine, (NSD-1015), a potent DDC inhibitor, it was found that partial inhibition by NSD-1015 led to potentiation of leucine inhibition. This is consistent with a mechanism in which leucine gave inhibition by stimulating DOPA efflux. It would not be predicted for inhibition via inhibition of influx. The above is corroborated by studies of DOPA transport in bulk striatal synaptosomes: at 2 uM DOPA in the medium (and saturating NSD-1015), steady state synaptosomal levels of DOPA are decreased by 37,50,68, & 74% at 10, 25,50, & 100uM leucine. Efflux of DOPA from preloaded synaptosomes is stimulated by leucine. Stimulation of efflux is thus both inferred from kinetic experiments, and measured directly in a heterogeneous preparation. In general, in carrier systems with strong exchange properties, trans stimulation of efflux by amino acids in the medium is expected. Inhibition of TH is also observed. At 2uM tyrosine, $^{14}CO_2$ release is inhibited by 15,21,33, & 47% at 25, 50,100, & 250uM leucine. At 0.5uM tyrosine, $^{14}CO_2$ is inhibited by 48% at 100uM leucine and 64% at 250uM while ^3H release is inhibited by 33% at 100uM and 47% at 250uM. The differential inhibition is consistent with stimulated efflux of DOPA from the synaptosomes. Thus, efflux of substrate and intermediate amino acids must be considered as potential regulatory steps in dopamine biosynthesis.

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1370 PERSISTENT ALTERATION IN BRAIN AMINO ACIDS FOLLOWING HYPOXIA AND LESIONS. A. S. Kimes and M. K. Shellenberger. Ralph L. Smith Research Center, Kansas Univ. Med. Center, Kansas City, KS 66103

We have previously reported persistent changes in the levels of catecholamines when adult rats were exposed to carbon monoxide (CO) and allowed to survive 4-6 weeks (Neurosci. Abst. 4:282, 1978). As part of an on-going study we have also examined the effects of CO-induced hypoxia and cortical lesions on the levels of amino acids (Asp, Glu, Gln, and GABA) suspected of being involved with neurotransmission.

Four groups of animals were used. The first group contained male and female adult rats (4 months old) which were exposed to CO (0.42% in air flow 4.5 l/min) until comatose and to a point near respiratory failure. In a second group of adult rats, a discrete area of frontal cortex was removed bilaterally by aspiration under light pentobarbital-ether anesthesia taking care to leave the olfactory tracts and saggital sinus intact. The last two groups were controls; one was of rats exposed to air in the chamber and a second control group consisted of sham operated animals. The brains of animals from these groups (N/group $\geq$ 6) were removed 4-6 weeks after the treatment and rapidly frozen. These brains were sectioned and the PCA soluble fraction analyzed for amino acid levels. The following sections were used: frontal cortex, striatum, midbrain-hypothalamus and pons-medulla. The amino acids were separated by ion exchange chromatography and quantitated fluorometrically.

CO exposure resulted in a significant elevation of Asp (>80%) and Gln (>100%) in the striatum ($p < .05$) in both sexes. Similar significant increases in striatal Asp and Gln were found in the sham operated animals suggesting that these animals experienced some hypoxia. Lesioning resulted in a significant reduction of Gln in the striatum while the Asp response was not consistent across sexes. Male animals showed an increase ($p < .05$) in Asp in hypothalamus and in the area surrounding the lesion in frontal cortex. On the other hand, females showed a significant decrease ($p < .05$) in striatal Asp with no alterations elsewhere.

No significant alterations were found in levels of the suspected neurotransmitters, Glu and GABA. However, the changes seen in Gln and Asp suggest that flux through the compartments of Glu and/or GABA has been altered but not to an extent large enough to be reflected in changes of levels.

In summary, exposure to CO-hypoxia and frontal cortical lesioning cause persistent alterations in amino acid levels as well as the levels of catecholamines. It remains to be determined whether these findings reflect altered metabolic or neurotransmitter functions.

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1371 NEUROTOXINS FROM BUNGARUS FASCIATUS VENOM. T.P.A. Kruck* and D.M. Logan* (SPON: J.D. Vickers), Dept. Biology, York University, Toronto, Canada, M3J 1P3.

The increasing cost and limited availability of Bungarus multicinctus venom has encouraged us to examine the venom of the related snake Bungarus fasciatus as a source of specific neurotoxins and in particular a substitute for α-Bungarotoxin. Crude venom was fractionated on several different column materials with optimal separation being obtained with CM30 (Bio Rad) which resolved the venom into 21 protein peaks, 19 of which could be identified separately by 2-dimensional gel electrophoresis. Several of the peaks demonstrated toxicity in mice and subsequent characterization identified both α (postsynaptic) and β (presynaptic) neurotoxin activities. Amino acid analyses of the major peaks showed that several of the peaks had amino acid compositions that were similar to but not identical with those reported by Hanley et al. (Biochem. 16:5840, 1977). In general, however, the molecular weights of the toxin components determined by SDS acrylamide gel electrophoresis and/or amino acid composition exceed the values reported for Bungarus multicinctus α neurotoxins. All identified Bungarus fasciatus toxin components have molecular weights in the range 12,700 to about 22,000, in contrast to α neurotoxins from Bungarus multicinctus (7,000 to 8,500 M.W.). N-terminal amino acid determinations of α neurotoxin fractions identified isoleucine and methionine at the N termini as reported for other elapid α neurotoxins. The cysteine content in these proteins is also very high as has also been found in other snake venom neurotoxins. Binding studies on the individual α neurotoxin fractions will be presented.

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1372 DEPLETION OF BRAIN TAURINE CONTENT BY GUANIDINOETHYL SULFONATE. Hugh E. Laird, Shirley Lippincott* and Ryan J. Huxtable*. Dept. of Pharmacology, University of Arizona, Tucson, Az 85721.

Further advances in our understanding of taurine in the brain await the development of pharmacological antagonists to taurine, and the finding of methods to modify taurine concentrations in the brain. We have found that the administration of guanidinoethyl sulfonate to rats, orally as a 1% solution, leads to a drop in taurine concentrations in all regions of the brain. For example, after 9 days of guanidinoethyl sulfonate therapy the concentration of taurine in the cerebellum, inferior colliculi, hypothalamus, midbrain,pons-medulla and cerebral hemispheres were reduced by 30, 30, 37, 51, 43 and 44%, respectively. After 20 days of guanidinoethyl sulfonate treatment taurine had been depleted by 55, 53, 49, 57, 66 and 59%, respectively in the same brain regions. Other free amino acids were not affected by the guanidinoethyl sulfonate. Furthermore, there is a correlation between the depletion of taurine and the accumulation of guanidinoethyl sulfonate. Guanidinoethyl sulfonate is the amidino derivative of taurine and is transported in place of taurine in the isolated perfused heart by a saturable process (Azari et al., Proc. West. Pharm. Soc. 22:, 1979). This transport is a one-component system with a Km of 153 μM and a Vmax of 65 nmole/g dry weight/min. By comparison, taurine is transported in the heart by a saturable process with a Km of 45 μM and a Vmax of 32 nmole/g dry weight/min. (Huxtable and Chubb, Science 198:409-411, 1977). Although the cardiac transport system for taurine has a lower affinity than the taurine transport system found in brain synaptosomes, the structural requirements for transport appear to be similar (Azari et al., vide supra; Hruska et al., Mol. Pharmacol. 14: 77-85, 1978). The experimental modification of taurine concentrations has proved to be difficult (see Huxtable, In: Taurine and Neurological Disorders, pp. 5-17, 1978, for discussion). We propose oral administration of guanidinoethyl sulfonate as a simple and effective method for the experimental manipulation of taurine concentrations in the brain and other organs.

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1373 CYCLIC GMP AND CYCLIC AMP INCREASES IN SPECIFIC BRAIN REGIONS FOLLOWING CENTRAL CHOLINERGIC STIMULATION. Robert H. Lenox, G. Jean Kant, and James L. Meyerhoff. Dept. of Medical Neurosciences, Walter Reed Army Inst. of Research, Washington, DC and Dept. Psychiatry, University of Vermont, Burlington, VT.

Evidence for cholinergic mediated increases of cyclic GMP has been demonstrated in a number of organ systems including the brain. Cholinergic agonists have been shown to increase the levels of cyclic GMP in the cerebellum of both rats and mice. Changes in reported levels of cyclic AMP have either been variable or nonexistent. We have proceeded to examine more closely the regional cyclic nucleotide response following central cholinergic stimulation in the brain of the rat.

Male albino rats WRC strain, weighing between 250-300 grams were maintained in a light cycled chamber and all experiments took place at the same time of day. Animals received an intraperitoneal injection of either oxotremorine (2 mg/kg), physostigmine (0.5 mg/kg), or saline 10 minutes prior to sacrifice by exposure to high power microwave irradiation. We used a 2.5 kilowatt, 2450 MHz, microwave inactivation system as modified in our laboratory. Animals receiving the cholinergic agonists were pretreated (30 min.) with methylatropine (0.5 mg/kg) to avoid excess peripheral cholinergic stimulation. Following sacrifice and decapitation, trunk blood was collected for radioimmunoassay for corticosterone (CS) prolactin (Prl) and growth hormone (GH), and 18 brain regions were dissected for radioimmunoassay for cyclic AMP and cyclic GMP.

Levels of cyclic GMP in the cerebellum of animals receiving oxotremorine increased significantly (0.98 ± .11 to 2.03 ± .18 pmoles/mg tissue) as did levels in several other regions, i.e., brainstem, midbrain, hippocampus and thalamus. Physostigmine only increased levels significantly in the midbrain and hippocampus. Oxotremorine increased the levels of cyclic AMP in several regions, e.g., the hypothalamus, substantia nigra and interpeduncular region, with the most dramatic increase in the pituitary (1.15 ± .13 to 14.63 ± 2.74 pmoles/mg tissue). There were no significant differences in CS or Prl levels among the groups. GH however, was significantly reduced following oxotremorine.

The regional pattern of increase in cyclic GMP levels in the animals receiving oxotremorine is similar to the response following locomotor activity (Meyerhoff et al., Life Sci., 1979) consistent with the behavioral observation of tremor. The increased levels of cyclic AMP, particularly in the pituitary and the GH response to cholinergic stimulation are presently undergoing further study.

1374 PARALLEL EFFECTS OF PHYSOSTIGMINE ON CHOLINERGIC ENZYMES IN BRAIN AND IN BLOOD. S.-C. Liang* and L. L. Hsu* (SPON: V. Davis) Texas Research Institute of Mental Sciences, 1300 Moursund Ave., Houston, Texas 77030.

Physostigmine, an anti-ChE drug known to reverse the central effects of atropine poisoning in man, has been clinically used to treat Parkinsonian conditions and extrapyramidal side effects resulting from long term therapy with phenothiazine and butyrophenone. Furthermore, it has been experimentally used in tardive dyskinesia and in the related condition of Huntington's Chorea. Recently, it has been described that I.V. and oral administration of physostigmine led to transient improvement in manics and in chronic schizophrenics. There has been evidence that CAT activity was significantly decreased in hippocampus and limbic lobe of schizophrenics. It has also been reported that during the treatment of paranoid schizophrenics with neuroleptics, there was significant reduction in RBC AChE activity and in plasma pseudocholinesterase (ChE) activity immediately preceeding the administration of anti-parkinsonism agents. There are no reports however, on the correlation between changes in cholinergic enzyme activity in brain and in blood. We have investigated such possible correlations in mice after I.P. administration of physostigmine. For this study, ten adult male DBA/2J mice were divided into two groups: five received 30 ul of physostigmine (0.3 mg/kg, i.p.) and 5 received 30 ul of distilled water (i.p.) injections. Fifty min. following the injections, mice were sacrificed by decapitation. Selected brain regions (corpus striatum, hippocampus and remainder of brain) were immediately dissected and homogenized in 0.05M Na-phosphate buffer (pH, 7.5) for determination of cholinergic Na-phosphate buffer (pH, 7.5) for determination of cholinergic enzyme activities (AChE, CAT and AcCoA hydrolase). Whole blood from each mouse was collected into 100 ul of ice cold 15% EDTA solution and fractionated into RBC and plasma and the RBC AChE plasma ChE, and AcCoA hydrolase activities were measured. Our results indicate that in the physostigmine treated mice: (a.) plasma ChE was significantly decreased (22%), RBC AChE was slightly but not significantly decreased (13%) and AcCoA hydrolase was not changed in whole blood. (b.) AChE was significantly decreased in corpus striatum (25%) and hippocampus (31%) but not changed in the remainder of brain, CAT was significantly decreased in corpus striatum but not changed in hippocampus or remainder of brain, and AcCoA hydrolase was not changed in any of the brain regions examined. In conclusion, we have observed a parallel reduction in plasma ChE and in specific brain regions, corpus striatum and hippocampus and CAT activity in corpus striatum. These observations may provide a basis for study of the possible cholinergic mechanism in schizophrenia.

1375 CHOLESTERYL ESTER CHANGES IN TISSUES OF CHICKENS WITH HEREDITARY MUSCULAR DYSTROPHY. D.M. Logan*, R. Battistella‡ M.P. Rathbone, and E.S. Werstiuk*, Dept. Biol. York Univ., Downsview, Ont., Canada, Dept. Neurosci., McMaster Univ., Hamilton, Ont., Canada.

Increased total cholesterol (TC) content at 9 days in ovo is the earliest biochemical change reported in muscles of chickens with hereditary muscular dystrophy (HMD) (Exp. Neurol. (1977) 57: 475). Subsequently, lipid infiltration of the degenerating muscles is a cardinal feature of the dystrophic process. The increased TC content of dystrophic muscle is due to an increased content of cholesteryl esters (CE). Dystrophic chickens are also hyperlipidemic (Neurosci. Letts. (1978) 8:151) and have a high liver TC content. We previously demonstrated that the TC content of the HMD muscle is neurally regulated irrespective of the cholesterol content of serum and liver (Ann. NY Acad. Sci. (1979) 317:594). As a first step in determining the molecular mechanisms underlying the abnormal neural influence on HMD muscles and the possible role of systemic alterations of lipid metabolism on the pathogenesis of HMD in chickens, we examined the nature of the CEs in muscles, livers, serum and brains of HMD and normal chicken embryos at 16 days in ovo. The cholesteryl esters were separated by thin layer chromatography, their fatty acids (FA) converted to methyl esters and separated by gas chromatography. The profiles of FAs derived from CEs differ in each of the tissues examined. There is also a difference in FAs between the corresponding normal and HMD tissues. In HMD serum the content of short, unsaturated fatty acids is reduced; 12:0 by 80%, 14:0 by 70%; the levels of 18:2, 18:3, 18:4 and 20:1 are also lower. In contrast, 18:1 is 150% higher in HMD serum. Differences between HMD and normal livers are less marked; 14:0 and 16:0 are approximately 40% lower in dystrophic livers and 18:1 is approximately 25% lower. In HMD brains 18:1 is reduced by 80%. The FA profile from thigh muscles, which are minimally affected by the dystrophic process, were identical in normal and HMD embryos. In contrast, in superficial pectoral muscles, which are severely affected, 18:1 was 350% higher in HMD. The increased 18:1 in the pectoral muscles is indicative of increased intracellular esterification of cholesterol, rather than increased uptake of CE from the plasma. Thus, the neural influence probably regulates CE synthesis in the muscles.

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1376 PHYLOGENETIC DISTRIBUTION OF [^{3}H]KAINIC ACID RECEPTOR BINDING SITES IN NEURONAL TISSUE. Edythe D. London, Nikolei Klemm* and Joseph T. Coyle, Lab. of Neurosciences, GRC, NIA, NIH, Balto., MD 21224; Depts. of Pharmacol. Exp. Therap. and Psychiatry and Behav. Sci., Johns Hopkins Sch. Med., Balto., MD 21205.

The phylogenetic distribution of specific binding sites for [^{3}H]kainic acid was determined in 14 species of invertebrates and vertebrates. The highest level of binding was observed in brains of the frog (Xenopus laevis), followed by the spiny dogfish (Heterodontus francisci), the goldfish (Carasius auratus) and the chick (Gallus domesticus). Significant specific binding was noted in some (e.g. Hydra littoralis), but not all of the phylogenetically lowest forms tested. In most cases, specific binding to both high and low affinity sites was detected; notable exceptions were the cockroach brain (Periplaneta americana), which had negligible high affinity binding, and the crayfish brain (Procamburus) which had negligible low affinity binding. In the spiny dogfish, the smooth dogfish and the chick, the highest level of binding occurred in the cerebellum; less occurred in the forebrain and the least in the medulla; in the mammalian species, the highest level of binding occurred in forebrain structures; less in the cerebellum and least in the medulla.

Eadie plots of the saturation isotherms for [^{3}H]kainic acid revealed similar kinetics of binding for frog whole brain, rat forebrain and human parietal cortex with two apparent populations of binding sites: K_{D1}=25-50 nM and K_{D2}=3-14 nM. While binding in the spiny dogfish forebrain and human caudate nucleus occurred exclusively at high affinity sites, binding was only to low affinity sites in the cerebella of chick, rat and man. In the three species studied most extensively, frog, rat and man, unlabeled kainic acid was the most potent inhibitor of [^{3}H]kainic acid specific binding. L-Glutamic acid was 20- to 200-fold less potent than kainic acid; D-glutamic acid was 4- to 2,500-fold less potent than its L-isomer. Reduction of the isopropylene side chain of kainic acid to form dihydrokainic acid decreased the affinity of the derivative 115- to 3,000-fold. While the Hill coefficient derived from these inhibition studies was 1.0 for unlabelled kainic acid, it was approximately 0.5 for L- and D-glutamic acids and dihydrokainic acid, compatible with negative cooperativity. These studies demonstrated a widespread distribution throughout the animal kingdom of specific binding sites for kainic acid in neural tissue. The characteristics of these receptor sites were remarkably similar from primitive vertebrates to man, suggestive of an endogenous neuronal system that has not changed appreciably through evolution. Supported by USPHS Grants NS 13584, MH 26684, RCDA Type II KO 2 MH 00125 and a grant from the Nat'l. Found'n. to JTC and USPHS Fellowship MH 0714202 to EDL.

1377 TAURINE INTERACTION WITH CENTRAL NERVOUS SYSTEM MEMBRANES. A.M. López-Colomé* and H. Pasantes-Morales* (SPON: G.H. Vázquez). Centro de Investigaciones en Fisiología Celular, U.N.A.M., México 20, D. F., México.

Taurine has been considered as a putative neurotransmitter in the Central Nervous System; evidence supporting this function is not yet conclusive and an alternative role as a modulator of nerve excitability has also been suggested (Mandel and Pasantes-Morales, Rev. Neurosci. Vol 3 pp. 157; 1978).

Binding to synaptic membranes has been a useful tool in the identification of postsynaptic receptors for neurotransmitter candidates. It has been considered that the interactions of neurotransmitters with postsynaptic receptors is a sodium independent process which becomes more apparent in frozen and thawed membranes, while the opposite is true for other kinds of receptors - uptake sites for instance.

In the present work, we studied the characteristics of [^{3}H]-taurine binding to membranes from the chick retina,from the rat cerebral cortex and dorsal root ganglia. Parallel studies were carried out for GABA, a recognized central inhibitory neurotransmitter. Membrane preparation and binding assay were carried out following the general procedure of Enna and Snyder (Brain Res., 100: 81; 1975).

Taurine binding to chick retinal membranes in the absence of sodium was extremely low (0.014 ± 0.004 p moles/mg protein); under the same conditions, GABA binding was of 0.494 ± 0.16 p moles/mg protein. In the presence of sodium, maximum taurine binding was 22.9 p moles/mg protein. In rat cerebral cortex membranes, taurine binding was extremely low in the presence of sodium and practically undetectable in the absence of sodium. GABA binding, although considerable in the presence or absence of sodium, was higher in the later condition. In membranes obtained from dorsal root ganglia, in which no synaptic terminals are present, no specific binding of [^{3}H]-taurine or [^{3}H]-GABA was observed to frozen and thawed membranes in the absence of sodium. In freshly prepared membranes in the presence of sodium, taurine binding could not be detected whereas GABA binding was consistently observed.

According to the present results, an interaction with the characteristics adscribed to postsynaptic receptor binding was not observed for taurine in any of the preparations studied.

1378 EVIDENCE FOR FUNDAMENTAL INVOLVEMENT OF RAT BRAIN NICOTINIC RECEPTOR DISULFIDE/SULFHYDRYLS IN THE RESPONSE TO ACETYLCHOLINE. Ronald J. Lukas and Edward L. Bennett, Chemical Biodynamics Division, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720.

The affinity of nicotinic acetylcholine receptors (nAChR) from muscle and electric organ for acetylcholine (ACh) is sensitive to modification of receptor SH/S-S residues. We earlier reported (Biochem. 18, in press, 1979) that the affinity of rat brain α-bungarotoxin (α-Bgt)/acetylcholine receptors for ACh is also sensitive to SH/S-S modification. It is of interest to determine whether alteration of the SH/S-S groups in question plays a fundamental role in agonist-stimulated function(s) of nAChR, or whether their experimental modification bears no relation to receptor activity, but merely affects ACh binding sterically.

Alkylation of dithiothreitol (DTT)-reduced rat brain membranes, whether with neutral N-ethylmaleimide (NEM) or iodoacetamide, or with anionic p-chloromercuribenzoate or iodoacetate, leaves nAChR in a low-affinity state with respect to agonist. That is, the ability of ACh to retard the rate of [^{3}H]-α-Bgt binding is diminished. As reported earlier for treatment of reduced nAChR with dithio-bis-nitrobenzoate (DTNB), treatment of native receptor with sodium bisulfite ($Na_2S_2O_5$), which cleaves S-S bonds and forms a mixed disulfide (nAChR-S-SO_3) with one of the freed sulfhydryls, leaves nAChR in a high-affinity state. Thus, the net charge or physical size of the modifying group bears no particular relation to its effect on affinity for ACh. Rather, modification effects are determined by the lability (and enhanced reactivity?) of the mixed S-S bond produced on DTT-DTNB or $Na_2S_2O_5$ reaction, relative to stable S-C or S-Hg bonds produced on alkylation. Further, reactivity of nAChR SH/S-S groups is modified by pretreatment of nAChR with agonist, but not antagonist. For instance, membranes treated with 10μM ACh for 30 prior to exposure to DTT-NEM, and then washed free of ACh, DTT and NEM, are susceptible to transformation to a high-affinity state on exposure to agonist. However, membranes treated with 1mM d-turbocurarine or with buffer only prior to DTT-NEM are "frozen" in a low-affinity state. Insofar as ACh does not block NEM-alkylation of DTT-reduced receptor, it is unlikely that ACh effects are steric in nature. Moreover, the ACh concentration dependencies of blockade of [^{3}H]-α-Bgt binding and alteration in SH reactivity are similar. Taken together, the results are consistent with a fundamental role of SH/S-S groups in ACh-stimulated nAChR function.

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1379 QUANTITATION OF GOLDFISH TECTAL PROTEINS IN TWO-DIMENSIONAL POLYACRYLAMIDE GELS. W. A. Lutin*, C. F. Kyle*, and J. A. Freeman (SPON: J. S. Lappin). Vanderbilt Univ., Nashville Tenn 37232.

Proteins from goldfish optic tectum were separated by high resolution two-dimensional polyacrylamide gel electrophoresis. The separation pattern obtained from gels stained with coomassie blue yielded two hundred polypeptide spots. Individual spots were quantitated by computer-automated integration of the optical density of films of the gels. This was accomplished by approximating the optical density surface representing the scanned film with a set of best-fit two-dimensional Gaussians. This method resulted in volume integrals accurate to ±5% in six minutes of processing time on a DEC 1099 computer.

Multiple gels of tectal homogenates were compared by a local pattern matching algorithm which uses the Gaussian parameters for the protein spots in each gel as input. This method of comparison singled out 5 micrograms of creatine kinase added to a tectal homogenate as the only difference between two otherwise identical samples. The application of this method to autoradiograms of two-dimensional gels of 35S- or 14C- labeled proteins from goldfish tectum, toad optic nerve, or human fibroblasts allowed the itemization of quantitative and qualitative differences between gels containing over five hundred spots. This technique is currently being employed to identify goldfish tectal membrane proteins which are altered in concentration during regeneration of the optic nerve.

1380 COMPARATIVE EFFECTS OF PENTOBARBITAL, KETAMINE, ENFLURANE AND HALOTHANE ON CEREBROSPINAL FLUID DYNAMICS IN THE RAT. J. D. Mann, S.L. Cookson*, E.S. Mann*, Departments of Neurology and Anesthesiology, University of North Carolina 27514.

Four different anesthetic agents were studied to determine their effects on mechanisms regulating intracranial fluid dynamics in adult albino Sprague Dawley rats. Comparable anesthetic depth was achieved with pentobarbital 40 mg/kg i.p.; ketamine hydrochloride 40 mg/kg/hr i.v.; enflurane, 2.5% in oxygen; and halothane 1.0% in oxygen. Following induction of anesthesia with one of these agents, animals were paralyzed with pancuronium and artificially ventilated. Body temperature was maintained at 37°C., and heart rate, arterial blood pressure and blood gases were monitored throughout the experiment. Artificial CSF buffered to pH 7.32, was then infused into the subarachnoid space at the cisterna magna through a 20 ga. spinal needle with continuous monitoring of pressure responses. Infusions were performed at multiple rates (between 5 and 30 ul/min) until steady state pressure was achieved for each infusion rate. A previously described mathematical model of CSF dynamics was used to assess intracranial compliance, CSF outflow resistance at the arachnoid villi, and CSF formation rate (J.D. Mann, Ann. Neurol. 3:156-165, 1978).

Blood pressures were comparable in all groups (102 ± 14 SD mm Hg) with the exception of the enflurane animals (78 ± 13 mm Hg). Resting CSF pressure was lowest for the pentobarbital anesthetized rats (35 ± 19 mm H_2O) and highest for the enflurane group (130 ± 48 mm H_2O). Compared to pentobarbital, there was a significant two-fold increase in maximum outflow resistance when either ketamine or enflurane was used. Analysis of outflow resistance characteristics for the ketamine animals revealed an impairment of low pressure, transcellular CSF transport, while for the enflurane rats, outflow resistance was increased over a broader range of pressures and to a greater extent at high pressures, representing delayed activation of large volume CSF transport mechanisms. Calculated CSF formation rates were in the normal range (2-4 ul/min) for animals anesthetized with either pentobarbital or ketamine (3.97 ± 1.24 SD and 3.29 ± 1.87 ul/min). However, CSF formation was enhanced 55% ($p < .05$) with halothane, and 85% ($p < .01$) in animals anesthetized with enflurane. Our results suggest a complex interaction between anesthetic agents and the various mechanisms which regulate intracranial fluid dynamics, including CSF formation rate and resistance to reabsorption at the arachnoid villi. The biochemical bases for these changes remain to be determined. This work was supported by NIH Grant NINCDS 1 K07 NS00244.

1381 RAT PHEOCHROMOCYTOMA TYROSINE HYDROXYLASE: CHARACTERIZATION OF THE BASAL AND PHOSPHORYLATED ENZYME. K. Markey*, S. Kondo*, L. Shenkman* and M. Goldstein. (SPON. R. Margolis). New York University Medical Center, Departments of Psychiatry and Medicine, New York, N.Y. 10016.

Tyrosine hydroxylase (TH) was purified from pheochromocytoma PC-12 clonal cells to homogeneity. The purified enzyme was used as an antigen for production of antibodies in rabbits. The specificity of the anti-TH was demonstrated by immunoelectrophoretic analysis as well as by specific inhibition of the enzyme activity. The rat pheochromocytoma anti-TH reduces the activity of the homologous enzyme more effectively than the activity of the heterologous enzyme.

The activity of purified TH is stimulated by a c-AMP dependent protein kinase phosphorylating system (PKP system), and the highest % of stimulation is obtained when the enzyme activity is measured at physiological pH's. The stimulation of the purified enzyme by the PKP system results in a reduction of the apparent K_M for the co-factor 6-MePH$_4$ and in an increase of the K_i for dopamine.

Incubation of purified TH with the PKP system and ^{32}P ATP, resulted in incorporation of radioactivity into the 62,000 subunit of the enzyme. Purified enzyme which has lost some enzyme activity upon storage has incorporated less ^{32}P than the enzyme with a higher specific activity. The incorporation was approx. 1 mole of ^{32}P/mole of TH (M.W. of TH 240,000). These results suggest that either one of the four subunits of TH is phosphorylated or that under our experimental conditions only 25% of the enzyme was phosphorylated. Further studies on the incorporation of ^{32}P into TH are in progress.

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1382 FRAGMENTATION OF SUBSTANCE P BY BRAIN AND PITUITARY CATHEPSIN B. Neville Marks and Alojz Suhar*. Center for Neurochemistry. Rockland Research Institute. Wards Island. N.Y. 10035

Neurosecretory regions of rat brain were found to contain high levels of cathepsin B relative to other areas suggesting a possible involvement in the processing of neuropeptides. In other tissues cathepsin B has been implicated in the activation of inactive precursors to yield active hormonal peptides. In addition, cathepsin B is considered to play a key role in the intracellular turnover of proteins but has not been studied extensively in the CNS. Enzyme, therefore, was purified from human pituitary and bovine brain over 400 fold using classical procedures yielding in some preparations an enzyme with a single protein band. Both tissues contained a family of related BANA hydrolases hydrolysing benzoyl-arginyl β-naphthylamide of which only one resembled the classical cathepsin B purified from other tissues (pH optima 6.5 in presence of -SH groups, inhibition by low concentrations of Ac-Leu-Leu-arginyl or leupeptin).

Purified enzyme from both sources cleaved a number of CNS components including histones, neurophysins, myelin basic protein, β-lipotropin and β-endorphin. The enzyme was selective since it did not cleave albumin, γ-globulin or caesin, and showed low or no activity with hemoglobin and polylysine. Surprisingly, among the best substrates tested was the unidecapeptide substance P (Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met.NH_2 Since cleavage products of substance P show a spectrum of activities dependent on the test system utilised then proteolytic enzymes may play a role in regulation. Among the products isolated after incubation of substance P with purified brain and pituitary cathepsin B was the tetrapeptide Phe-Phe-Gly-Leu indicating probable cleavage at the Gln-Phe and Leu-Met.NH_2 bonds. Cleavage at the first bond would generate a C-terminal pentapeptide that would retain ability to contract guinea pig gut in vitro but would lose the property of depolarisation of frog spinal motoneurons. Loss of Met.NH_2 from the pentapeptide or substance P would lead to inactivation. Cathepsin B of brain and pituitary can be added to the list of enzymes known to degrade substance P and which include aminopeptidases, neutral endopeptidases, and a specific prolyl endopeptidase.

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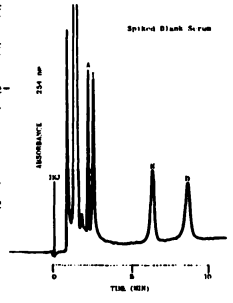
1383 A RAPID, SENSITIVE METHOD FOR THE SEPARATION AND MEASUREMENT OF TRICYCLIC ANTIDEPRESSANTS AND THEIR PSYCHOACTIVE METABOLITES IN HUMAN SERUM BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY. D. M. Martin*, A.L.C. Pottash*, and M. S. Gold. Yale University School of Medicine, New Haven, CT 06520, Psychiatric Institutes of America and Psychiatric Diagnostic Laboratories of America, Summit, NJ 07901

Recent studies indicate that the measurement of serum levels of tricyclic antidepressants (TCA) and their psychoactive metabolites may facilitate the effective pharmacological management of endogenous depression. Existing High Performance Liquid Chromatographic (HPLC) techniques employing both normal and reverse phase technologies are limited by column deterioration or complex mobile phase formulation re-respectively, making their routine application in the clinical laboratory difficult.

Tricyclics were extracted from one (1.0) ml of patient serum into a basic organic phase, freeze dumped and taken to dryness under a stream of nitrogen. The residue was injected into an isocratic system of 0.025% butylamine in methanol flowing at 1.4 ml/min. through a column of fully porous silica. Detection was in the ultraviolet at 254 nm, Amitriptyline (A), Imipramine (I), Nortriptyline (N) and Desipramine (D) respectively, were separated in a single injection(Fig). Unknowns were quantitated by comparison of peak height ratios to standard curves generated against spiked blanks run in parallel. Over five hundred (500) patient samples have been run using this technique yielding a mean day-to-day coefficient of variation of less than 7%. As little as 5 ng of each drug can be reliably detected in one (1.0) ml serum. Recovery of spiked blanks ranged from 65-75% increasing with concentration as reported in the literature. The precision, reproducibility, ease of sample preparation and simplicity of instrumentation make this technique exceptionally well suited for therapeutic monitoring of tricyclic antidepressants.



1384 2'3'-CYCLIC NUCLEOTIDE-3'PHOSPHOHYDROLASE (CNP) ACTIVITY IN NERVOUS TISSUE OF TEMPERATURE ACCLIMATED GOLDFISH (CARASSIUS AURATUS L.). D.F. Matheson*, R. Oei* and Betty I. Roots, Dept. Zool. and Erindale College, University of Toronto, Toronto, Ont., Canada.

Nervous tissue rich in myelin possesses high activity of CNP (EC 3.1.4.37). Consequently CNP activity has been accepted as a myelin index.

Previous findings from our laboratory have established that the distribution spectrum of axon diameter in the optic nerve of goldfish varies with temperature of acclimation; at 5°C there is a greater proportion of large axons greater than 0.9μm in diameter than at 25°C (cf. 71% and 22%). In order to relate these morphological observations to a biochemical assessment of myelin, CNP activity was determined in the optic nerve of 5° and 25° acclimated fish, and compared with the activity in other myelin-rich preparations (spinal cord myelin, brain myelin). As expected, the optic nerve possesses significantly higher CNP activities in the 5° fish compared with the 25°. It was found also that brain myelin has higher activities at the lower acclimation temperature. In the optic tectum, a tissue containing little myelin, there is no difference in the CNP activity with acclimation temperature. Other biochemical indices of myelination were determined in optic nerve and tectum; e.g. molar ratios of phosphatidyl ethanolamine/phosphatidyl choline (PC), and galactolipid/PC. With elevation in acclimation temperature these ratios decrease in nerve and rise in tectum. An interesting observation is that the CNP activity/μmoles galactolipid ratio is lower at higher acclimation temperatures in all myelin-rich preparations but remains the same in the optic tectum.

These findings not only confirm the findings from morphological studies that the amount of myelin changes during temperature acclimation but indicate that there may also be a change in the composition.

The significance of these findings will be discussed.

1385 CHANGES IN γ-AMINOBUTYRIC ACID RECEPTOR BINDING IN THE POST-MORTEM CAT CENTRAL NERVOUS SYSTEM. G. Keith Matheson and Godfrey Tunnicliff*. Evansville Center, Indiana University School of Medicine, Evansville, IN 47732.

Postmortem GABA receptor binding is becoming a useful measurement in the investigation of the role of GABA in diseases of the nervous system. One of the problems of studying human postmortem brains is the lack of standardized procedures for their storage. This investigation was undertaken to determine if postmortem changes in GABA binding occur when brains are stored for different periods of time before the membranes are prepared. GABA receptor binding in fifteen regions of cat central nervous system was investigated immediately postmortem, and at twenty-four and seventy-two hours postmortem. A two-fold increase in binding was observed after twenty-four hours in the cerebellum, the sensorimotor cortex, the visual cortex, and the amygdala. Substantial increases were also noted in the thalamus, caudate nucleus, hippocampus, and hypothalamus. At seventy-two hours postmortem further increases in GABA binding were seen. Membranes from the pons and spinal cord did not exhibit appreciable changes in GABA binding. These findings suggest that interpretation of GABA binding data obtained from human brains that have not been treated in a similar postmortem manner should be made with reservation.

REGION	HOURS POSTMORTEM	
	0	24
Cerebellum	263±34	594±83
Visual Cortex	136±19	322±41
Sensorimotor Cortex	130±21	314±56
Amygdala	141±33	323±39
Caudate Nucleus	110±15	166±22
Hippocampus	80±18	131±16
Thalamus	72± 9	118±13
Hypothalamus	71±10	94±14
Pons	26± 8	34± 9
Spinal Cord	24± 5	32± 7

These values are the means ±S.E.M. of GABA binding (f moles/mg protein). Each determination was done in triplicate. Three animals were used in each experiment.

1386 PUTATIVE NEUROTRANSMITTER RECEPTOR SITES IN RAT HIPPOCAMPUS. Dee Ann Matthews, Paul Salvaterra and Renee Foders*. Div. of Neurosciences, City of Hope Nat. Med. Center, Duarte, CA 91010.

The hippocampus is well suited for studies of synaptic transmission mechanisms in CNS by reason of its simple laminated organization, defined connections and variety of transmitter systems. In order to study the quantitative relationship among various neurotransmitter systems in rat brain hippocampus, the levels of several putative neurotransmitter receptors were studied by radiolabeled ligand binding techniques. The levels and affinities of the various ligands used were determined by Scatchard analysis of binding isotherm data using crude membrane preparations. All ligands showed only a single high affinity site. Data is presented in the table below. Data were also analyzed by Hill plots and all ligands had slopes near 1, indicating the absence of any cooperative interactions. Pharmacological characterization of the ligand binding sites indicated properties consistent with their identification as functional neurotransmitter receptors. The rank order of the total number of putative receptor binding sites (gabaergic>cholinergic>adrenergic) is in agreement with current estimates of the relative number of terminals contributed by each system. Supported by NIH NS 12116.

Ligand	Putative Receptor	B_{max}[a] (fmoles/mg)	K_D (M)
[^{3}H]Muscimol	GABA	1920[a]	5.47 x 10^{-8}
[^{3}H]Quinuclidinyl-benzilate	muscarinic-Cholinergic	863	2.21 x 10^{-9}
[^{3}H]Dihydroergo-cryptine	α-Adrenergic	474	6.9 x 10^{-9}
[^{3}H]Dihydroal-prenolol	β-Adrenergic	67.8	2.72 x 10^{-9}
[^{125}I]αBungaro-toxin	nicotinic-Cholinergic	43.2	1.19 x 10^{-9}

[a] Values are derived from Scatchard plots of two sets of binding isotherm data using 6 different concentrations of ligand in triplicate.

1387 BRAIN CYCLIC NUCLEOTIDE LEVELS IN RATS SENSITIVE TO AUDIOGENIC SEIZURES. James L. Meyerhoff, G. Jean Kant, T. Daryl Hawkins* and Robert H. Lenox. Dept. of Medical Neurosciences, Walter Reed Army Inst. of Research, Washington, DC.

The subjects were seventh generation Wistar-derived male rats selectively bred for sensitivity to audiogenic seizure (AS). Groups of adult AS-strain rats and adult control (C) male Wistar rats of equal size and from identical maintenance conditions were subjected to audiogenic challenge. None had ever previously been exposed.

Rats were placed singly in a metal grid chamber (size 33x24x13 cm) with a sound horn affixed to the top of the chamber. Auditory challenge was produced by an amplifier emitting white noise within 11.0 - 14.0 KHz range at an intensity of 105 ± 1 db at floor level. Each sound exposure was 1 min in duration.

Of the 15 AS rats challenged, five (33%) responded with wild running followed by clonic-tonic seizures. None of the control strain responded. Under similar testing procedures at three weeks of age, rats from the AS strain show a 95% incidence of clonic-tonic seizure. In different groups from the control strain, subjected to audiogenic challenge at 3 to 52 weeks of age, no clonic-tonic seizures have ever been observed.

Separate groups of AS (N=6) and control rats (N=6) were sacrificed for neurochemical studies. Average body weight in each group was 321 grams. Neither group was ever subjected to audiogenic challenge. Sacrifice was accomplished by a 5 second exposure to 2.5 KW microwave irradiation at 2450 MHz. Brains were dissected into 25 regions and assayed for cAMP and cGMP by radioimmunoassay and for gamma-aminobutyric acid by enzymatic-fluorimetric assay. Brain regions assayed included lateral cerebellum and vermis, midbrain, brainstem, corpus striatum, substantia nigra, hypothalamus, thalamus, olfactory bulb, olfactory tubercle, nucleus accumbens, septal region, hippocampus, amygdala, inferior colliculus, superior colliculus, medial geniculate, lateral geniculate, frontal cortex, and areas 2,17,24,29,41, and 51. The pineal and pituitary were also assayed.

In rats of the audiogenic seizure sensitive strain, cyclic GMP was elevated in the hippocampus, hypothalamus and vermis of the cerebellum.

1388 BIOCHEMICAL CORRELATES OF SELECTED ANTIDEPRESSANT DRUGS: β-RECEPTOR SENSITIVITY, MONOAMINE UPTAKE AND MAO INHIBITORY PROFILES. Laurence R. Meyerson, Wendy R. Simko*, Wayne W. Petko* and Daniel B. Ellis*. Dept. of Biochemistry, Hoechst-Roussel Pharmaceuticals, Inc., Somerville, NJ 08876.

Mechanistically the mode of action of antidepressant drugs may be related to (1) increased neuronal stores of biogenic amines with resultant augmented synaptic concentrations of specific neurotransmitters via inhibition of oxidative deamination pathways (MAO), (2) inhibition of presynaptic monoamine reuptake leading to increased neurotransmitter concentrations at receptors and enhanced activity of neuronal circuits, (3) β-adrenoceptor subsensitivity as indicated by a decrease in norepinephrine-induced accumulation of cAMP (Vetulani *et al*, Arch. Pharmacol. 293: 109, 1976) or cortical [^{3}H]-dihydroalprenolol binding (Banerjee *et al*, Nature, 268: 455, 1977; Sarai *et al*, Biochem. Pharmacol. 27: 2179, 1978). These biochemical parameters were utilized to evaluate the mode of action of a series of selected antidepressant and psychoactive agents. Extremely weak *in vitro* inhibitory effects on rat brain mitrochondrial MAO-type A or B were observed with HRP-197 and HP-505, both 3-aryl-spiroisobenzofuranpiperidines, P77-2984 a 3-aryl-spirobenzothiophenepiperidine derivative, LM-5008 an indolylethylpiperidine, desipramine, nisoxetine and P76-2543, a novel heterocycle possessing antidepressant characteristics. As anticipated, deprenyl showed potent substrate selective inhibition of MAO type B. The kinetics (B_{max} and K_D) of [^{3}H]-dihydroalprenolol binding were also studied following chronic administration of these same drugs (10 mg/kg, b.i.d.). After a 10-day treatment regimen, heterogeneous results were obtained in that some compounds elicited changes in receptor density and dissociation constant while others such as nisoxetine produced no kinetic alterations. Additionally, these same agents were tested as inhibitors of temperature dependent-high affinity synaptosomal monoamine uptake. Again, heterogeneous results were observed in terms of the selectivity of uptake blockade of either norepinephrine, dopamine or serotonin into intact synaptosomal preparations. While present biochemical antidepressant tests as utilized in this study are designed to evaluate modulations of aminergic systems in terms of neurotransmitter availability, fluxes in concentration and attendant receptor recognition site sensitivities, underlying mode(s) of action at the cellular level still remains to be elucidated.

1389 THE RELATIONSHIP BETWEEN THE INTENSITY OF THE STIMULUS AND THE METABOLIC RESPONSE IN THE VISUAL SYSTEM OF THE RAT. M. Miyaoka*, M. Shinohara*, M. Batipps*, K. D. Pettigrew*, C. Kennedy, and L. Sokoloff. Laboratory of Cerebral Metabolism, National Institute of Mental Health, Bethesda, MD 20205.

A wide variety of neurophysiological and psychological studies in sensory systems have demonstrated a direct relationship between the magnitude of the response and the logarithm of the intensity of stimulation. This is known as the Weber-Fechner Law. The recently developed [^{14}C]deoxyglucose method for measuring local cerebral glucose utilization (LCGU) has revealed a close relationship between local functional activity and local energy metabolism in the central nervous system. The present studies were undertaken to determine the quantitative nature of this relationship. The [^{14}C]deoxyglucose method was employed in pigmented Norway rats exposed to six levels of light intensity: darkness, 0.3, 1.4, 7.0, 700, and 7000 lux. LCGU was measured in the stratum griseum superficialis of the superior colliculus, the dorsal and lateral nuclei of the lateral geniculate body, the postero-lateral thalamic nucleus and the visual cortex of four rats at each level of light intensity. Mean values for LCGU (μmoles/100g/min) plotted against light intensity (lux) on a semilog scale revealed a direct linear relationship between the metabolic rate and the logarithm of the stimulation intensity in all of the above structures, except for the visual cortex. In the visual cortex a similar relationship appeared to be present up to a light intensity of 7 lux, but the slope was less than that found in the other structures and not statistically significantly different from zero. Values for LCGU in 14 non-visual structures also measured at each level of light intensity were not significantly different from the mean value obtained when the animal was in total darkness. Similar experiments were also carried out in Sprague-Dawley albino rats. In these a similar relationship was found in the structures of the visual pathway, but this was confined to a narrower range of light intensity (0-7.0 lux), above which the metabolic response decreased with increasing intensity of stimulation. These results demonstrate that the response in energy metabolism in the visual system to variations in intensity of stimulation conforms, like behavioral and perceptual responses, to the Weber-Fechner Law.

1390 THE OLFACTORY MARKER PROTEIN (OMP) DURING DEGENERATION AND RECONSTITUTION OF THE OLFACTORY SENSORY NEURONS. G.A. Monti Graziadei* and David W. Samanen* (SPON: P.P.C. Graziadei). Dept. Bio. Sci., Florida State University, Tallahassee, FL 32306.

Section of the fila olfactoria induces degeneration of the olfactory sensory neurons which is followed by differentiation of new neuronal elements from the basal cells of the neurepithelium. The disappearance of the olfactory marker protein (OMP) after axotomy, and its return to normal values after thirty days, has been shown to correlate with the process of degeneration and reconstitution of the olfactory neurons (Harding, et al., Brain Res., 1977). We used immunohistochemical techniques (Monti Graziadei, et al., J. Histochem. Cytochem., 1977) to observe the morphological changes of OMP content in the neurepithelium following axotomy. Adult mice (60 - 100 days) were unilaterally axotomized at the cribiform plate. The animals were then sacrificed at regular intervals (5 to 64 days) and serial section of the entire head was performed. Alternate sections were stained respectively with iron hematoxylin and peroxidase-antiperoxidase methods.

At five days survival time, the mucosa was almost completely devoid of OMP. In hematoxylin stained sections all the mature neurons had disappeared; only a few immature elements were present. By the 12th day the neuronal population had reconstituted and contained only young maturing neurons. A few mitoses remained, the peak having already occurred between the 8th and 11th days (Monti Graziadei and Graziadei, J. Neurocytol., 1979, in press). The OMP was present only in a few scattered neurons and in very fine bundles of axons leaving the epithelium. At twenty days survival time, the content of OMP had increased and larger bundles of positive axons were present beneath the epithelium. At 30 days the mucosa, which in hematoxylin stained sections appeared normal, had regained a full population of neurons. However, the OMP was not evenly distributed. Many regions of negativity were still distinguishable as well as zones where all cells possessed full positivity. All basal cells remained unlabelled. At 64 days the operated side was indistinguishable from controls, both in hematoxylin and peroxidase-antiperoxidase sections. (Supported by BNS grant 77/16/37 and NIH grants NS 08943 & 5/T/32 07010).

1391 MITOCHONDRIA AND SARCOPLASMIC RETICULUM AS MODEL TARGETS FOR NEUROTOXIC AND MYOTOXIC PHOSPHOLIPASES A. Ronald H. Ng, Tod Brown*, Herman H. Higa*, Gail A. Wong* and Bruce D. Howard, Biol. Chem. Dept., Sch. Med. UCLA, Los Angeles, CA 90024.

Certain snake venoms contain neurotoxic phospholipases A that inhibit acetylcholine release from nerve terminals. Some of these toxins are also myotoxic in that they damage skeletal muscle independently of their action on neurons. Although the phospholipase A (PLA) activity of the toxins appears to be required for their toxicity, there is no simple correlation between enzyme activity and toxicity. Most well known phospholipases A are not neurotoxic or myotoxic in spite of having greater specific enzyme activity than the toxic enzymes. We previously reported that one of these toxins, β-bungarotoxin, initially acts to depolarize synaptosomes. This activity correlates well with the neurotoxicity of β-bungarotoxin and is not simply due to the non-specific hydrolysis of membrane lipids by the toxin's PLA activity. The ion fluxes involved in the toxin-induced depolarization are unidentified but β-bungarotoxin may depolarize by altering the flux of Ca^{2+} across synaptosomal membranes. In order to facilitate study of the effect of PLA toxins on Ca^{2+} transport we have turned to two Ca^{2+}-transporting structures that are simpler and better characterized than synaptosomes. These are mitochondria (mito) and sarcoplasmic reticulum (SR). We found that several neurotoxic and myotoxic phospholipases A have the ability to inhibit Ca^{2+} uptake by brain mito and SR and this ability appears to be a biologically relevant correlate of their toxicity. β-Bungarotoxin and notexin had 5% and 50% respectively of the PLA activity of IVa PLA (a non-neurotoxic enzyme); however, β-bungarotoxin and notexin each had greater ability to inhibit Ca^{2+} uptake by brain mito. β-Bungarotoxin lost its neurotoxicity but not its PLA activity after modification with ethoxyformic anhydride in the presence of lecithin. The modified toxin also lost most of its ability to inhibit Ca^{2+} uptake by brain mito. The ability of a PLA to inhibit Ca^{2+} uptake into SR correlated well with its myotoxic potency. Each of the myotoxic enzymes substantially inhibited Ca^{2+} uptake into SR, notexin being the most potent. A 10 min incubation with 1 nM notexin caused an 80% inhibition of Ca^{2+} uptake. Two non-myotoxic phospholipases A at 10 nM had only a small effect on Ca^{2+} uptake although one of them had twice the PLA activity of notexin. The effect of notexin on SR was Ca^{2+}-dependent, temperature-dependent and time-dependent. Notexin treatment caused a slight activation of the SR Ca^{2+}-ATPase activity and an efflux of previously accumulated Ca^{2+}. SR vesicles reconstituted from solubilized SR accumulate Ca^{2+} and retain their sensitivity to notexin.

1392 KAINIC ACID AND CEREBELLAR GLUTAMATE METABOLISM IN VITRO. William J. Nicklas, Barbara Krespan* and Soll Berl. Dept. of Neurology, Mt. Sinai School of Medicine of C.U.N.Y., New York, New York, 10029.

The mechanism of neurotoxicity of kainic acid (KA) may involve presynaptic inhibition of glutamate (GLU) uptake (hence, increased extracellular levels of GLU) and/or receptor-mediated post-synaptic depolarization. The cerebellum (CB) was chosen to examine this problem in vitro because the slice has an intact glutamergic neuron, the granule cell. In 0.3mm thick parasagittal CB slices, KA caused a dose-dependent decrease in ATP levels; the effect was maximal at 1 mM KA, a 30-40% reduction. 2-Amino-4-phosphonobutyrate did not block this change. No such alterations in ATP levels were noted with striatal slices even at 10 mM KA. Treatment of CB slices for 30 min with 1 mM KA caused decreases in tissue levels of GLU, aspartate (ASP) and glutamine (GN) (15-20%, 15-35% and 60-70%, respectively). Concomitantly, levels of GLU and ASP increased in the incubation medium by 2 to 4-fold. Medium GN levels did not change; hence, total GN was markedly reduced. Metabolic studies with ^{14}C-glucose/^{3}H-acetate, ^{14}C-GLU, ^{14}C-GN and ^{14}C-GABA were consistant with an inhibition of GLU uptake and a decreased GN turnover. Striatal slices treated with KA showed only minimal metabolic alterations. Synaptosomal preparations of cerebral cortex, striatum and CB showed moderate inhibition by KA of high affinity GLU uptake with no concomitant net release of intra-synaptosomal GLU. Thus, the altered flux of GLU and GN between neurons and glia may play a role in the neurotoxic action of KA. The data are consistent with the findings of McGeer et al. (Brain Res., 139: 381, 1978) that an intact glutamergic neuron may be required to produce the biochemical changes associated with the neurotoxicity of KA.
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1393 THE ACTIVATION OF ADENYLATE CYCLASE AND PHOSPHODIESTERASE IN C-6 GLIOMA CELLS BY THE CALCIUM-DEPENDENT REGULATOR PROTEIN. Jon A. Norman. Friedrich Miescher-Institut, P.O.Box 273, CH-4002 Basel, Switzerland.

C-6 astrocytoma cells provide a homogeneous cell type for studying the role of Ca^{++} in regulating the enzymes involved in cyclic nucleotide metabolism. Adenylate cyclase and cyclic nucleotide phosphodiesterase, the enzymes responsible for cAMP synthesis and degradation, respectively, are present in different isozyme forms in C-6 glioma cells. The calcium-dependent regulator (CDR), a calcium-binding protein, is also present in C-6 cells and this protein, when complexed with Ca^{++} can bind to and activate one form of both adenylate cyclase and phosphodiesterase. A procedure has been developed for the partial purification of CDR-sensitive phosphodiesterase using affinity chromatography with CDR attached to sepharose. The CDR-sensitive form of phosphodiesterase will bind to the CDR-sepharose column in the presence of Ca^{++} and will elute from the column in the presence of EGTA. This procedure utilizes a preliminary chromatographic step on DEAE cellulose which removes CDR and modulator-binding protein from phosphodiesterase. The modulator-binding protein is an inhibitor of CDR-stimulated phosphodiesterase and will copurify with CDR-sensitive phosphodiesterase in other CDR affinity chromatography procedures. This two step procedure allows the partial purification of CDR-sensitive phosphodiesterase in high yields from C-6 cells to allow the final purification of the enzyme by sephacryl S-200 chromatography. The activation of CDR-sensitive phosphodiesterase under physiological ionic conditions is presently being investigated in addition to an analysis of the subunit structure of this enzyme by SDS gel electrophoresis. A similar purification procedure for adenylate cyclase is being developed.

1394 IN VITRO INHIBITION OF ACETYLCHOLINESTERASE BY SEROTONIN AND BY AN UNIDENTIFIED SEROTONIN-DERIVED COMPOUND(S) AS STUDIED AT PHYSIOLOGICAL SUBSTRATE LEVELS. B. Oderfeld-Nowak*, J.R. Simon, L. Chang*, and M.H. Aprison. The Institute of Psychiatric Research Depts. of Biochemistry, Pharmacology and Psychiatry, Indiana U. School of Medicine, Indianapolis, IN 46223.

Inhibition of acetylcholinesterase activity (AChE) by serotonin (5-HT) and by an unknown compound(s) derived from 5-HT was investigated under conditions employing biological concentrations of acetylcholine (ACh) as substrate (10^{-5}M). Freshly prepared solutions of 5-HT (10^{-5}M) were found to inhibit AChE from rat striatum as well as purified AChE from bovine red blood cells. The degree of inhibition was dependent on the ratio of inhibitor to enzyme as well as on preincubation of the enzyme with inhibitor. Inhibition was not dependent on ACh concentration nor on the duration of the enzymic reaction. Inhibition of AChE by freshly prepared solutions of 5-HT was found to be concentration-dependent in the range of 10-100 μM 5-HT with resulting inhibition of approximately 10-30%. Kinetic analysis of the observed inhibition indicated it to be of a noncompetitive nature with a K_i of approximately 0.2 mM. When 5-HT solutions were exposed to air and light at room temperature ("exposed 5-HT"), greater inhibition of AChE was observed than when freshly prepared solutions were employed. Inhibition obtained with "exposed 5-HT" solutions also revealed noncompetitive inhibition; however, the K_i was reduced by 50% (0.1mM). The enhanced inhibitory potency of the "exposed 5-HT" solutions suggested the formation of some additional compound(s) derived from 5-HT under conditions of exposure to light and air. Analysis of these solutions employing the techniques of high performance liquid chromatography and thin layer chromatography indicated this to be the case. Following the time course of production of the unknown compound(s), a progressive increase in peak height was observed. A concomitant increase in inhibition of AChE was noted up to 12 days of exposure. The maximum peak height of the unknown corresponded to approximately 5% of the 5-HT peak height. At this time, the inhibition of AChE was 60% suggesting that the unknown was a better inhibitor than 5-HT. Preliminary checks of factors which influence the production of this unknown inhibitor indicated that (a) both oxygen and light enhance this production, and (b) these two factors can act synergistically. The inhibitory effects of 5-HT and/or"exposed 5-HT"on AChE appeared to be highly specific since no effects were observed on other cholinergic parameters such as high affinity uptake of choline, choline acetyltransferase or muscarinic receptor binding. The process by which the unknown in this in vitro model might be produced in brain becomes important to elucidate from both the biological and clinical point of view.(Grant MH03225-20 and Fogarty Fellowship (BON))

1395 LOCALIZATION OF GLUTAMATE AND CYSTEINE SULPHINATE DECARBOXYLASE SUBUNITS ON TWO-DIMENSIONAL ELECTROPHORESIS BY USE OF A RADIOACTIVE SUICIDE SUBSTRATE ANALOGUE. Wolfgang H. Oertel*, Donald E.Schmechel*, John W. Daly* and Irwin J. Kopin. Lab. Clin. Science, NIMH, and Lab. Bioorganic Chem., NIAMDD, Bethesda, Md. 20205

Glutamic acid decarboxylase (GAD) has been reported to be purified from mouse, bovine, rat and human brain and, when tested, to contain cysteine sulphinate decarboxylase (CSD) activity throughout all stages of purification. In the present study a partially purified rat brain GAD/CSD preparation was analyzed by high resolution two-dimensional electrophoresis (2DE). Since the enzyme activity is lost completely during this procedure, a radioactively labelled irreversible inhibitor of GAD was used to localize the enzyme. (^{3}H)-γ-Acetylenic GABA (4-amino-hex-5-ynoic acid, GAG) was synthesized to a specific activity of 3 mCi/mM. GAG is an irreversible inhibitor of GAD (Jung et al., Biochem. 17:2628,1978) acting as a "suicide substrate" by covalent bond formation at the active site. GAG inhibits in parallel both CSD and GAD activity in brain. After reacting the enzyme with ^{3}H-GAG two radioactive bands of identical pI (5.3-5.6, 9M urea) and of molecular weight of 54000 and 58000 (±1500) are found in the 2DE pattern of the GAD/CSD preparation. These two radioactive areas are enhanced by reacting the radioactive inhibitor with GAD/CSD-antiGAD/CSD immune complex and using the immune precipitate for 2DE. These two areas found on 2DE represent the presumptive catalytic site bearing subunits of GAD/CSD. It is, however, still not possible to answer the question of whether GAD and CSD represent the same active site and/or are located on the same molecule as suggested by Davison (Biochim.Biophys. Acta 19:66, 1956). The use of labelled suicide substrate analogues permits unequivocal localization of enzymatically active subunits even after the denaturation which attends 2DE. In the case of GAD/CSD separate immunization using the proteins present in the two areas may provide definitive evidence for the non-identity of GAD/CSD-enzymes or support the view that the enzyme activities are the result of catalytic sites on the same enzyme molecule. This procedure should prove particularly useful for enzymes which are difficult to purify.

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1396 RESPIRATION IN DISSOCIATED NEONATAL RAT BRAIN PRIMARY CELL CULTURES. James E. Olson and David Holtzman*. Depts. of Neurology and Pediatrics*, Stanford Univ. School of Medicine, Stanford, CA.

Astrocytes are believed to possess certain unique properties of metabolism and respiration important in brain physiology. We have utilized the methods of Booher and Sensenbrenner (Neurobiol. 2:97, 1972) to obtain a relatively pure preparation of astrocytes in culture. This affords the possiblilty of measuring the respiratory parameters in a controlled environment, uncontaminated with neurons or endothelial components.

Neonatal rat cortical brain tissue was dissociated by treatment with trypsin followed by filtration through an 80 μm mesh. The cells were grown to confluence (two to eight weeks). 90% of the cells stained positively for the astrocyte-specific glial fibrillar acidic protein (GFAP). At various times after culturing, the nutrient medium was replaced in some culture dishes with a medium lacking fetal calf serum, but containing 0.25 mM dibutyryl cyclic-AMP (dbc-AMP). The cells in dbc-AMP treated cultures took on the morphological appearance of mature, multipolar astrocytes typically seen in vivo. This morphological transformation persisted as long as the dbc-AMP medium was present.

Cells grown to a monolayer were removed from the culture dish with a mild trypsin exposure, suspended in a phosphate-buffered saline and placed in a sealed oxygen chamber at 37° C for polarographic measurement of O_2 utilization. The rate of respiration was constant over a wide range of pO_2. The average basal rate was measured at 11.6±0.1 natom·min^{-1}·mg protein^{-1}. Addition of oligomycin (approximately 2 nmol/mg protein) reduced this to 42.4±0.5% of the basal rate. Additions of dinitrophenol (40 μM DNP) could overcome this inhibition. The maximal DNP-stimulated rate was 104±3% of the basal rate in dbc-AMP treated cells compared to 148±3% in untreated cells ($p<0.01$). With DNP added directly to the respiring cells (without prior oligomycin addition) there was no difference in the relative rates of treated (155%) and untreated cells (149%). Increased osmolality (produced with KCl, NaCl or sucrose) causes a decrease in basal rate and an increase in the rate in the presence of DNP for both treated and untreated cells.

These results will be compared with observations on brain slices and isolated brain mitochondria.

1397 EFFECT OF INORGANIC LEAD ON RAT BRAIN MITOCHONDRIAL RESPIRATION IN VITRO. J.J. O'Neill, B.R. Melamed*, D. Epstein*, K. O'Neill*, Depart. of Pharm., Temple Univ. Medical School, Phila.,Pa. 19140.

Inorganic lead, added to the diet of suckling rats in high doses has been shown to produce a cerebellar encephalopathy, and to inhibit the respiration of subsequently isolated cerebellar and, to a lesser extent, cerebral mitochondria (Holtzman and Shen Hsu, Pediat. Res. 10:70-75, 1976). Little data exists on the in vitro effects of lead on mitochondria isolated from the brains of naive rats, largely due to the problems of Pb^{2+} chelation and precipitation by EDTA and inorganic phosphate.

Mitochondria were isolated from rat brain (cerebellum removed) by a modification of the method of Nicklas and Clark (JBC 245:4724, 1970). The mitochondrial pellet was washed twice with an EDTA free medium containing mannitol 0.3 M, BSA 0.1%, HEPES 10 mM, pH 7.4, and suspended in the same medium. Omission of EDTA from the incubation medium produced a large decrease in the respiratory control ratio with pyruvate (10 mM) plus malate (2.5 mM) as substrates. Pb-acetate (5uM) when added following the addition of substrate but 2 min prior to the addition of inorganic phosphate (5mM) completely blocked the transition to State 3 respiration on the addition of ADP, 0.5uM Pb-acetate produced a 25% inhibition of State 3 respiration. The amount of lead bound to the mitochondria was determined by anodic stripping voltometry following digestion of the filter in nitric:sulphuric: perchloric acid (24:1:24). The present experiments demonstrate a dose-response relationship between lead and inhibition of mitochondrial respiration.

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1398 POTASSIUM INDUCED SWELLING OF A GLIAL ENRICHED PREPARATION: THE FROG FILUM TERMINALE. D. J. Packey, T. Richie, M. C. Trachtenberg, and B. Haber. Div. of Neurosurgery, Marine Biomedical Institute, and Depts. of Neurology, Physiology & Biophysics and Human Biol. Chem. & Genetics, University of Texas Medical Branch, Galveston, TX., 77550.

The phenomenon of potassium (K+) induced swelling of neural tissue has to date been largely studied in a variety of preparations containing both neurons and glia. The frog filum terminale (FT), which contains largely glia and myelinated fibers, offers a unique opportunity to study the same aspects of K+ induced swelling in a normal glial preparation devoid of neurons. In these experiments the extracellular space was measured by incubating excised portions of FT in the presence of ^{3}H-inulin in an oxygenated bicarbonate buffered frog ringer at 15^{o}C containing various concentrations of K+ (Na+ was covarred to maintain isotonicity). Parallel experiments were performed using excised spinal cord (SC), which contains both neurons and glia. Results (extracellular space, ECS) are expressed in microliters per mg. wet weight tissue.

The FT, which has been shown to have a lower cellular density than the SC on the basis of DNA measurement, has a correspondingly larger ECS (58%). Increases in the extracellular K+ greater than 30 mM result in changes in the ECS of both FT and SC. The magnitude and rate of swelling are similar in both preparations. The enzyme, carbonic anhydrase, has been implicated in K+ induced swelling in mammalian neural tissues. However, addition of acetazolamide, an inhibitor of carbonic anhydrase, does not change the extent of K+ induced swelling in the frog FT or SC. Furthermore, the range of K+ concentrations necessary to induce swelling is far higher in the frog than those previously reported for mammalian preparations. In other experiments, frog FT and SC are shown to have relatively low levels of carbonic anhydrase activity. Taken together, these data indicate that K+ induced swelling in amphibian neural tissue, and particularly in glia, is mediated by carbonic anhydrase independent mechanisms, in contrast to those seen in mammalian brain. The specific ionic dependencies of K+ induced swelling of amphibian neural tissue is under further investigation.

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1399 IMMUNOCHEMICAL EVIDENCE FOR INDUCTION OF CHOLINE ACETYLTRANSFERASE IN THE MEDIAL PREOPTIC AREA OF RAT BRAIN ELICITED BY ESTRADIOL. Dong H. Park, Victoria N. Luine, Tong H. Joh, Bruce S. McEwen and Donald J. Reis. Laboratory of Neurobiology, Cornell Univ. Medical College, and Rockefeller University, New York, New York 10021.

The circulating ovarian hormone estradiol (E_2) is accumulated and retained with regional selectivity in brain, notably in the medial preoptic area and hypothalamus. These brain regions contain binding sites for the hormone in nuclear and cytosol fractions. In the preoptic area of ovariectomized (OVX) rats, E_2 increases the activity of choline acetyltransferase (CAT)(Endocrin. 100:903, 1977), the enzyme catalyzing acetylcholine synthesis. We sought to establish by immunochemical titration using a specific antibody to CAT (Nature 275:324, 1978), whether the increase of CAT activity elicited by E_2 is due to an increase in enzyme molecules or to change in its catalytic activity. In the first experiment, thirty days after removal of ovaries and adrenals, female rats (Long-Evans) received estradiol benzoate (EB)(100ug/220g body weight, s.c.) dissolved in sesame oil or sesame oil alone. The rats were sacrificed 24h later, the preoptic area removed and CAT activity measured. E_2 increased CAT activity to 125% of control (15.5 to 19.3nmol/ug protein/10min). In the second experiment, three groups of female rats (Sprague-Dawley CD strain) were ovariectomized and 14d later received the following agents, were sacrified 3h after the last injection, the preoptic area removed, and assayed for CAT. The injection schedule and CAT activity were:

	d1	d2	d3	d4	CAT activity (nmol/ug/10min)	
Group A	v*	v	v	v	7.71	100%
Group B	v	v	v	100ugE$_2$	7.33	102%
Group C	30ugEB	30ugEB	30ugEB	v	10.20	132%

*sesame oil as vehicle

This experiment shows that E_2 increases CAT activity in preoptic area after chronic but not acute treatment. Immunochemical titration of CAT showed that the equivalence point was shifted to the right by approximately 30%, demonstrating that the increases in CAT activity are entirely attributable to increases in CAT protein. The study demonstrates for the first time that: (a) the activity of CAT, like catecholamine synthesizing enzymes can be increased by the accumulation of the enzyme protein (induction), and (b) estradiol induces CAT regionally in rat brain.

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1400 A COMPARISON OF CHRONIC PRENATAL AND POSTNATAL HALOTHANE EXPOSURE ON BRAIN MYELIN SYNTHESIS. Patsalos, P.N.*, Rigor, B.M.* and Wiggins, R.C.; Dept. Neurobiology and Anatomy, Univ. Texas Medical School, Box 20708, Houston, TX 77025.

Long-Evans rats were exposed prenatally or postnatally to 0.5% halothane for 8 hours/day during the specified age interval. Synthesis of brain subcellular membrane proteins (myelin, nuclear, synaptosomal, mitochondrial, and microsomal) was examined by a double isotope procedure as we have described previously. Typically, experimental rats were injected intraperitoneally with ^{3}H-leucine; controls, with 1-^{14}C-leucine. Brains of experimental and control pups were combined, subfractions prepared, and delipidated. ^{3}H/^{14}C ratios (test/control) were determined for each subfraction and the myelin, synaptosomal, nuclear, and mitochondrial values for each pair of rats were normalized to the corresponding microsomal value for that preparation. The resultant percentage is a measure of relative synthesis for each subfraction (Wiggins et al., 1976), normalized to test and control microsomal synthesis.

Table [1]Relative myelin synthesis in halothane exposed rats (percentage)

	Exposure regime[2]	(17)	Postnatal Age (20-22)	(25)	(30)
I	5d, G - Birth	97±3	96	103±2	
II	Birth - 5d, P		93±7		
III	Birth - 10d, P		87±14		92±3
IV	Birth - 15d, P		92±5		

[1]Mean ± S.E.M. (N=3); [2]G=gestational age; P=postnatal age.

Results show that the myelin ratio was consistently low at 20-22 days, the period of most rapid myelin synthesis, reflecting a relative decrease in synthesis (about 8%, $P<0.05$, paired t-statistic). Isotope reversal showed that the relatively low myelin values are not artifacts of isotope utilization. Prenatal exposure had comparatively little effect on subsequent brain myelin synthesis. All intervals of postnatal exposure produced about the same degree of myelin reduction, and the effect persisted at 30 days, well past peak myelination. Thus, the decrease is not the result of a delay in maturation. Synthesis of nuclear membrane protein was also decreased (6%, $P<0.01$, paired t-statistic) at 20-22 days. Relative synthesis of the synaptosomal, mitochondrial, and microsomal protein fractions were identical to one another. (This research was supported by Public Health Service Grant NS-14355.)

1401 GABA LEVELS IN CEREBROSPINAL FLUID OF NEUROLOGICAL PATIENTS. A.L.N. Prasad* and S. Fahn (SPON: S.P. Mahadick). Department of Neurology, College of Physicians and Surgeons, Columbia University, New York, NY 10032

Recently we have developed a simple and sensitive assay for the measurement of GABA in fluids and tissues by high performance liquid chromatography (Proc. International Soc. Neurochem. 6, 644, 1977). The only sample preparation required is deproteinization and usually 100 µl of CSF is all that is required for a reliable determination of GABA. We have measured GABA CSF levels in patients with various neurological disorders as well as in a few control specimens. The control values, around 300 µM, agree with published results. Patients with Parkinson's disease or Huntington's chorea had CSF GABA levels ranging from 26-150 µM with occasional high values and are in general agreement with those reported by Enna et al. (Arch. Neurol. 34, 683, 1977).

GABA CSF levels in patients with myoclonus, stroke, dystonia and other miscellaneous neurological disorders ranged from 50-600 µM, without any recognizable pattern. From the results obtained thus far, low levels of CSF GABA are not necessarily limited to patients with Huntington's chorea or Parkinson's disease.

1402 AVOIDANCE OF L-DOPA DECARBOXYLATION BY METAL CHELATION - IN VITRO STUDIES. K. S. Rajan, M. Robinson*, IIT Research Institute, Chicago, IL 60616, and John M. Davis, Ill. State Psych. Inst., Chicago, IL 60612

Consistent with a metal chelation approach, in vivo studies on the administration of Cu(II)- and Zn(II)-L-DOPA chelates to experimental rats have shown up to 200% increased replenishment of DOPA (and its metabolites) in the whole brain compared to the unchelated L-DOPA controls. (Rajan, K. S., et al, Brain Res. 107, 317 (1976) and Rajan, K. S., et al. Pharmacologist, 20, 216 (1978), Neuroscience abstracts #164.5 (1978)). This approach is based on a theoretical speculation that the pyridoxal phosphate-dependent decarboxylation of the aromatic amino acid, L-DOPA, might be largely avoided if its aminocarboxylate group is chelated to a suitable metal ion prior to its in vivo administration. In Vitro studies have since been carried out to investigate the activity of the DOPA-decarboxylase on the ^{14}C-L-DOPA chelates of Cu(II) containing equimolar amounts of citric acid, dipyridyl and ATP. Warburg-technique was used to determine the $^{14}CO_2$ released from the decarboxylation reaction. The chelate substrates investigated were: ^{14}C-L-DOPA-Cu(II)-citric, ^{14}C-L-DOPA-Cu(II)-dipyridyl, ^{14}C-L-DOPA(II)-ATP (1:1:1) and ^{14}C-L-DOPA-Cu(II) (2:1). In the range of concentration of 0.1 to 0.4 m moles of the substrates, a substantial inhibition of the decarboxylase activity was observed, i.e. two to five fold decrease in the catechol amines produced as compared to ^{14}C-L-DOPA controls. Kinetic analyses of the saturation of binding curves for the chelates and the control were undertaken. The inhibitory activities of the chelate substrates are examined in the light of the metal L-DOPA binding strengths. The potential clinical significance of this approach to Parkinsonism is discussed.

1403 HEXOSAMINIDASES IN HUMAN BRAIN AND TUMORS OF THE NERVOUS SYSTEM. Robert B. Ramsey. Department of Neurology, Saint Louis University, St. Louis, MO. 63104.

Total hexosaminidase activity of nervous system tumors has been found to be consistently greater than that of normal brain. Studies utilizing tissue homogenates for determination of apparent Km values have suggested that hexosaminidase derived from tumor tissue differs from that in brain in other respects as well. Since this enzyme may serve as a key enzyme in maintaining the neoplastic condition, we have sought to further characterize the hexosaminidase of nervous system tumors. Using a resuspended ammonium sulfate derived from the 37,000 x g supernatant of a homogenate of lyophilized brain and tumor preparations, it could be shown that initial gel filtration on G-150 Sephadex yielded two peaks of enzyme activity; the lesser peak of activity was associated with the void volume (peak I), the greater activity with an approximate MW of 135,000 (peak II). In normal brain tissue, peak I represented 35% of the total activity in grey matter, and 15% of the total activity of white matter. In comparison to normal white matter, the void peak was significantly reduced ($P<.01$) in preparations of glioblastomas. Other tumors which have been analyzed (meningiomas, astrocytomas and tumors metastatic to the nervous system) also demonstrate a reduction in peak I. DEAE-cellulose chromatography of the two peaks derived from gel filtration demonstrated that peak II was dominated by hexosaminidase A in normal white and grey matter. Isozyme A constituted 85% of the total eluted activity of human grey matter and 92% of white matter activity. Only 13% of the total eluted activity of grey matter was isozyme B. It represented 6% of the white matter activity. The remainder of the activity was derived from isozyme I. In normal brain tissue, peak I contained only isozymes B and I. Analyzing glioblastoma samples in the same manner, it was found that DEAE-cellulose chromatography of peak II yielded a significant ($P<.01$) increase in the relative content of the B isozyme, compared to normal white matter, and a significant ($P<.01$) reduction in the relative A isozyme content. Isozyme I was not always present in these tumors. Peak I activity from these tumors could not generally be eluted from DEAE-cellulose using a 0-.3M NaCl gradient. Comparable isozyme patterns were observed in tumor preparations when p-nitrophenyl N-acetyl-β-D-galactosaminide was used instead of *p-nitrophenyl N-acetyl-β-D-glucosaminide as substrate.* Although specific properties of the isozymes derived from these tumors have yet to be defined, it is already obvious the alterations in total enzyme activities also reflect changes in isozyme content as a result of neoplasia.

1404 DECREASED AMINO ACID CONCENTRATIONS IN BRAIN OF PATIENTS WITH LESCH-NYHAN SYNDROME. D. K. Rassin, K. G. Lloyd and I. Fox.* Dept. Human Dev. & Nutr., Inst. Basic Res. Ment. Ret., S.I., NY, Dept. Neuropharmacol., Synthelabo, LERS, Paris, France, and Dept. Int. Med., Univ. Mich., Ann Arbor, MI.

Lesch-Nyhan Syndrome (LNS) is an X-linked recessive disease associated with a deficiency of the enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPRT). Increased uric acid production has been found in patients with this disease. Glutamine and glycine are associated with the affected metabolic pathway but have not been indicies of the state of the disease.

In an effort further to understand the pathogenesis of LNS we have analyzed amino acids in autopsy material obtained from five patients and six controls. The free amino acid pool of the occipital cortex, limbic cortical area, cerebellar cortex, hippocampus and putamen was measured with an automatic amino acid analyzer after precipitation of protein with TCA. Amino acids in brain areas from the LNS patients were usually lower in concentration; most dramatically decreased were threonine, serine, valine, isoleucine, leucine, lysine and arginine. Only glutamine and urea were higher than controls. Glutamate, gamma-aminobutyrate and cystathionine were essentially unaffected. High concentrations of glycine have been found in cultured mutant mouse cell lines of neural origin that lack HGPRT (Skaper and Seegmiller, J. Neurochem. 26:689, 1976; 29:83, 1977). Glycine was slightly decreased in the brain areas analyzed in the current study. Some of the results are given in the following table:

	Occipital Cortex		Hippocampus	
	LNS	Control	LNS	Control
	(µmoles/100 g)			
Threonine	54±12*	82±5	55±4*	97±7
Serine	115±15*	174±13	161±23*	227±18
Valine	53±11*	80±5	65±3*	94±7
Isoleucine	24±6*	40±4	28±5*	53±6
Leucine	57±13*	90±10	70±13*	112±12
Glutamine	664±94	473±44	828±375	373±29
Urea	756±386	241±36	653±141*	214±38
Glutamate	1563±194	1601±202	951±100	1118±95
Cystathionine	75±47	82±23	62±12	31±7
Glycine	229±34*	315±11	261±66	332±11

*p <0.05

Our findings suggest that patients with LNS have a generally lower free amino acid pool in brain. This decreased pool may limit the capacity of the brain to make proteins essential for normal function and, thus, be related to the pathogenesis of the disease.

1405 SPECIFICITY OF ASSOCIATION OF A Ca^{2+}/Mg^{2+} ATPase WITH CHOLINERGIC SYNAPTIC VESICLES FROM TORPEDO ELECTRIC ORGAN. Joan E. Rothlein* and Stanley M. Parsons* (SPON: Harry J. Carlisle). Marine Science Institute and Department of Chemistry, University of California, Santa Barbara, CA 93106.

Purified cholinergic synaptic vesicles from the electric organ of Torpedo californica have been subjected to analytical scale separation techniques not utilized in the isolation procedure, and the ATPase activity of separated fractions determined. Most of the ATPase activity migrated with the vesicles. Sensitivity of the ATPase activity to 16 potential inhibitors also was determined. Most of the ATPase activity was inhibited by low concentrations of 4-chloro-7-nitrobenzo-oxadiazole (NBD-Cl) and dicyclohexylcarbodiimide (DCCD), but not by a water soluble carbodiimide. Chlorpromazine also inhibited significantly. The close association of the ATPase with the vesicles and the pattern of inhibition obtained provide further support for the authentic presence of a membrane bound Ca^{2+}/Mg^{2+} ATPase in the cholinergic synaptic vesicle.

1406 REGIONAL DISTRIBUTION OF MULTIPLE BENZODIAZEPINE RECEPTORS IN RAT BRAIN. M. C. Sano*, B. Beer and A. S. Lippa*. Dept. of Central Nervous System Research, Medical Research Division, American Cyanamid, Pearl River, NY 10965.

Brain specific benzodiazepine receptors appear to mediate the pharmacological properties of benzodiazepines. Multiple classes of benzodiazepine binding sites have recently been reported and triazolopyridazines have a high affinity for one class of these binding sites (Type I) (Squires et al., Proc. Natl. Acad. Sci., in press). In the present studies, we have used CL 218,872 (3-methyl-6-[3-(trifluoromethyl)phenyl]-1,2,4-triazolo[4,3b]pyridazine) to investigate the regional distribution of multiple benzodiazepine binding sites. In confirmation of prior reports, diazepam displaced specifically bound ^{3}H-diazepam (1.5 nM) with equal potencies in frontal cortex, cerebellum, striatum and hippocampus (IC_{50} ranged from 4-6.5 nM). Hill coefficients for diazepam approached unity in all regions suggesting that diazepam has equal affinity for the various classes of binding sites. In contrast, the potency of CL 218,872 to displace specifically bound ^{3}H-diazepam (1.5 nM) varied as a function of region: cerebellum (IC_{50} = 25 ± 7 nM); frontal cortex (IC_{50} = 113 ± 28 nM); striatum (IC_{50} = 167 ± 24 nM); hippocampus (IC_{50} = 238 ± 36 nM). The Hill coefficients for CL 218,872 also varied as a function of region: cerebellum (α = .82); frontal cortex (α = .69); striatum (α = .63); hippocampus (α = .71). The findings that CL 218,872 is very potent in cerebellum, and its Hill coefficient approaches unity, suggest that benzodiazepine receptors in cerebellum may be relatively homogeneous and of the Type I class. Calculations suggest that approximately 78% of ^{3}H-diazepam binding in cerebellum represents Type I receptors; while 64, 52 and 34% of binding in frontal cortex, striatum and hippocampus represents Type I receptors in these regions. Scatchard analysis of cerebellar ^{3}H-diazepam binding in the presence of different concentrations of CL 218,872 demonstrates the competitive nature of this inhibition.

1407 THE ECC SYNDROME: POSSIBLE CHOLINERGIC INVOLVEMENT IN AN ANIMAL MODEL OF HYPERKINESIS. A.C. Sconzert, B. Haber, and S. Gabay. Marine Biomedical Institute, UTMB, Galveston, TX 77550, V.A. Hospital, Brockton, Mass.

The ECC syndrome is characterized by behavioral excitation, circling, and choreiform head and neck movements. This syndrome is induced in rats by the administration of β-β'-iminodipropionitrile (IDPN) and is permanent once established. Thus the ECC rat is a potentially useful model system in which to explore some of the neurochemical parameters underlying hyperkinetic behavior. Evidence to date indicates involvement of dopamine, serotonin, and GABA systems in the behavioral expression of this syndrome. In the present studies, female ECC rats were sacrificed 15 months post injection of IDPN, and the brains analyzed for choline acetyltransferase (CAT) and acetylcholinesterase (ACE) activity on a regional basis (cortex, striatum, hippocampus, hypothalamus, cerebellum, and brain stem). Enzyme activities were determined radioenzymatically. In IDPN treated rats, CAT activity was depressed in striatum and cerebellum (81 and 60% of control, respectively). ACE activity was depressed in all regions except cerebellum. ACE activity was particularly depressed in cortex (65.2% of control, $p<0.05$) and striatum (77.4% of control, $p<0.001$). The changes in the activity of CAT, the rate limiting enzyme to acetylcholine synthesis, parallels the depression of acetylcholine levels in some, though not all, brain areas examined. These changes in the cholinergic enzymes CAT and ACE are additional to those previously shown by us in the GABAergic system in the ECC rat. These long term effects of this drug, which persists for fifteen months post injection, make it unlikely that they reflect direct inhibitory effects of IDPN on CAT and ACE per se. It is possible that IDPN results in the destruction of either GABA or acetylcholine containing neurons, particularly in the striatum, as has been shown in other movement disorders such as Parkinson's disease and Huntington's disease. The examination of these possibilities and the relationships of multiple neurotransmitter pathways in the development and maintenance of the ECC syndrome are currently underway.

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1408 PLASMA AMINO ACIDS AND OTHER METABOLITES FROM NORMAL AND DEPRESSED PATIENTS UNDER DIETARY MONITORING. P.A. Shea, M. DeMyer*, and H.C. Hendrie*. The Institute of Psychiatric Research and Depts. of Biochemistry and Psychiatry, Indiana University Medical School, Indianapolis, IN 46223.

Eighteen unipolar depressed (drug free) patients and ten normal controls were voluntarily hospitalized for six days and five nights. Under controlled conditions dietary effects on particular biochemical measurements were monitored. Using standard biochemical procedures the following measurements were made on blood drawn after a 12 hour fast on days two, three, and five: (a) plasma levels of free and total tryptophan, (b) twenty other amino acids, (c) choline, (d) cortisol and (e) free fatty acids. Total protein, fat, and carbohydrates consumed with each meal were measured by a nutritionist. Each patient was rated daily for severity of depression using Hamilton and Beck questionnaires. In general, there were no significant effects of dietary protein, fat or carbohydrate on any of the plasma biochemical measurements performed. It was noted that although both groups consumed the same amounts of protein and carbohydrates, depressed patients consumed significantly higher amounts of fat. No differences were noted in the levels of plasma metabolites between the two groups except for tyrosine and phenylalanine which were significantly higher in depressed patients when expressed as the mean ± S.D. Thus tyrosine levels in the depressed group were 60.4 ± 9.4 nmol/ml and 51.3 ± 12.9 nmol/ml for controls. Phenylalanine in depressed patients was 56.0 ± 12.2 whereas controls were 47.6 ± 8.2. Significant differences ($p < .04$ and $p < .06$, respectively) were obtained using a student t-test based on the sum of the means of the three day blood measurements. There were no differences in the ratios of either free or total tryptophan to the sum of the five competing amino acids (valine, leucine, isoleucine, phenylalanine and tyrosine). These neutral amino acids have been shown to block the uptake of tryptophan into the CNS. The data on ratios follow: Free tryptophan/sum five amino acids: depressed patients 0.023 ± 0.005, control 0.024 ± 0.005; total tryptophan/ratio: depressed patients 0.089 ± 0.02, control 0.101 ± 0.03. Further, these ratios showed no significant differences in both groups irrespective of the concentrations of either free fatty acids and cortisol in plasma or dietary amounts of protein, fat and carbohydrate in the diet. The increased levels in the two competing neutral amino acids tyrosine and phenylalanine found in depressed patients suggests that tryptophan transport into the CNS of these patients may be affected. (Supported in part by Indiana Dept. Mental Health 178-679-005 and Indiana Attorney Generals Fund, 1979).

1409 PREFERENTIAL LOCALIZATION OF γ-GLUTAMYL TRANSPEPTIDASE IN GLIA. H.D. Shine, L. Hertz, J. deVellis, and B. Haber. Marine Biomedical Institute, UTMB, Galveston, TX 77550, UCLA School of Med., Los Angeles, CA.90024, Univ. of Saskatchewan, Canada.

γ-glutamyl transpeptidase (γ-GTP) is an enzyme which may have a role in transport of amino acids and peptides across biological membranes. High levels of γ-GTP activity found in the brain suggest that this enzyme may well have such transport functions within neural tissues. We observe that in the central nervous system levels of enzymatic activity are greatest in the capillaries, followed by white matter and are significantly lower in gray matter. The higher levels of γ-GTP activity in white matter suggest a preferential association of γ-GTP with glia. To determine the cellular localization of γ-GTP in neural tissues we have employed cultured cells of neuronal and glial origin as models of neurons and glia in vivo. γ-GTP activity was measured using a highly sensitive fluorometric assay which employed the synthetic substrate 7-γ-glutamylamido-4-methyl coumarin and high pressure liquid chromatography or fluorescent spectroscopy. Enzymatic activity in clonal cell lines of glial origin uniformily expressed greater activity than did a variety of neuroblastoma cell lines. Furthermore the γ-GTP activity of normal non-transformed rodent astrocytes and oligodendrocytes was also higher than neuronal levels. No significant difference in γ-GTP activity was found between the normal astroglia and oligodendroglia. Taken together this data strongly suggest that the bulk of CNS γ-GTP activity is primarily associated with astroglia, oligodendroglia and CNS capillaries.

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1410 **ACTIONS OF LEAD (PB) ON GABAERGIC NEUROTRANSMISSION: DISCREPANCIES BETWEEN IN VIVO AND IN VITRO EFFECTS.** **E. Silbergeld, R. Hruska, J. Lafferman*, NINCDS, NIH Bethesda, MD 20205.**

Exposure to Pb in vivo produces convulsions and, at lower levels of exposure, appears to sensitize animals to convulsant agents (Neurosci. Abstr. (1977) 3:1039). This effect can be produced by chronic exposure of rats from birth (to 10 mg/ml Pb acetate in drinking water) or by intraperitoneal injection of Pb acetate (7.58 mg/kg) for 3 days. Doses producing convulsions are decreased and duration of clonus is increased in both chronic and acute Pb rats challenged with mercaptopropionic acid or bicuculline. Neurochemical studies were done on synaptosomes and synaptic membranes prepared from caudate (CN), cortex (CX), cerebellum (CB) and substantia nigra (SN) of acute and chronic Pb rats. Na-Dependent high affinity ^{14}C-GABA uptake, release of ^{14}C-GABA (in the presence of 5 mM or 35 mM K+), and Na-independent specific ^{3}H-GABA binding were studied. In Pb rats, GABA uptake was inhibited in CN, CB, and SN. Kinetic analyses demonstrated that in these regions Vmax was reduced without significant effects on Km. Release of GABA was also inhibited under conditions of spontaneous and K+-stimulated release. No changes were observed in any region for Na-independent GABA binding. The results suggest that in vivo Pb exposure primarily affects presynaptic elements of GABAergic function, which may reflect either a selective destruction of GABA-releasing and transporting elements or a blockade by Pb of presynaptic GABA cycling. We attempted to study mechanisms of action of Pb by exposing synaptosomes and synaptic membranes from these regions to Pb in vitro. However, at concentrations as high as 100 uM, Pb in vitro has no effects on GABA uptake, release, or receptor binding, even when long exposure times are used. The discrepancy between in vivo and in vitro data suggests that the actions of Pb in vivo on GABA uptake and release may result from indirect effects of Pb on other neurotransmitter systems or from the action of a toxic metabolite. In vivo, Pb can decrease CNS acetylcholine release and increase CNS dopamine release (Life Sci (1977) 20:309). A possible toxic metabolite affecting GABAergic function is δ-aminolevulinic acid (ALA); concentrations of ALA in tissue and plasma are greatly elevated after Pb exposure in vivo. However, ALA in vitro (as high as 1 mM) has no effects on synaptosomal GABA uptake or release; ALA inhibits GABA receptor binding but only weakly (IC50 = 1 mM). The mechanisms of action of Pb in vivo on GABAergic function are at present still unclear.

1411 ISOENZYME FORMS OF ACETYLCHOLINESTERASE IN RAT AND MOUSE TISSUE. K.A. Skau* and W.S. Brimijoin, Mayo Fdn., Rochester, MN 55901.

Acetylcholinesterase (AChE) is present in rat skeletal muscle in at least three isoenzymic forms identified by sucrose density gradient centrifugation as 4S, 10S and 16S (Hall, J. Neurobiol. 4:343,1973; Vigney et al, J. Neurochem. 27:1347, 1976). The 16S form has been called the endplate specific form as it is absent from non-innervated regions of the muscle and disappears from endplate regions after denervation. The 4S and 10S forms are present in endplate-free sections as well as in endplate-rich sections and decrease upon denervation. We have studied the pattern of isoenzyme forms in various rat and mouse tissues to determine whether there are species or tissue differences with respect to these isoenzymes.

Five to 20% linear sucrose density gradients were used to investigate the isoenzyme forms of detergent solubilized muscle extracts. Denervated extensor digitorum longus muscles (EDL) or hemidiaphragms were produced by sectioning the sciatic nerve in the mid-thigh region, or the phrenic nerve in the thoracic cavity at least 6 days prior to sacrifice.

	% of Total AChE					
	Control			Denervated (6 days)		
Rats	4S	10S	16S	4S	10S	16S
Hemidiaphragm	21	52	15	21	63	5
EDL	43	29	18	10	90	0
Mouse						
Hemidiaphragm	30	no peak	46	51	no peak	12
EDL	30	32	34	30	30	37

There were large species differences as well as differences between muscles within a species. Of particular significance was the persistence of the 16S form in mouse EDL after denervation. The 16S form was a major peak in this tissue up to 27 days after denervation whereas in the rat EDL the 16S form disappeared within 6 days. Another striking observation was the lack of a 10S peak in mouse hemidiaphragms.

These species and tissue differences lead us to question the specific localization of the 16S form of AChE. In related studies we detected small but significant 16S peaks in a nerve segment just proximal to a ligature of rat vagus nerve and in mouse cardiac tissue. These results indicate that the pattern of AChE isoenzyme forms is more complex than previously proposed and that the 16S form is not restricted to endplate regions of skeletal muscle. (Supported by NIH Grants NS 11855 and NS 14304. W.S.B. is a recipient of Research Career Development Award NS 00119 from NIH.)

1412 Ontogeny of γ-Hydroxybutyrate in Human and Rhesus Monkey Brain. O. Carter Snead, III, and Barbara J. Morley. Dept. Pediatrics and the Neuroscience Program, Univ. Alabama in Birmingham Sch. Med. B'ham. Al.

Gamma Hydroxybutyrate (GHB) is a GABA metabolite that produces age specific EEG and behavioral effects in experimental animals that resemble human petit mal epilepsy (Snead, Neurology 28:643, 1978). We have used an electron capture gas liquid chromatographic assay method (Doherty, Snead & Roth, Anal. Biochem. 69:268, 1975) to determine the regional distribution of GHB in infant and adult rhesus monkey brain as well as discrete regional concentrations in post mortem brain from humans, ranging in age from 4 weeks gestation to 57 years old (Table 1). GHB is present in human brain at 4 weeks gestation in a concentration of 9 nmol/g. It gradually increases in concentration until 14-22 weeks gestation when it peaks in cerebellum at 50-100 nmol/g. Concentrations fall postnatally in cerebellum but rise in the striatum. The highest concentration of GHB in children under ten was found in globus pallidus I, caudate and putamen. Similarly in the rhesus monkey, the neonatal brain concentrations of GHB were twice those of adults with the highest concentration occurring in neonatal cerebellum.The marked elevation of GHB in immature human brain, the propensity of this substance to produce age specific absence seizures in primates experimentally, and the younger age predominance of petit mal seizures in human all point toward the possibility that GHB may play a role in the pathogenesis of human petit mal epilepsy.

TABLE 1
CONCENTRATION OF GHB IN HUMAN BRAIN* (NMOL/G±S.E.)

	AGE			
	12-14 Wk. Gest.	4 Years	14 Years	50 Years
Cortex	17.15±6.32	11.12±1.2	14.27±2.31	5.59±1.85
Striatum	32.21±7.42	26.01±3.4	27.23±5.03	10.82±3.82
Cerebellum	109±13.3	16.75±1.2	17.25±1.2	8.56±2.45

* The protocol for procurement of human tissues was approved by the Institutional Review Board of The University of Alabama in Birmingham.

1413 PHARMACOLOGICAL CHARACTERIZATION OF DIFFERENT TYPES OF DOPAMINE RECEPTORS: STUDIES WITH ERGOT DERIVATIVES AND SUBSTITUTED BENZAMIDES. P. F. Spano* & M. Trabucchi* (SPON: J.P. Schwartz) Depts. of Pharmacol., Universities of Cagliari & Brescia, Italy

Many lines of evidence have recently indicated the presence of multiple receptors sites for dopamine (DA) in mammalian central nervous system. In this report we will show that two classes of drugs, dopaminergic ergot derivatives and substituted benzamides interact with DA receptors in a way which is basically different from the classical DA-mimetic and DA-antagonist agents such as apomorphine and neuroleptics, respectively. In fact, DA-stimulated adenylate cyclase activity in cell free preparations from rat striatum is not blocked by DA ergot drugs such as bromocriptine and lisuride. Moreover, sulpiride and other substituted benzamides endowed with antipsychotic activity are not able to inhibit DA stimulated adenylate cyclase either in vitro or in vivo. However both classes of drugs can displace ^{3}H-haloperidol from putative DA binding receptor sites in membranes prepared from rat striatum. Experiments performed using ^{3}H-sulpiride have shown a specific binding for this ligand in dopaminergic regions of the brain. Our data provide strong indication that two distinct populations of DA receptors, one coupled and the other uncoupled to adneylate cyclase, are present in rat striatum. Thus, we have defined as D_1 and D_2 respectively these DA receptor sites.

1414 ALTERATIONS IN MYELIN BASIC PROTEIN AND MYELIN-ASSOCIATED GLYCOPROTEIN IN JIMPY MICE. Nancy H. Sternberger*, Richard H. Quarles*, Steven R. Cohen*, Kathryn Winchell* and H. deF. Webster. NIH, Bethesda, MD 20205.

To study the distribution of myelin basic protein (MBP) and myelin-associated glycoprotein (MAG) during development, 10 to 25-day jimpy mice and littermate controls were perfused with a $HgCl_2$-formaldehyde fixative. Vibratome sections of the brainstem and anterior commissure were immunostained with antiserum to MBP or MAG according to the peroxidase-antiperoxidase technique. In littermate controls, a normal distribution of MBP staining was found in oligodendroglial cytoplasm, oligodendroglial processes, and in compact myelin. In contrast, jimpy MBP staining had the following characteristics: 1) oligodendroglial cytoplasm and processes were stained and there were collars of staining surrounding axons, 2) the staining around axons was more frequent than could be accounted for by the small amount of myelin seen in phase and electron microscopic sections of jimpy brainstem, 3) the axonal collars were easily and intensely stained throughout development, suggesting that they were not compact myelin sheaths but were oligodendroglial processes containing mesaxons and/or loosely wrapped myelin spirals, 4) the number of stained axonal collars decreased with development, 5) staining was less than in littermate controls and irregularly distributed, 6) during the earlier stages of myelination, as in the 10-day anterior commissure, little difference was found between jimpy and littermate MBP staining but with further development, the rapid increase in MBP staining seen in littermate controls was not present in the jimpys. Littermate controls stained with MAG antiserum also showed a normal distribution of staining in oligodendroglia, processes and myelin. However, in sections of jimpy brainstem and anterior commissure stained with MAG antiserum, we observed that 1) only a few stained oligodendroglia were present; they were stained much less intensely than oligodendroglia in littermate control sections, 2) many fewer collars of axonal staining were seen than had been observed in jimpy sections stained with MBP. These observations suggest that in jimpys, axonal ensheathment by oligodendroglia is less severely affected than the subsequent formation and growth of compact sheaths. The results also indicate that the deficit in oligodendroglial synthesis of MAG is much greater than that of MBP.

1415 EVIDENCE FOR NEURONAL SYNAPSES ON THE BASEMENT MEMBRANE OF CEREBRAL CAPILLARIES. R.L. Suddith, K.E. Savage*, P.S. Baur*, J.S. Crawford*, and H.M. Eisenberg. Division of Neurosurgery, Shriner's Burns Institute and Department of Surgery, University of Texas Medical Branch, Galveston, Texas 77550.

While innervation of large cerebral vessels by adrenergic and cholinergic nerve fibers have been shown, similar innervation of the cerebral microvasculature has not been demonstrated. In this study isolated cerebral microvessels were examined for presence of neuronal synapses. The microvessels were isolated from the cerebral cortex of mature Sprague-Drawley rats by a process of serial filtration and differential centrifugation. The isolate contained numerous capillary segments and fewer segments of small arterioles and venuoles.

Transmission electron microscopy showed that the capillary segments were circumferentially complete, the endothelial cells being surrounded by an intact basement membrane. Electron dense areas containing numerous uniform vessicles were found external and adjacent to this membrane. Morphologically these areas were similar to neuronal terminals found on larger vessels. Using histochemical techniques the presence of monoamine oxidase was found in association with capillaries and using radioenzymatic assay techniques the microvessel isolate was shown to contain this enzyme.

These studies show that electron dense bodies present on the basement membranes of cerebral capillaries have the appearance of neuronal synapses and that the enzyme monamine oxidase is present in the cerebral microvessel preparation. If these structures are functional synapses they may then have a role in the control of cerebral blood flow and vascular permeability.

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1416 CHARACTERIZATION OF ^{3}H-SPIPERONE BINDING TO BRAIN MEMBRANES OF THE RAT. R. H. Sundermann*, C.-H. Cheng*, and G. F. Wooten. Depts. of Neurology and Pharmacology; Washington University School of Medicine; St. Louis, MO 63110

The biochemical properties of ^{3}H-Spiperone binding were defined in vitro using a crude homogenate of rat corpus striatum and separation by filtration. In this system saturable binding constituted greater than 90% of the total binding. Scatchard analysis revealed a high affinity site with a K_d of 1.3 nM and a low affinity site with a K_d of 62 nM. B_{max} for the high affinity site was 175 f moles/mg of tissue. The binding was stereospecific in that (+)-butaclamol was 1000 times as potent as (-)-butaclamol in displacing bound spiperone. The binding demonstrated other properties of a specific protein receptor. ^{3}H-Spiperone binding was pH dependent, being maximal at pH 7.6 to 7.8. Thermal inactivation of specific binding was demonstrated at temperatures greater than 50°C. Incubation at 60°C for 5 minutes resulted in 50% loss of specific binding, while 5 minutes of incubation at 70°C caused total loss of specific binding. Extremes of pH and temperature did not affect non-specific binding. The rate of association of the ligand with the high affinity binding sites was rapid at 37°C, specific binding being half maximal in less than 30 seconds. The concentration of the cations Na^{+}, Ca^{++}, Mg^{++} and Mn^{++} had no significant effect on the amount of saturable ^{3}H-Spiperone binding to the high affinity site.

The potencies of several neurotransmitters and pharmacological agents in blocking specific high affinity ^{3}H-Spiperone binding were determined. Of the neurotransmitters, dopamine was the most potent with an IC_{50} concentration of 2×10^{-5}M. The IC_{50} for serotonin was 1.8×10^{-4}. Epinephrine and norepinephrine were much less potent, both with IC_{50} concentrations greater than 10^{-2}M. The inhibitory potencies of apomorphine and three other novel, putative dopamine receptor agonists were studied. Bromocryptine was the most potent inhibitor, followed by, in decreasing order, apomorphine, lergotrile and piribedil.

Nine regions of the rat brain were assayed for ^{3}H-Spiperone binding and the affinities and concentration of binding sites were quantified by Scatchard analysis. The concentration of binding sites in each region varied directly with the dopamine concentration.

1417 **SEROTONIN BINDING PROTEINS IN RAT PLATELETS.** H. Tamir, R. J. Bebirian*, D. Casper*, and F. Muller*. Division of Neuroscience, N.Y. State Psychiatric Institute and Department of Psychiatry, Columbia University, New York, NY 10032.

A protein with high binding affinity for serotonin is present in homogenates of both CNS and PNS (myenteric plexus). This protein is enriched in brain synaptic vesicles. When enteric neurons are stimulated both serotonin and serotonin binding protein are released by a Ca^{++}-dependent mechanism. The protein binds newly synthesized serotonin; serotonin binding is inhibited by reserpine. We now wish to report the properties of serotonin binding protein in the high speed supernatant of rat platelets. Platelet rich plasma, obtained by centrifuging rat blood drawn by cardiac puncture, was frozen and thawed, and the resulting suspension was centrifuged (100,000g; 60 min) to obtain the high speed supernatant. The binding of serotonin to the protein in this supernatant was highly specific (92%), dependent on Fe^{++}, trypsin sensitive and partially heat stable (42% decrease of activity at 100°). Two proteins with serotonin binding capacity were present: a glycoprotein and albumin. Both were also present in platelets sedimented and washed to remove the plasma. They were purified using $(NH_4)_2SO_4$ fractionation, Sephadex sieve chromatography and affinity column chromatography. The two proteins differed in most characteristics. However they showed two similar properties: sensitivity to trypsin (85% loss of binding activity with 0.2 mg trypsin/ml at 28° for 20 min) and enhanced binding (5 to 10 fold) in the presence of total brain lipid (25 µg lipid/25 mg protein). The proteins exhibited several differences: 1) migration on 5% acrylamide gel of the complex [protein-Fe^{++}-^{3}H-serotonin]: glycoprotein, Rf 0.41, albumin Rf 0.76. 2) heat stability (100°, 15 min); glycoprotein unstable, albumin stable. 3) molecular weight (SDS gels): glycoprotein 100,000; albumin 64,000. 4) carbohydrate reaction: glycoprotein stains strongly with Schiff's reagent after periodate oxidation; albumin, no reaction. 5) binding capacity and binding constants: glycoprotein 0.02 nmoles/mg protein; Scatchard plot, complex; albumin 0.125 nmoles/mg protein, two dissociation constants $K_{D1}=2.0\times10^{-8}$M and $K_{D2}=50\times10^{-8}$M. The properties of both the glycoprotein and albumin differ considerably from those of the serotonin binding protein of brain. It appears from these results that the storage form of serotonin in platelets is different from that of the brain.

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1418 DEACYLATION-REACYLATION ACTIVITY OF MOUSE BRAIN PHOSPHOLIPIDS DURING CARBAMYLCHOLINE INDUCED CONVULSION. Wilson Tang* and Grace Y. Sun. Sinclair Research Farm and Biochemistry Dept., Univ. Missouri, Columbia, MO 65211.

Brain microsomes and synaptosomes were prepared from placebo controls and from mice after becoming convulsive by injecting intracerebrally with carbamylcholine (CC) (4 µg/brain). When the membrane samples (app 800 µg protein) were incubated with labeled oleoylCoA (0.1 µCi in 5 nmol), a large portion of the label was hydrolyzed by the acylCoA hydrolase to form free fatty acids, but a small portion of the oleoyl group was transferred and subsequently incorporated into the phospholipids. The acylCoA hydrolase activity was lower (12%) in synaptosomes isolated from CC-stimulated mice as compared to controls. CC-induced convulsion gave rise to a 3-fold increase in oleoyl transfer to diacylglycerophosphocholines (GPC) in the synaptosomes. The most rapid increase was observed during the initial 5 min after onset of convulsion. Among other phospholipids in synaptosomes, the diacyl-glycerophosphoinositols (GPI) also indicated an increase in acylation (app 1-fold) with respect to CC-induced convulsion, but the effect was not readily discernable due to low incorporation activity. The increase in acylation activity was pertaining only to synaptosomal phospholipids, suggesting that specific types of lyso-phosphoglycerides were released in synaptosomes during CC-induced convulsion. Calcium (4 mM) inhibited acylCoA hydrolase activity by app 25% but enhanced the acyl transfer into phospholipids, especially diacyl-GPC and diacyl-GPE (ethanolamine). The calcium-enhanced acyl transfer to diacyl-GPC and diacyl-GPE in synaptosomes was further stimulated during CC-induced convulsion. Some increase in acylation of phospholipids due to calcium was also observed in microsomes but among them, the increase in oleoyl transfer to diacyl-GPS (serine) was most dramatic, giving a 3-fold difference with respect to CC-induced convulsion. The calcium-associated increase in oleoyl transfer to membrane is attributed to the release in lyso-phosphoglycerides due to a stimulation of the phospholipase A_2. Apparently, the sensitivity of calcium-stimulated phospholipase A_2 towards diacyl-GPC and diacyl-GPE in synaptosomes and diacyl-GPS in microsomes is altered with respect to convulsion. Results of this experiment have demonstrated for the first time an involvement of deacylation-reacylation mechanism in membrane phosphoglycerides during CC-induced convulsion. The increased deacylation activity with respect to brain stimulation may be mediated through an unknown mechanism of interaction between calcium and phospholipase A_2. (Supported in part by NS-12960 from NIH and BNS 76 24338 from NSF.)

1419 COMPARATIVE ASPECTS OF CARBONIC ANHYDRASE ACTIVITY: POSSIBLE FUNCTIONAL SIGNIFICANCE. M. C. Trachtenberg and D. J. Packey. Div. Neurosurg. and Dept. Physiol. and Biophys., Univ. Texas Med. Bra., Galveston, TX 77550.

It has been suggested that in the neuroepithelia of warm blooded animals the demand for protons and bicarbonate requires the presence of the enzyme carbonic anhydrase (CA). Thus the choroid plexus of mammals exhibits high CA activity. In contrast, the choroid plexus of a cold-blooded species such as frog is devoid of the enzyme. To test the idea of a relationship between body temperature and CA activity we have measured the CA activity in a number of poikilotherms and homeotherms with emphasis on nervous system strucutres. In nervous tissue CA is specific to neuroglia and absent from neurons.

We observe that in general poikilotherms exhibit much lower CA activity than do comparable tissues in homeotherms. There are exceptions to this observation, however. The blood of poikilotherms has quite appreciable CA activity - almost 14,000 U/mg ww for the saltwater catfish. The lower brainstem (LBS) of the saltwater catfish exhibits as much CA activity as does LBS of the rat and more than is present in LBS of the cat, for example (1532, 1602 and 906 U, respectively). An interspecies comparison of nervous system CA activity shows that for each species retina and choroid consistantly display maximal CA activity. The retina/choroid of homeotherms however, have 12-40 times more activity than do those of poikilotherms. In the saltwater catfish and sting ray LBS CA activity is as high as that in their respective retinae. These animals have a well developed lateral line system with synaptic centers in LBS. This observation may suggest a functional relationship between neuronal metabolism and glial CA activity. This supposition would be supported by a decrease in CA activity in neuropil poor tissues. The neural filum terminale (FT) of the frog is a structure devoid of neuronal somata but enriched in glia, surrounded by axons. CA activity in the frog FT is less than half that of the sacral or lumbar segments of the spinal cord of this animal. In contrast, the FT of all of the other species studied is about equal to that of the remaining spinal cord.

Our data suggest that the relationship between a requirement for protons and bicarbonate and body temperature is not straight forward. A functional relationship based on ion movement and acid-base balance requirements might better explain our observations. As for the nervous system distributions, it might be tempting to speculate that structures central to the functioning of the animal may have higher metabolic needs or require finer regulation of extracellular ionic concentrations and therefore such regions require greater CA activity.

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1420 **CYCLIC NUCLEOTIDES AND β-ADRENERGIC BINDING IN THE CEREBELLUM OF pcd (PURKINJE CELL DEGENERATION) MUTANT MICE DURING PURKINJE AND GRANULE CELL DEGENERATION.** **Lewis L. Truex*, Bernardino Ghetti and Michael J. Schmidt (SPON: J. Clemens). Eli Lilly and Company and Indiana University School of Medicine, Indianapolis, IN 46206.**

Norepinephrine-stimulated accumulation of cyclic AMP (cAMP) was studied in vitro in the cerebellum of pcd mice before, during and after total degeneration of Purkinje cells. The cerebellar concentration of cAMP in the presence of norepinephrine in pcd mice was 300 pmoles/mg protein compared to 80 pmoles/mg protein in controls. First evidence of hyperresponsiveness was detected at 24 days of age, the time when ataxia developed and Purkinje cells started to degenerate. The difference in hormonal response persisted even when all Purkinje cells had disappeared, as documented by histologic examination of pcd cerebellum. The accumulation of cAMP was greater in sections of the cerebellar cortex than in regions containing the deep nuclei. The difference observed between mutant and normal mice in the magnitude of cAMP accumulation was maximal between 30 and 130 days of age and then lessened until there was no detectable difference by the age of 270 days. At 1 yr norepinephrine elicited a smaller cAMP accumulation in pcd mice than in unaffected controls. After 6 months of age a progressive and substantial loss of granule cells was noted.

The differences in cAMP accumulation were not accompanied by changes in ^{3}H-dihydroalprenolol binding, indicating that the increased response to norepinephrine was not due to an increase in the number of beta adrenergic receptors in pcd cerebellum. Adenylate cyclase activity was similar in control and pcd mice. Theophylline, an adenosine receptor blocking agent, did not attenuate the cAMP accumulation in pcd cerebellum. This suggests that the exaggerated accumulation of cAMP seen in slices from pcd mice following NE-stimulation could not be explained through the phenomena of adenosine synergism. The exaggerated accumulation of cAMP was also found in the cerebellum of "nervous" mice in which 80% of the Purkinje cells are lost. Therefore, the phenomenon is not restricted to the pcd mutation.

The data confirm that cAMP synthesis and beta adrenergic receptors occur in the cerebellum in cells other than Purkinje cells. We speculate that the exaggerated norepinephrine-elicited accumulation of cAMP in pcd mutants might be due to astrocytosis or changes in granule cells in the cerebellar cortex.

1421 EFFECTS OF 3,4-DIHYDROXYPHENYLETHANOL AND ITS QUINONE ON THE UPTAKE AND RELEASE OF ^{3}H-CATECHOLAMINES IN A RAT BRAIN CRUDE SYNAPTOSOMAL FRACTION. Anthony D. Vanker, Stephanie J. Prevost*, and Frank L. O'Brien*. Depts. of Biology and Chemistry, Georgia State University, Atlanta, Georgia 30303.

The inhibitory effects of 6-hydroxydopamine (6-OHDA) on catecholamine uptake into presynaptic elements are well known. There is evidence that at least some of the effects of 6-OHDA are due to its oxidation to the corresponding quinone which then undergoes nucleophilic reactions. Similar quinones can be formed from normal catecholamine metabolites such as 3,4-dihydroxyphenylethanol (DHPE). Previously we reported on the use of controlled potential coulometry as the means of oxidizing DHPE and the effects on the uptake of ^{3}H-norepinephrine (^{3}H-NE) (Soc. Neurosci. Abts. 4, 1041). Crude synaptosomal fractions of rat forebrain were isolated and mixed with a TES salts buffer (NEP).

Different portions were then used in each of the following experiments: 1) NEP + stirring 2) NEP + stirring + DHPE 3) NEP + stirring + DHPE + electrolysis. The time interval for each experiment was 10 min. Immediately following each experiment, standard procedures were used to measure the uptake of ^{3}H-dopamine (^{3}H-DA) into synaptosomes found in the NEP. The DHPE quinone inhibited uptake in a 'step-function' manner with essentially no inhibition at 10^{-6}M and complete inhibition at 10^{-5}M. DHPE itself exhibited a weaker but potent inhibition in an apparent competitive manner. This inhibition of ^{3}H-DA uptake obtained with DHPE and its quinone is similar to but more pronounced than that reported previously for ^{3}H-NE. In some experiments a brain mince was incubated in the TES salts buffer with either ^{3}H-DA or ^{3}H-NE to 'preload' presynaptic elements prior to homogenization and differential centrifugation. The preloaded crude synaptosomal fraction was resuspended and treated as before to determine whether DHPE or its quinone act as releasing agents. DHPE did not cause the release of ^{3}H-DA or ^{3}H-NE at any of the concentrations used in the uptake study. The quinone was a weak releasing agent (25% for both ^{3}H-DA and ^{3}H-NE) only at the highest concentration used. Electrochemical data indicated substantial secondary oxidation reaction occur in the presence of synaptosomes. (Supported in part by NS-14338).

1422 CALCIUM-DEPENDENT ACTIVATION, STABILIZATION, AND DESTABILIZATION OF TRYPTOPHAN HYDROXYLASE FROM RAT MIDBRAIN. Anthony Vitto and Arnold J. Mandell. Dept. Psychiatry, Sch. Med., UCSD, La Jolla, Ca 92093

Inactivation of tryptophan hydroxylase from rat midbrain was followed at 37°C in the presence and absence of various pathway-related ligands and other compounds. Control studies indicate a multiphasic inactivation characterized by a rapid initial loss within 15 minutes of 30-40% of the initial enzyme activity. After 15 minutes, the rate of inactivation declined until stability of 40-50% of the initial activity was achieved at about one hour. This pattern of inactivation was unaltered by tryptophan (0.1, 0.25, 1.0 mM), tetrahydrobiopterin (0.1 mM), reduced nicotinamide adenine dinucleotide (0.1 mM), dithiothreitol (0.1 mM), ethanol (1.0%), and phenylmethyl sulfonyl fluoride (0.1 mM); however, the effects of combinations of the above were not studied.

In the presence of greater than 200 uM calcium, loss of activity was rapid and followed classical first-order kinetics. At low and intermediate levels of calcium (200-800 uM), first-order loss of activity commenced after an initial period of stability lasting as long as 40 minutes. With higher levels of calcium, rapid loss of activity proceeded with no or minimal periods of stability. The range of calcium concentrations at which stabilization and destabilization are observed parallels that for the previously reported calcium activation of tryptophan hydroxylase (Life Sci. 16: 1583, 1975), suggesting that the molecular events underlying these three phenomena are intimately related, if not identical. The possibility of a calcium-activated protease, which mediates these events through an activating limited proteolysis followed by an inactivating proteolysis, is being examined. Enzyme preparations stored in the frozen state for several days, when thawed, showed slightly higher initial activity but exhibited rapid first-order loss of activity when incubated at 37°C. The slow release of endogenous calcium and/or activation of a proteolytic system may explain the time-dependent appearance of a destabilized enzyme population.

Preliminary observations indicate that tryptophan hydroxylase from rats housed at 30°C shows marked instability at 37°C when compared to the enzyme from rats housed at 22°C. We speculate that modulation of enzyme stability by a system, proteolytic or otherwise, sensitive to ambient temperature may represent a mechanism for the apparent role of the serotonergic system in thermoregulation.

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1423 INHIBITION OF RAT BRAIN AND SCIATIC NERVE ENOLASE ACTIVITY BY ACRYLAMIDE. Ivy L. Vyas*, Richard D. Howland*, and Herbert E. Lowndes (SPON: J.J. McArdle). Dept. Pharmacol., CMDNJ, N.J. Med. Sch., Newark, N.J. 07103.

Acrylamide may produce peripheral nerve degeneration by interference with axonal energy metabolism (Spencer and Schaumburg, in Waxman, S.G.(ed.): Physiology and Pathobiology of Axons, N.Y., Raven Press, 1978). The effect of acrylamide on the glycolytic enzyme, enolase, was investigated in rats in vitro and in vivo since inhibition of the neuron-specific form (Marangos et al., J. Neurochem. 31: 727, 1976) could offer an explanation of the relative specificity of acrylamide for initially producing degeneration of distal portions of peripheral long axons. Enolase was isolated in the soluble fraction of rat whole brain homogenates (WBH). Activities were determined from initial velocity measurements. Homogenates were taken to 80% $(NH_4)_2SO_4$ saturation according to the method of Marangos et al., (Biochem. Biophys. Res. Commun. 68(4): 1309, 1976) and the P80 fraction was obtained. Enolase in WBH and in the P80 fraction was inhibited in vitro by acrylamide (I_{50} 4.0 mM and 3.7 mM, resp.). When the P80 fraction was incubated at 50°C for one hour, approximately 20% of the total enolase activity remained. This fraction was termed HP80 and was assumed to reflect neuron-specific enolase activity (Marangos et al., J. Neurochem. 31: 727, 1978). Enolase activity in the HP80 fraction was inhibited by acrylamide in vitro (I_{50} 3.3 mM). Double reciprocal plots of data obtained on WBH showed a complex inhibition pattern. Hexanedione, another neuropathic agent, caused no inhibition at a 10 mM concentration. For in vivo experiments, rats were treated with acrylamide (50 mg/kg/day i.p.) to a cumulative dose of 350 mg/kg or 550 mg/kg. Enolase activity was determined in brains and sciatic nerves of control and treated animals. In rats receiving 350 mg/kg or 550 mg/kg brain weights were decreased and soluble fraction protein content was decreased when compared to controls. Enolase specific activity was increased. Enolase activity was decreased but protein content unchanged in the soluble fraction of sciatic nerve homogenates. No enolase activity was detectable in the HP80 fraction from sciatic nerve preparations from 550 mg/kg acrylamide treated rats, suggesting that in vivo inhibition of the neuron-specific form of enolase by neurotoxic doses of acrylamide may be involved in the etiology of this distal axonopathy.
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1424 BLOOD-BRAIN BARRIER PERMEABILITY TO CYSTINE AND CYSTEINE. Lester A. Wade, Helen M. Brady* and Delynne J. Myers*. Dept. Physiology, Tulane Univ. Sch. Med., New Orleans, La. 70112.

Cysteine, a sulfur-containing amino acid in the reduced form, exists in equilibrium with cystine, the oxidized form. We measured the degree of blood-brain barrier penetration by cysteine and cystine using the carotid injection technique of Oldendorf in the rat. A mixture of a radioactively-labeled amino acid and ^{3}H-H_2O was injected into the carotid artery, the rat was decapitated at 5 or 15 seconds, and the brain dissected into 5 regions before counting. When ^{35}S-cystine was injected, we found BUI values that were very close to the background values of this technique. The addition of non-radioactive cystine (200-600 µM) had no effect on the uptake of two neutral amino acids, ^{14}C-cycloleucine (25 µM) and ^{14}C-methionine (50 µM) or of the basic amino acid, ^{14}C-arginine (10 µM). In carotid injections using ^{35}S-cysteine (10 µM), we found that this amino acid was readily taken up (BUI of 20). When non-radioactive cycloleucine (4 mM) and alanine (4mM) were added to the injectant, the uptake of ^{35}S-cysteine was inhibited by 35% and 30%, respectively. Although cystine and cysteine are chemically related, their transport characteristics do differ. For the disulfide cystine, we were unable to demonstrate any significant degree of blood-brain barrier transport at the concentrations tested. Cysteine, the sulfhydryl form, did penetrate the blood-brain barrier. Cycloleucine's inhibition of cysteine uptake suggests that the leucine (L) neutral amino acid transport system accounts for a componed of cysteine's total uptake. Another possible componed of cysteine's uptake is the alanine-serine-cysteine (ASC) neutral amino acid transport system, which remains to be further evaluated.

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1425 DE NOVO SYNTHESIS OF NEURON SPECIFIC ENOLASE IN A RABBIT RETICULOCYTE TRANSLATION SYSTEM PROGRAMMED BY POLY(A)-RNA FROM RAT BRAIN. William A. Walker* and Claire Zomzely-Neurath. Dept. Biochem., Roche Institute of Molecular Biology, Nutley, N.J. 07110.

Poly(A)-RNA prepared from the brains of 30 day old, male rats has been shown to direct the synthesis of neuron specific enolase (NSE) in a cell-free system derived from rabbit reticulocytes. Rat brain RNA was obtained by treating total polysomes with EDTA and SDS followed by LiCl precipitation. Poly(A)-RNA was then isolated by oligo(dT)-cellulose chromatography.

The reticulocyte lysate was treated with Staphylococcal nuclease to render the system completely dependent on exogenous m-RNA. The ability of the lysate to translate Poly(A)-RNA from 30 day old rat brains was optimal at 1.5 mM magnesium and 100 mM potassium while spermidine had no stimulatory effect. The cell-free reactions were immunoprecipitated by either anti-NSE antisera or preimmune sera coupled to cyanogen bromide activiated Sepharose. Analysis of the immunoprecipitates on sodium dodecyl sulfate-polyacrylamide slab gels and autoradiography indicated the synthesis of a product that co-migrated with purified NSE and was recognized only by the anti-NSE antisera. Similar immunoprecipitation of reticulocyte lysates programmed with total RNA derived from embryonic chick heart failed to indicate the synthesis of NSE. These results show that the m-RNA coding for NSE contains a poly(A) sequence and that brain specific factors are not required for its translation. This is the first demonstration of the de novo synthesis of a neuron-specific protein which is 1% of the total soluble protein and whose biological function is known.

1426 VOLTAGE-SENSITIVE CALCIUM INFLUX ACTIVATES cAMP-DEPENDENT PROTEIN KINASE (cDPK) IN ADRENAL MEDULLARY CELLS. J.C. Waymire and R.E. Boehme. Dept. of Psychobiology, Univ. of Ca., Irvine, Irvine, Ca. 92717 and Dept. of Neurobiology, Univ. of Texas Med. Sch., Houston, Tx. 77025.

We have characterized adrenal medullary cell cDPK and instituted procedures to analyze, in the crude extract, the activation of cDPK following stimulation of intact cells. These procedures indicate that 1) adrenal medullary cell cDPK is predominantly type II and 2) the activation of cDPK occurs through a voltage-sensitive influx of calcium.

Treatment of isolated medullary cells with ACh in the presence of Ca^{++} produces a time- and dose-dependent increase in the cDPK activity index which appears to be nicotinic in nature; being both mimicked by nicotine and blocked by hexamethonium. At high ACh (100 uM), the increase in cDPK index is rapid, but less prolonged; reaching a maximal level in 5 min and decaying by 20 min. At lower ACh (10 uM) the activation proceeds more slowly, but is maintained longer; reaching a similar maximum in 10 min and decaying by 30 min. Other agents, known to depolarize medullary cells, veratridine, KCl and angiotensin II, produce a similar calcium-dependent activation. In all cases, theophylline potentiates the activation by the above agents by about 33%, but has no influence alone. The involvement of extracellular Ca^{++} in this depolarization induced response is indicated by the observation that no alteration in cDPK index occurs in its absence. Further, 5 uM A23187, although not inducing an activation in the absence, produces a marked increase in cDPK in the presence of 1.5 mM extracellular Ca^{++}.

A possible explanation of these results, that Ca^{++} is directly activating cDPK, is unlikely since Ca^{++} does not activate isolated cDPK when added directly to the in vitro enzyme incubation. Another possibility, that Ca^{++} indirectly stimulates cDPK by elevating cAMP through the secretion of catecholamines (CA), which in turn stimulate an auto receptor-linked adenylate cyclase, is also unlikely since α or β receptor blocking agents were found not to prevent the Ca^{++} dependent cDPK activation. Also CA themselves would not produce an activation of cDPK. Unless some presently unknown agent, secreted in response to Ca^{++}, is stimulating adenylate cyclase, a likely explanation of these results is a Ca^{++} interaction with calmodulin to modulate chromaffin cell cAMP through sequential activation of adenylate cyclase and phosphodiesterase. The shapes of the time response curves at different agonist concentrations supports this conclusion. Supported by USPH NS-11061 and AG 00538.

1427 P2 PROTEIN FROM BOVINE PNS MYELIN - NH_2-TERMINAL SEQUENCE AND NEURITOGENIC ACTIVITY. M. J. Weise*, D. L. Hsieh*, S. Levit*, P. M. Hoffman*, J. M. Powers* and S. W. Brostoff* (SPON: B. B. Wannamaker). Departments of Neurology and Pathology (Neuropathology), Medical University of South Carolina, Charleston, S.C. 29403 and V.A. Medical Center, Charleston, S.C.

Three peptides denoted CN1, CN2 and CN3 are generated by CNBr digestion of bovine P2 protein. Overlapping peptides provided by trypsin digestion and BNPS-skatole cleavage of the tryptophan at residue 8 enable us to order these peptides in the P2 protein. CN3 is at the NH_2 terminus, followed by CN1 with CN2 at the COOH terminus. The sequence of the first 65 residues of the protein which overlap CN3 and CN1 is:

NH_2-Ac-Ser-Asn-Lys-Phe-Leu-Gly-Thr-Trp-Lys-Leu-Val-Ser-Ser-Glu-Asn-Phe-Asp-Glu-Tyr-Met-Lys-Ala-Leu-Gly-Val-Gly-Leu-Ala-Pro-Arg-Lys-Leu-Gly-Asn-Leu-Ala-Lys-Pro-Arg-Val-Ile-Ile-Ser-Lys-Lys-Gly-Asp-Ile-Ile-Thr-Ile-Arg-Thr-Glu-Ser-Pro-Phe-Lys-Asn-Thr-Glu-Ile-X-Phe-Lys-COOH.

When tested for their ability to produce disease in guinea pigs, rabbits and Lewis rats, CN3 proved inactive while CN2 produced disease in all three species. Experimental allergic neuritis (EAN) was produced in Lewis rats by peptide CN1 as well, with this peptide being more active than CN2 in this species.

CN2, which is neuritogenic in all species tested, is a disulfide-linked dipeptide accounting for approximately 18 residues at the COOH terminal end of the P2 protein. In the intact isolated protein, adjacent regions of the single amino acid chain near the COOH terminus appear to be covalently linked through a disulfide bond, which remains intact after cleavage of the two methionines resulting in CN2. The presence of this intra-chain disulfide bond in the native, membrane bound P2 protein and its effect on the disease-inducing properties of the P2 protein remain to be determined.

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1428 SYNTHESIS AND TRANSPORT OF A SPECIFIC PROTEIN BY ASTROCYTES. Fredric P. White, Faculty of Medicine, Memorial University of Newfoundland, St. John's, Newfoundland A1B 3V6.

When rat telencephalon slices are used to label protein with radioactive amino acids and these slices are subsequently fractionated by rate and density gradient centrifugation, two fractions, nuclei and blood capillaries, are found which have very high specific activities of radioactively labeled protein (White, 1979). Only the blood capillary fraction, however, appears to be transporting some of this newly synthesized protein to other sites on a translocation system which is inhibited by colchicine, vinblastine, and low Ca^{++} concentrations (White, 1976; 1979). SDS-PAGE of those fractions and others shows that more than 10% of the radioactivity in the capillary fraction is in a protein of molecular weight 71,000 daltons, and that this protein is exported from particles in this fraction, and imported into particles in a myelinated axon fraction, microsomal fraction and soluble/cytosol fraction. Present studies were undertaken to identify the particle responsible for this specific synthesis and subsequent transport.

Rat telencephalon slices were incubated in a Krebs Ringer phosphate solution containing [^{3}H] leucine for one hour. Some of the slices were fixed immediately after incubation while others were homogenized in 2.0 M sucrose containing 1 mM $MgCl_2$. A nuclei and capillary fraction was prepared by centrifugation in sucrose density gradients. Autoradiographs were prepared from these fractions and from 25 μ transverse sections of the tissue slices. After developing the radiograph all sections were stained with either thionin blue or Basic Fuchsin. Results show that the vast majority of the radioactivity found in the capillary fraction is located over the capillaries themselves. The radioactivity in the nuclear fraction is associated with both the light staining (neuronal and astrocyte) nuclei and dark staining nuclei. However, the grain count is much higher over the light staining nuclei, and the grain densities appear to be associated with material adhering to the nuclei (possibly Nissl substance). The autoradiographs of the tissue slices confirm that the high grain densities are associated with blood capillaries. The pattern of labelling over the capillaries shows that the astrocyte end feet are probably responsible for the synthesis of large amounts of protein in the brain slice. This data suggest that astrocytes synthesize a 71,000 dalton protein in their end feet and transport this protein to other sites away from the capillaries on a vinblastine sensitive translocation system. This research was supported by grant MA-5404 from the MRC of Canada.

White, F.P. (1976) J. Neurochem. 27, 1543-1545.
White, F.P. (1979) J. Neurobiol. (In Press).

1429 REGULATION OF PINEAL HYDROXYINDOLE O-METHYLTRANSFERASE (HIOMT) BY ADRENAL CORTICAL HORMONES. Dona L. Wong*, Alfred W. Sandrock, Jr.* and Roland D. Ciaranello. Dept. of Psychiatry & Behavioral Sciences, Stanford University Sch. Med., Stanford, CA 94305.

Recent work from this laboratory has shown that adrenal glucocorticoids control the steady-state levels of adrenal medullary phenylethanolamine N-methyltransferase (PNMT) by inhibiting the in vivo proteolysis of this enzyme. Further investigation disclosed that glucocorticoids were not acting directly, but through S-adenosylmethionine (SAM), an important constituent in the PNMT reaction. Hypophysectomy reduced the levels of glucocorticoids and of S-adenosylmethionine. This, in turn, resulted in a loss of stability of PNMT to in vivo proteolysis. SAM is a critical methyl donor in a variety of methylation reactions involving biogenic amines. Our current research shows that pineal hydroxyindole O-methyltranferase, like adrenal PNMT, is under glucocorticoid regulation. Hypophysectomy reduces the levels of HIOMT, while dexamethasone administration restores them. SAM, both in vitro and in vivo stabilizes HIOMT against proteolytic degradation, resulting in a restoration of enzyme levels. Thus the levels of two important biogenic amine methyltransferases, HIOMT and PNMT, are under glucocorticoid regulation through mechanisms involving SAM stabilization of the enzyme against proteolytic breakdown. (Supported by MH 25998, NSF PCM 78-14183 and MH 00219).

1430 RELATIONSHIP BETWEEN FUNCTIONAL ACTIVITY AND GLUCOSE UTILIZATION IN THE RAT SUPERIOR CERVICAL GANGLION IN VIVO. P. Yarowsky, J. Jehle*, D. H. Ingvar*, and L. Sokoloff. Laboratory of Cerebral Metabolism, NIMH, Bethesda, MD 20205.

The rat superior cervical ganglion (SCG) was studied as a model system for correlating in vivo glucose utilization, as measured by the deoxyglucose method (SOKOLOFF et al. J.Neurochem. 28:897, 1977), with electrical activity. The SCG is particularly suitable for such a study. One can modulate and monitor both the preganglionic input as well as the output of the postsynaptic neurons. Three different experimental conditions have been studied: normal controls, unilateral decentralization, and electrical stimulation. In normal conscious rats, mean (± S.E.M.) glucose utilization of the SCG in vivo was 35 (± 1) µmoles/100 g/min (n=20) with no significant side-to-side difference. This value is higher than the in vitro glucose utilization at 37° of the SCG, 23 µmoles/100 g/min, reported by Horowitz & Larrabee (J. Neurochem. 9:407, 1962). Glucose consumption was not uniform throughout the SCG. In the rostral portion of the SCG the rate of glucose utilization was 3 times higher than the rate in the rest of the ganglion. This region of high metabolic activity corresponds to the anatomical distribution (demonstrated by the horseradish peroxidase technique) of postganglionic neurons which give rise to the axons of the internal carotid nerve (Bowers & Zigmond, J.Comp.Neurol. 288:227, 1979). In unilaterally decentralized animals, the left cervical sympathetic trunk (CST) was severed at least 1 cm from the SCG, and a piece of the nerve was removed 7-9 days prior to the measurement of glucose utilization. Following the decentralization, the animals developed both miosis and ptosis unilaterally. The mean (± S.E.M.) glucose utilization of the decentralized SCG was reduced to 22 (± 2) µmoles/100 g/min (n=8), and the region of high activity was obliterated. Glucose utilization in the contralateral control SCG was the same as that found in the normal animals. Experiments in which the CST was electrically stimulated were carried out under urethane (1 g/kg) anesthesia. The CST was placed on platinum electrodes and stimulated via a stimulus isolation unit. Stimulation was initiated 5 min prior to the onset of measurement of glucose utilization. In all cases stimulation caused ipsilateral mydriasis and widening of the palpebral fissure. With stimulation frequencies of 5, 10, and 15 hz, there was an apparent frequency-dependent increase in the mean rate of glucose utilization of the SCG above control levels under urethane anesthesia. The region of high utilization was still present and enhanced. These studies suggest that the glucose consumption of the SCG is modulated by functional and/or electrical activity.

NEUROCYTOLOGY

1431 NUCLEUS PARAGIGANTOCELLULARIS LATERALIS IN THE RAT: ANALYSIS WITH GOLGI IMPREGNATIONS. Joseph A. Andrezik. Department of Anatomical Sciences, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190.

The nucleus paragigantocellularis lateralis (PGCL) lies lateral to the medullary pyramid and extends caudally from the trapezoid body through the rostral one-third of the inferior olivary nucleus. At least eight neuronal types of the PGCL can be distinguished in Nissl preparations, and some of these are distributed preferentially within the nucleus. On the basis of cytoarchitecture and the distribution of afferent fibers, the PGCL may be divided into rostral and caudal subdivisions (Andrezik and Chan-Palay, Anat. Rec. 187:524, 1977).

Brains of adult Sprague-Dawley rats were impregnated with the rapid Golgi, Golgi-Cox, or several variants of the Golgi-Kopsch technique. The brains were sectioned in coronal, horizontal, or sagittal planes for examination.

At caudal levels, the dendrites of most PGCL neurons are oriented in the coronal plane with the longest dendrites extending in the medial to lateral direction. Examinations of sections cut in horizontal or sagittal planes reveal a few categories of neurons whose arborizations are nearly equal in transverse and longitudinal extent. One type of arborization is essentially planar - dendrites emanate from a spherical or fusiform perikaryon either directly opposite or at right angles to one another, often resulting in a cruciate appearance. Another type exhibits a dendritic tree forming three cones, each commencing from an apex of the pyramidal-shaped perikaryon. Dendrites of some other neurons form a sphere with the perikaryon at the center. At more rostral levels, PGCL neurons are situated medial to the facial motor nucleus. A striking feature in this area is that dendrites of PGCL neurons follow the contours but do not penetrate the borders of the facial nucleus. The converse is not true.

Most PGCL neurons have relatively unbranched dendrites and a smooth surface. Some, however, exhibit spines on the perikaryon and dendrites, but these are rare. Spines usually are simple in form, being either sessile or pedunculated. Dendritic bundles are found in the PGCL and are formed by 5-7 dendrites each from a different neuron. The bundles course between fascicles of passing fibers and are most easily visualized in sections cut in coronal or horizontal planes. The various forms of PGCL neurons probably reflect the number and types of afferents which contact them and ultimately influence their activity.

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1432 A NEW DESIGN FOR ULTRA-RAPID FREEZING OF NERVE-EFFECTOR CELL JUNCTIONS. Robert T. Bailly*, Jack McC. Baggett*, Åsa Thureson-Klein and Richard L. Klein. Department of Pharmacology and Toxicology, Univ. Miss. Med. Ctr., Jackson MS 39216.

An ultra-rapid freeze apparatus has been designed to study vesicle dynamics at peripheral noradrenergic terminals in thin strips of blood vessels and vas deferens. The apparatus consists in linear sequence of (1) a warmed superfusion chamber for stimulation and recording, (2) two closely opposed copper blocks pre-cooled to liquid nitrogen temperature, (3) a similarly pre-cooled tissue vice specially constructed to mount in a Denton freeze fracture apparatus, and (4) a taught spring.

A 0.1-0.2 mm thick tissue strip is placed within an opening in a fabric belt. The tissue and belt are enclosed in a teflon carrier. The belt fits snugly in a channel with dimensions of 1.0 cm x 0.2 cm x 15.0 cm which is continuous through the surface of one of the opposed copper blocks. The belt is attached at the end to the taught spring. About 2.0 cm of teflon and 3.0 cm of open space insulate the carrier from the pre-cooled copper freezing blocks. The entire unit is flushed with dry nitrogen gas. During superfusion the taught spring is released. The fabric belt, tissue and carrier combination is drawn toward the copper freezing blocks where the teflon carrier stops. The belt and tissue continue through the channel and between the copper freezing blocks leading to the vice, where the tissue comes to rest some 2 msec later.

Premature cool-down of tissue has been essentially eliminated by the use of a warm teflon carrier enclosing the tissue and separated from the freezing blocks until the moment of rapid freeze. Freezing block warm-up has been rendered insignificant by providing for the continuous movement of the tissue across the surface of the freezing blocks with a traverse time of 2 msec. The design of operation provides uninterrupted electrical stimulation up until and during rapid freezing. This may be mandatory to study vesicle dynamics during exocytosis.

Upon completion of the freezing process, the vice is tightened on the tissue, packed in freon 22 snow, unscrewed from its copper mounting post and transferred to the Denton freeze fracture device after which routine processing can procede.

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1433 PSEUDOPODIAL INVASIONS OF NERVE TERMINALS IN RAT AMYGDALA. Alan F. Boyne and Sally B. Tarrant* Depart. of Pharmacology, Northwestern Medical and Dental Schools, Chicago, Ill 60611.

Stimulation-induced exocytosis has been shown to cause pseudopodia to grow from nerve terminals in Torpedine ray electric organ (Boyne & McLeod, Neuroscience 1979). They invade adjacent, abutted terminals and can then be pinched off. We are exploring the possibility that a similar process could occur in electro-convulsed mammalian brain.

The central nucleus of the amygdala was chosen for study because its encephalinergic-opiate receptor system may (1)utilise abutted, axo-axonal presynaptic inhibitory synapses and (2) it may regulate the depression-euphoria mood spectrum (Snyder, Sci. Amer. 1977).

We have been surprised to find that pseudopodial invasions of nerve terminals are present in the control, unstimulated amygdala. They are present amongst abutted terminals synapsing with dendrites in the neuropil adjacent to large neurons. They are found in terminals containing a mixed population of small clear and large dense-cored vesicles. The penetrating element generally contains clear vesicles but dense-cored vesicles have also been seen. We have not found synaptic specializations associated with the penetrating element.

Questions we are now trying to approach include: (1) What is the origin of the pseudopodia ? (2) Does the ultrastructural relationship imply a physiological interaction ? (3) Are either or both elements encephalinergic ? (4) Are the invasions detached during seizures ?

This work was supported by NIH grant # 13043.

1434 MYELINATED DENDRITES AND NEURONAL PERIKARYA IN THE MOUSE OLFACTORY BULB. Gail D. Burd and Aldo Rustioni. Neurobiology Program and Depts. of Anatomy and Physiology, UNC, Chapel Hill, N.C. 27514.

Myelinated dendrites have been reported in the olfactory bulb of the human (Braak, et.al., 1977), monkey (Pinching, 1971), and the cat (Willey, 1973). In addition, myelinated neuronal perikarya were observed in the olfactory bulb of man (Braak, et.al., 1977) and the rat (Diaz-Flores, et.al., 1977). This is the first report of myelinated dendrites in the olfactory bulb of rodents and of myelinated neurons in the mouse olfactory bulb. Observations were focused on the glomerular and external plexiform layers of control and experimental mice with olfactory nerve lesions, but mitral cell, internal plexiform, and granule cell layers were also examined. Mice were perfused with mixed aldehydes. Olfactory bulbs were cut into 1-2mm blocks, osmicated, dehydrated, and embedded in Spurr-Epon. Thin sections were stained with UA and LC. Only a very small population of dendrites and perikarya of the olfactory bulb were myelinated, and no difference was observed in the relative frequency of these myelinated structures in control vs experimental mice. Large dendrites with 6-10 lamellae of myelin were observed in the periglomerular region of the glomerular layer and external plexiform layer. Identification of the myelinated dendrites as mitral cell dendrites or large tufted cell dendrites was based on the large diameter and cytological detail of the myelinated dendrites. Myelinated dendrites contained: rough endoplasmic reticulum, free ribosomes, mitochondria, smooth endoplasmic reticulum and microtubules. On one myelinated dendrite cut in longitudinal section, synaptic contacts were present on a portion of the dendrite devoid of the myelin sheath. A thinner layer of myelin (2-5 lamellae) was observed to surround partially the sparse myelinated neurons present in the periglomerular region and the external plexiform layer. From a comparison of the cytological detail of these neurons with the descriptions of normal neurons in the olfactory bulb by Pinching and Powell (1971), these myelinated neurons appear to be small tufted cells or superficial short-axon cells. The cell bodies contained: moderate amount of rough endoplasmic reticulum, free ribosomes, mitochondria, stacks of Golgi, slightly indented nucleus, and frequently, a well defined nucleolus in the nucleus. The origin of the myelin sheaths cannot be determined for all the myelinated profiles. Oligodendrocytes may contribute myelin lamellae to the myelinated dendrites and perikarya, but this was not observed. However, in a few instances, myelin lamellae surrounding small myelinated axons were observed to leave the axon and contribute myelin lamellae to the myelinated dendrite or perikarya.

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1435 FINE STRUCTURAL ANALYSIS OF THE MOTOR TRIGEMINAL NUCLEUS OF THE RAT. John P. Card* and Robert Y. Moore (SPON: J.C. Sipe). Department of Neurosciences, UCSD, La Jolla, California 92093.

Recent histofluorescence examination of the monoamine innervation of various brainstem nuclei of the rat (Levitt and Moore, 1979) have demonstrated that the motor trigeminal nucleus (MTN) receives a heavy noradrenergic innervation from lateral tegmental cell groups. The present investigation was therefore undertaken to examine the ultrastructural organization of this nucleus as a basis for further fine structural analysis of the pattern of catecholaminergic innervation of this area. Adult female rats anesthetized with Nembutal (42mg/kg) were subjected to intracardiac perfusion fixation with glutaraldehyde-paraformaldehyde solutions. The motor trigeminal nuclei were then dissected from the brainstem and prepared for transmission electron microscopic examination using conventional procedures. Large (40-60 μm) motor trigeminal neurons were a prominent feature among the numerous large dendritic trunks and myelinated axons that characterized the neuropil in this region. The cytoplasmic constituents of these cells were typical of those generally described in neurons throughout the neuraxis. However, of primary interest in the present study was the large number of axosomatic contacts which were routinely observed on the perikarya of all cells examined. In some instances, as much as 94% of the cell soma was covered by axon terminals. Such terminals could be subdivided into categories on the basis of axoplasmic density and vesicle shape. The presence of either clear or dense axoplasm within the endings provided an easily recognized morphological criterion for subdividing axosomatic terminals into two distinct populations. In addition, analysis of vesicle morphology within endings indicated that both clear and dense terminals could be further subdivided on the basis of their vesicular content. In each case, endings were observed which contained either exclusively lucent spherical vesicles (0.04 μm) or mixed populations of spherical and flattened (0.06 μm) vesicles. Thus, four morphologically distinct terminal types have been identified in synaptic contact with the soma of MTN neurons. In addition ultrastructural analysis of the neuropil of the MTN revealed that the numerous dendritic profiles characteristically present in this area were also contacted by the same types of terminals observed on the soma. Whether or not the distinct morphological differences in axosomatic and axodendritic endings reflect differences in neurotransmitter content cannot be determined at the present time. However, studies are presently underway to determine if the dense noradrenergic innervation of this nucleus can be correlated with one or more of the demonstrated terminal morphologies. Supported by USPHS Grant NS 12080.

1436 APPLICATION OF HIGH MAGNESIUM CONCENTRATION PERFUSION-FIXATION TO RAT VISUAL CORTEX. Fen-Lei Chang, Mary Kay Floeter* and William T. Greenough, Dept. Psychol. and Neural & Behav. Biol. Prog., Univ. of Ill., Champaign, IL 61820.

Birks (J. Neurocytol., 1974,3:133) suggested that artifically low vesicle density may be seen in synaptic boutons with conventional glutaraldehyde fixation procedures due to the activation of transmitter release by glutaraldehyde. He used a high Mg^{+2} concentration (110 mM) solution during glutaraldehyde perfusion of cervical ganglion to block synaptic transmission and found dense packing of vesicles in about 2% of synapses. McKinlay & Usherwood (J. Ultrastructure Res., 1978,62:83) demonstrated that glutaraldehyde can increase the frequency of miniature EPSPs at locust neuromuscular junction, and that addition of Mg^{+2} attenuates this effect. They also used high Mg^{+2} concentration during fixation and found a very dense vesicle packing in synaptic boutons.

We applied Birks' method to rat visual cortex. Three concentrations of Mg^{+2} ($MgCl_2$) were used - 110, 160 and 210 mM. Adult rats were anesthetized with pentobarbital and perfused through the heart first with 200 ml of a high Mg^{+2} solution (besides the $MgCl_2$, KCl 5.0 mM, Hepes buffer 10 mM at pH 7.0), and then with 500 ml of the same vehicle with glutaraldehyde added to a final concentration of 3%. After fixation in the same fixative for 1 hour, 1 mm tissue blocks were dissected and post-fixed for 1 hour with 1% OsO_4 in sodium phosphate buffer. Tissue was embedded in Epon, sectioned in silver to grey range (about 50 - 75 nm) and stained with uranyl acetate, and lead citrate.

Preliminary results using this technique indicate that 1) High Mg^{+2} perfusion does appear to result in fixation in which a higher packing density of vesicles is present in presynaptic terminals. There is a tendency in some, but not all cases, for vesicles to be aggregated in the center of the terminal (away from membrane other than the synaptic apposition). Whether absolutely greater numbers of vesicles per terminal are present with Mg^{+2} perfusion remains unclear (quantitative studies are in progress). 2) 110 mM Mg^{+2} appears to be sufficient concentration for increased vesicular packing density. 3) With higher concentrations there are some indications of increased extracellular space and/or shifts of cytoplasmic structures other than vesicles, but the aggregation of vesicles seems not to be accounted for in terms of shrinkage of presynaptic terminals. Work is in progress to evaluate the effects of a wider range of Mg^{+2} concentrations as well as chelating agents and other divalent ions.

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1437 THE CELLULAR AND SYNAPTIC ORGANIZATION OF THE INTERMEDIOLATERAL NUCLEUS OF THE RAT SPINAL CORD: A COMBINED FM AND EM STUDY. Frank D.H. Chen*, Jean Y. Jew and Terence H. Williams, Dept. Anat. Univ. Iowa Col. Med., Iowa City, IA 52242

The intermediolateral nucleus (IMLN) of the thoracic cord in the rat was studied by using fluorescent and electron microscopic techniques. As visualized with the fluorescence microscope, the IMLM somata primarily are surrounded by dense axonal varicosities and terminal boutons. At regular intervals these somata aggregate to form "islands". These aggregations are bridged by fluorescent preterminal fibers and presumed dendritic processes. At an early stage (2½ days) after animals were treated with 6-hydroxydopamine (6-OHDA) or 5,7-dihydroxytryptamine (5,7-DHT with desipramine pretreatment), those varicosities surrounding somata became less dense, and the preterminal fibers bridging the aggregated cell bodies were usually interrupted or less dense.

As visualized with the electron microscope, the IMLN somata have a fusiform shape, a high nucleus to cytoplasm ratio, an extremely light nuclear chromatin, and a paucity of cytoplasmic organelles. Dendritic elements are long and intertwine among unmyelinated axonal bundles in a parallel and longitudinal fashion. This longitudinal arrangement of axonal and dendritic plexuses and terminals demarcates the IMLN zone and is a characteristic feature of this nucleus.

The synaptic arrangement in the neuropil is mainly axo-dendritic which could consist of one axonal terminal contacting several dendrites or vice versa. There are also axo-somatic,axo-spinous, and axo-axonic contacts, but these are comparatively rare. Sometimes these contacts form serial synapses. In general, approximately 60% of the terminals in the IMLN form morphologically identifiable synaptic junctions.

The majority of monoaminergic terminals in the IMLN, which are very similar to those reported in other areas of the CNS,contain large granular vesicles (about 900 Å) and numerous small, round, clear visicles (about 500 Å). If 5-OHDA was injected, small, round, clear vesicles in some boutons were tagged and thus appeared electron dense.

At an early stage (2½ days) after 6-OHDA or 5,7-DHT treatment, terminals in the IMLN showed morphological changes. In the selective administration of 6-OHDA these changes included (1) a reduction in the number of synaptic vesicles, (2) the presence of degenerated electron dense elements, and (3) the increasing presence of dense granules in vesicles within some terminals similar to the appearance after 5-OHDA. Dense granules were visible also in small vesicles (about 500-650 Å) found in neuronal cell bodies and processes. It is inferred that some neurons in IMLN are monoaminergic in nature.

1438 NERVE FIBERS IN THE IMMATURE SHEEP THIRD VENTRICLE. Penelope W. Coates and Steven L. Davis*. Dept. Anat., Texas Tech Univ., Sch. Med., Lubbock, TX 79430 and Dept. Animal Sci. Univ., Idaho, Moscow, ID 83843.

The third ventricle of normal ewe and ram lambs (four - six months of age) was examined by scanning (SEM) and transmission electron microscopy (TEM). SEM of the ventrolateral floor (VF) revealed that tanycytes were covered predominantly by microvilli in the ewe lamb. The ram lamb VF had a more patchy appearance due to fewer surface features on some cells. Distinct fibers were not observed on the VF. The dorsolateral wall (DW) was thickly ciliated. Underlying ependymal surfaces could not be observed except in places where the cilia were parted, revealing a surface studded with microvilli and occasional protrusions. Even in these more exposed areas, SEM images which might be interpreted as nerve fibers on the DW were rare. Yet when the same regions on the opposite side of the third ventricle were examined with TEM many nerve profiles were revealed along the ventricular surface of the DW under the canopy of cilia. These small caliber unmyelinated nerve fibers contained small clear vesicles, occasional small dense core vesicles, neurotubules and mitochondria, and were interpreted as axons seen in section. Occasional attachment plaques between axons and the ependyma, and microvilli partially wrapped around some nerve fibers were noted. Single fibers as well as groups of fibers suggesting plexus-type organization were present. Possible myelinated fibers were rare. A noteworthy, though rare finding under the DW cilia were large dendrite-like profiles interpreted as sensory endings filled with mitochondria, neurotubules and other organelles. In comparison to the abundance of nerve fibers along the DW, TEM of the VF revealed few nerve fibers, although similar fine caliber unmyelinated nerve fibers were found close to the lumen of the third ventricle in spaces between tanycytes of the VF. Few such fibers were observed between ciliated ependymal cells of the DW. Taken together these data suggest the following: Nerve fibers, both axons and possible dendrites, are present in the third ventricle of immature sheep. There appears to be regional variation in the distribution of intraventricular fibers. Cilia tend to obscure their detection with SEM on the DW. These new observations in sheep extend previous observations on the presence of intraventricular nerve fibers in mammals. Of special note are possible dendritic sensory terminals. The apparent scarcity of nerve fibers on the VF of lambs contrasts with SEM and TEM data on the monkey and other species. The origin, destination and role of intraventricular nerve fibers (axonal or dendritic) in the immature sheep third ventricle remains to be determined. (Supported by USPHS Grant HD 12833 from the National Institutes of Health.)

1439 PERSISTENCE OF BASAL LAMINA DEFECT IN CULTURES OF DYSTROPHIC MOUSE SCHWANN CELLS IN CONTACT WITH NORMAL MOUSE NEURONS. M. Cochran,* C. Cornbrooks, F. Mithen,* AND R. P. Bunge. Dept. Anat. & Neurobiology, Wash. Univ. Sch. Med., St. Louis, Mo.

Dystrophic mice (C57 BL dy 2J/dy 2J) have patchy discontinuities of the basal lamina of Schwann cells in the dorsal and ventral roots and the sciatic nerve (Madrid et al., '75). This abnormality is expressed in organotypic cultures of sensory ganglia from newborn dystrophic mice (Okada et al., in preparation), and also in cultures containing only Schwann cells and neurons. Basal lamina production by cultured Schwann cells ceases when neurites are removed, and resumes when the axon-Schwann cell relationship is restored (Williams et al., '76). The basal lamina defect in the dystrophic mouse could therefore be the result of an abnormality of the Schwann cell and/or the neurite.

To determine which of these possibilities exist, cell recombination experiments were carried out in tissue culture. Dorsal root ganglia from newborn dystrophic and normal C57 BL mice were explanted into collagen-coated Aclar dishes. Using methods adapted from Wood ('76), we were able to produce cultures containing only neurons (N-DRG) and cultures containing only neurons and Schwann cells (SN-DRG). Small patches of the substrate with Schwann cells from the SN-DRG cultures were transferred to neuritic regions of the N-DRG cultures. Such recombinant groups included: 1) dystrophic Schwann cells onto normal neurites, 2) normal Schwann cells onto dystrophic neurites and 3) dystrophic Schwann cells onto dystrophic neurites.

By five weeks after transplantation, Schwann cells had proliferated and migrated to occupy a substantial region of the neuritic outgrowth. At this time, the cultures were fixed and examined by electron microscopy. Schwann cells derived from dystrophic mice had a characteristic patchy basal lamina when related to either normal or dystrophic neurites. Schwann cells derived from normal mice produced a thick, uniform basal lamina when cultured on dystrophic mouse neurites. The basal lamina abnormality in tissue culture is therefore an expression of an abnormality of the dystrophic Schwann cell rather than of the neurite.

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1440 SUPRAEPENDYMAL AND EPIPLEXUS CELLS IN RODENTS. Perry Cohn (SPON: Ruth Bleier). Dept. Neurophysiol., Univ. Wis., Madison, WI 53706

Abundant monocyte and macrophage-like supraependymal (SE) and epiplexus cells were observed with transmission and scanning electron microscopy in neonatal rats, mice and hamsters. In white Holtzman rats, which were most extensively studied, their numbers are elevated from 3-20 days of age. Some display microprocesses, ridges and ruffled and spreading lamellipodia, others have smoother surfaces and lengthy, branching pseudopodia and still others are rounded. Their ultrastructural characteristics include an extensive and varied group of lysosome-like organelles, a prominent Golgi zone, a nucleus possessing clumped, marginated chromatin and often a number of lucent 500-2000 nm vacuoles. Cilia are often deeply invaginated into SE cells. Occasional cells resemble lymphocytes and ependymal cells.

The macrophage-like SE cells are usually found in the relatively unciliated areas of the ependymal walls. The remaining descriptions are of the well studied third ventricle. In the fetus and the newborn SE cells can be seen throughout the ventricle, which at this time is mostly unciliated. In suckling rodents more than a few days old, the relatively unciliated area is restricted to an area roughly overlying the hypothalamic ventromedial, infundibular and premamillary nuclei and harbors about 75-100 SE cells per side. Most are found rostrally of caudally in this area. In adult rats the few SE cells (10-20 per side) congregate near the border between the ciliated and unciliated ependyma.

In suckling rats macrophage-like cells are also associated with capillaries that cross the ventricular lumen and with clusters of cells on the floor of the ventricle. The ultrastructure of the clustered cells includes a euchromatic nucleus, a prominent nucleolus, an active Golgi zone, a smooth surface and occasional synaptic endings on their surface. They are often embedded in a mat of fibers. As many as three separate clusters may be present.

The ependymal cells overlying the infundibular nucleus display many rounded excrescences (commonly known as blebs) in suckling female rats over 2 days old. Some male rats 3 to 6 days of age also have blebs. In adult rats preliminary investigation of the estrus cycle revealed little predictable variation. SE cells are not observed particularly over ependyma displaying blebs, though they are usually located nearby.

1441 NEUROSECRETORY TRACT IN THE CRICKET. William R. Colquhoun* SPON: R. Oesterreich, Biology Department, SUNY at Albany Albany, New York 12222

This communication describes the morphology of a neurosecretory tract in the cricket having exceptional properties for experimental study. A small (17μ) nerve emerges from the anterior end of the last abdominal ganglion in the cricket, *Acheta domesticus*. This nerve courses anteriorly between paired connectives and inserts into the next to last ganglion between connectives. Two-thirds of the way along this tract two branches emerge and extend laterally. These branches ramify just short of the cuticle and may function in spiracle opening.

Electron microscopy shows that this nerve contains approximately 18 axons most of which are densely filled with neurosecretory vesicles. Because of the great accumulation of vesicles in this nerve, it is highly refractile and easily distinguished from other nerves by phase contrast optics. Cobalt backfills have localized 11 of the cell bodies contributing axons to this tract from the last abdominal ganglion. These occur in three groups of three soma each lying along the anterior dorsal and anterior ventral mid-line. Two other cell bodies are laterally disposed. One group of three cells occurs at the point of departure of the connectives from the ganglion and should be easily sampled by electrode and E.M. sectioning techniques. Cobalt fills have also identified four cells in the next to last ganglion which contribute posteriorly coursing axons to this tract. The extraordinary accumulation of neurosecretory vesicles in this nerve (not just its endings) and the ability to easily locate several of the neurosecretory cell bodies makes this system especially amenable to experimental study.

1442 MORPHOMETRIC DETERMINATION OF MEMBRANE DENSITIES IN NORMAL AND 'GLIAL' OPTIC NERVES OF NECTURUS. Mary K. Dolack*, C-M. Tang* and Paula M. Orkand* (SPON: R.K. Orkand). Dept. Physiol. & Pharmacol., Sch. Dent. Med., Univ. Pennsylvania, PA 19104.

To measure the density of membrane sodium channels with radiolabelled saxitoxin and specific electrical properties of glial membranes one requires a determination of membrane areas of axons and glial cells. Intersection counting morphometry was used to determine axon and glial membrane densities from electron-micrographic montages of entire cross sections of Necturus optic nerves. Normal nerves and ones in which all the axons had degenerated due to enucleation of the eye 2 months previously were studied.

In 8 normal nerves, the cross sectional area was 5600 μ^2 (range: 3000 - 7700 μ^2). Axon diameters ranged from 0.2 - 2.0 μ. The cellular volume was 61% axon and 39% glia. Axonal membrane densities in the whole normal nerve were about 5 μ/μ^2 while those of glia were about 2 μ/μ^2. Following enucleation the cross sectional area decreased to 4500 μ^2 (range: 2500 - 5900 μ^2) and the density of glial membrane increased about two-fold.

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1443 DOUBLE-WALLED COATED VESICLES IN MITRAL CELL BODIES OF THE MOUSE OLFACTORY BULB: INCREASED NUMBERS DURING POSTNATAL DEVELOPMENT. Maryellen F. Eckenhoff and J.J. Pysh. Department of Anatomy, Northwestern Univ. McGaw Medical Center, Chicago, Ill. 60611

Previously, we have found a massive and transient increase in the formation of double-walled coated vesicles (DWCV's) from surface membranes of the cerebellar glomerulus during late postnatal development in the mouse.[1] In an attempt to determine whether this was specific to the cerebellum, or represented a more general phenomenon during brain development, we have examined another brain region. Since similar structures were reported in adult mitral cell bodies of the olfactory bulb we examined these cells. The purpose of this study was to describe the distribution and frequency of occurrence of DWCV's in the mitral cell bodies during postnatal development.

Mice were perfused through the left ventricle with dilute Karnovsky's fixative at 1, 5, 10, 16, 20, 26, 37 and 70 days of age. The olfactory bulbs were removed and prepared for electron microscopy.

We frequently observed DWCV's in random electron micrographs of immature mitral cell bodies. DWCV's consist of a coated invagination of the plasmalemma of the mitral cell body and an inner evagination of the plasmalemma of either an adjacent granule cell axon or mitral cell body. Also, coated invaginations of the plasmalemma of the granule cell axon were observed with inner evaginations of the plasmalemma of the adjacent mitral cell body. In addition, DWCV's were observed free in the cytoplasm of the mitral cell bodies and granule cell axons. A morphometric analysis revealed that the highest numbers of DWCV's were found at 37 days, (.055 DWCV's per μ^2 of mitral cell cytoplasm), some 23 fold more than at 1 day and 8 fold more than at 70 days of age. A lesser peak was found at 20 days, (.032 DWCV's per μ^2 of mitral cell cytoplasm), some 13 fold more than at 1 day and 5 fold more than at 70 days of age. In an attempt to determine whether there was a relationship between the occurrence of DWCV's and synaptogenesis, the number of synaptic terminals per μ of mitral cell body were counted. We found the number of synaptic terminals on the mitral cell bodies reached adult levels by the 20th day. The higher frequency of occurrence of DWCV's at 20 and 37 days of age follows synaptogenesis, as we observed in the cerebellum.

Thus, DWCV's are present in highest numbers during late postnatal development in at least two brain regions. It is possible that the formation of DWCV's may provide a mechanism for removal of specific surface constituents present on immature membranes. (Supported by NIH Grants #NS 10657 and 11325.)

[1] M.F. Eckenhoff and J.J. Pysh, J. Neurocyt. (in press) (1979).

1444 THE MONKEY CLAUSTRUM: AN ELECTRON MICROSCOPIC ANALYSIS Lawrence R. Edelstein and Frank J. Denaro*. Dept. of Psychol., SUNY At Stony Brook, Stony Brook, NY 11794.

While the claustrum has been studied for several years on both a light- and electron-microscopic level in several mammals, there is in fact a paucity of research in terms of the monkey claustrum. Of late, the use of HRP has aided investigators in the study of the connections of the monkey claustrum (Riche & Lanoir, JCN 177: 435, 1978). However, to our knowledge, the only published EM study done previous to this report on the monkey claustrum was carried out by Norita (Acta Anat. Nippon. 49: 47, 1974), who gave a brief report on the claustrum of Macaca irus. Therefore, we offer the following preliminary report on our study of the claustrum of Macaca arctoides.

While under deep Nembutal anesthesia, the subject was initially perfused with physiological saline at body temperature, followed by a second perfusion with a cold (~3°C) mixture of 2% EM Grade glutaraldehyde and 1% paraformaldehyde in a 0.12M phosphate buffer (pH 7.4). Postfixation of tissue cores from the body of the claustrum (taken with the "punch" technique using a glass pipette) was carried out with a 2% osmium tetroxide solution in 0.12M phosphate buffer (pH 7.4) and allowed to sit overnight. Following ethanol dehydration, the cores were flat-embedded in Spurr's resin and ultrathin sections were stained with uranyl acetate and lead citrate. Sections were viewed with a JEM 100B electron microscope.

The predominant cell type in the sections studied was slightly elongated (~10μm), displaying a large invaginated nucleus (containing chromatin clumps) with a thin perikaryon containing poorly organized ribosomes and a fair number of round and elongated mitochondria. Axo-somatic synapses were noted.

With respect to synaptic morphology, axo-somatic, axo-dendritic and axo-axonic synapses were observed, with a predominance of the axo-dendritic variety. Most of these were of the asymmetric type, and contained clear, spherical vesicles of a regular diameter (~600Å).

1445 AN EM AND HVEM STUDY OF NEURONS AND SYNAPSES IN THE FELINE DORSAL COLUMN NUCLEI. Leland C. Ellis, Jr. and Aldo Rustioni, Depts. of Anatomy and physiology, UNC, Chapel Hill, N.C. 27514

Previous EM studies on the synaptic organization of the feline DCN have focused on the identification of terminals of afferent projections, e.g., primary, cortical, and non-primary afferents (Rustioni and Sotelo, 1974; Rustioni and Ellis, 1978). As part of an ongoing study of the intrinsic organization of the DCN, the present study has concentrated on the identification and characterization at the ultrastructural level of the cell types in the "clusters region" of the feline DCN.

Light microscopical studies (Ellis et al, 1979) have demonstrated that greater than 90% of cells in the clusters are labelled with HRP following injection of HRP (30%, Boehringer) in the contralateral ventrobasal complex. For EM, cats with such an injection were perfused with mixed aldehydes, and 40um vibratome sections of the caudal medulla were reacted with Hanker-Yates substrate, osmicated, dehydrated in graded ethanols, and wafer-embedded in Spurr (1969). In thin sections (60nm) examined at 80kv, and in thick sections (.25um) at 1Mev (at the HVEM facility at Madison, Wisconsin), the thalamic relay neurons (TRN) labelled with HRP are typically round, average approx. 25um in diameter, and have a round nucleus with a small nucleolus. The HRP reaction product is localized in membrane bound, lysosome-like, organelles in the cytoplasm surrounding the nucleus. Profiles labelled with HRP, and interpreted as dendrites of TRN, were also seen in .25um sections at 1Mev. Stereo pairs demonstrate that these processes are postsynaptic to large terminals. In animals after dorsal rhizotomy, examination of serial .17um sections at 1Mev using computer reconstruction techniques demonstrates that these large terminals are primary afferent terminals distributed along the surface of the dendrites of TRNs.

The neurons unlabelled after HRP injection have distinct cytological characteristics. They are located at the periphery of clusters of HRP-labelled TRNs. They are typically smaller (average approx. 10um in diameter) than the TRN, fusiform, and have a nucleus that is often highly indented or lobulated. Membrane specializations, identified as puncta adherentia in thin sections and in stereo pairs of thick sections at 1Mev, have been observed between the TRN and these small cells. These small cells are also found away from the clusters, surrounded by bundles of myelinated fibers. For their cytological characteristics, these small cells are identical to the neurons identified in a LM study using HRP and Golgi techniques as interneurons (Ellis et al, 1979). Further study of these cell types and their synapses, using EM-Golgi techniques, is currently in progress. Supported by USPHS NS 12440 to A.R. and NIH RR-570 to the HVEM facility at Madison, Wisconsin.

1446 ASTROCYTE FORM IN HUMAN CEREBRAL CORTEX. Martin L. Feldman. Dept. of Anat., Boston Univ. School of Med., Boston MA 02118.

The purpose of the present study was to examine, in rapid Golgi preparations, the form of astrocytes in human temporal neocortex and hippocampus. Six blocks from 3 epilepsy patients (ages 22-35) were surgically removed and placed, within 60 sec, in EM fixative. Although in one patient histopathological evidence of a low-grade astrocytoma was present in the amygdala, the Golgi findings were similar in all 6 blocks. The major findings were also confirmed in a separate surgical block from human frontal cortex and in normal EM-perfused macaque area 17 and hippocampus.

The two major astrocytic forms encountered are radiate (R) and shrubby (S). R astrocytes have numerous (15-100/cell) primary processes, each ~50μm in length, which radiate out in relatively linear fashion from the soma. Branching is rare. These processes are rather heavily studded with small spinelike and irregular excrescences and usually taper to pointed tips. A small number of them, however, which frequently extend somewhat beyond the typical primary process length, give rise to distal fanlike endfeet about 6-10μm in diameter and of highly irregular form. In addition, a small number of primary R processes continue as long (up to ~ 750μm), radially oriented axon-like processes (ALPs). ALPs are unbranched, lack excrescences, and terminate distally in small (2-4μm) end knobs. S astrocytes have discrete spherical territories, ~ 100μm in diameter. Primary processes branch frequently and form thick perisomatic tangles. The processes are quite densely studded with excrescences and terminate bluntly. A small number of S processes, of somewhat greater length, bear endfeet similar to those of R cell primary processes. ALPs are lacking.

Both R and S forms are found in white and grey matter and at the pial surface, where many R cell ALPs course in a surface-parallel orientation and contribute to the glia limitans. In all cases, ALP knobs and R and S endfeet are in contact with vascular walls; the remaining primary processes do not typically exhibit this relationship. R cell ALPs commonly project to distant vessel segments which are very much closer to other R and S perikarya. While both R and S primary processes occasionally display a diffuse red margin, this lacks the multilocular character and definitiveness of the velamentous astrocytic appendages seen in macaque cerebellum. For these and other reasons, such cells are not considered to be the velate-type astrocytes of Palay & Chan-Palay.

A final astrocytic form, seen only with extreme rarity, corresponds to the classical smooth protoplasmic astrocyte. These cells have thin branched processes with numerous kinks. (supported by NIH Career award AG00016 and NIH grant AG00001).

1447 CLASSIFICATION OF SYNAPSES IN ELECTRON MICROGRAPHS BY MORPHOMETRY AND COMPUTER ANALYSIS. Victor L. Friedrich, Jr. and Gary E. Korte*. Dept. Biobehavioral Sci., Univ. of Connecticut, Storrs, CT 06268

We recently identified three types of synaptic boutons in cat superior vestibular nucleus, based on our impressions of their synaptic vesicles. Unilateral destruction of the vestibular ganglion resulted in loss of one type ipsilateral (lesioned side) but not contralateral (control side) to the lesion. In addition, we measured synaptic vesicles and found that the three putative types differed.

We have since analyzed the data on synaptic vesicles with two widely available statistical 'package' programs which perform classification of cases from numerical data: DISCRIMINANT, Statistical Package for the Social Sciences; and BMDP2M, cluster analysis, Biomedical Computer Programs.

The mean size and some other statistics on the vesicles in each bouton were entered to the discriminant program. In the 'training' phase, the program compared the three groups, as defined by us; in the 'classification' phase, each ending was reclassified solely from the vesicle data and without reference to our subjective label.

Agreement between our classification and that of the discriminant program program was better than 95%. When trained on the control side data only, the program repeated to 90% agreement our tabulation of the endings on the lesioned side and confirmed the preferential loss of the putative primary afferent type.

The cluster program associated the endings into a hierarchy of progressively larger and more heterogeneous groups; the pattern agreed with our subjective classification and indicated the presence of subclasses which we had not detected visually.

The discriminant analysis described here required the prior imposition of subjective labels to each ending, an undesirable feature. Subjective labeling may be unnecessary when techniques such as axonal transport, intracellular injections or cytochemistry are employed.

Our experience shows that these two programs, which are examples of statistical pattern recognition analysis, can classify synapses as well as human observers, when given good data. Furthermore, even in our apparently simple paradigm, one program detected patterns unnoticed visually. The approach of morphometry and statistical pattern recognition analysis may be generally useful in the analysis of synaptic connections by electron microscopy.

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1448 REVERSIBLE DEPLETION OF SYNAPTIC VESICLES INDUCED BY A SINGLE-GENE MUTATION OF DROSOPHILA MELANOGASTER. Kazuo Ikeda and Kogaku Saito*. Div. of Neurosciences, City of Hope National Medical Center, Duarte, CA 91010, USA.

It has been shown that a reversible, temperature-dependent blockage of neuromuscular transmission occurs in the single-gene mutant, shibire^{ts1} (shi^{ts1}). The neuromuscular junction of the dorsal longitudinal flight muscle was observed by electron-microscope. shi^{ts1} and the control, wild-type Oregon-R, were raised at a constant temperature (17° C). Four-day old adult flies were dissected in a phosphate buffer and fixed in a para-formaldehyde-glutaraldehyde mixture. The temperature was under precise control during these procedures. Electronmicroscopy revealed the following results: At 19° C, where normal transmission is confirmed by observing the postsynaptic potential, both shi^{ts1} and Oregon-R showed normal-looking synapses with clear vesicles gathered around the presynaptic dense body. The mitochondria, pre- and postsynaptic membrane, and other structures also appeared normal in both shi^{ts1} and Oregon-R. At 29° C, which is the critical temperature where complete disappearance of postsynaptic potentials occurs in shi^{ts1}, the shi^{ts1} synapse showed almost complete depletion of vesicles; and in addition, many cisternae-like structures appeared. The Oregon-R synapse remained the same as at 19° C. In order to see the reversibility of the temperature effect, shi^{ts1} was exposed to 29-30° C for 20 min. prior to dissection. The dissection and fixation were then done at 19° C. The structure of the shi^{ts1} synapse was returned to normal, i.e., the cisternae-like structure disappeared and the vesicles reappeared. Since high temperature (27-29° C) causes motor output to this muscle in shi^{ts1}, the depletion of vesicles might be caused by temperature-induced synaptic input. To determine this, the nerve innervating this muscle was cut before exposure to high temperature. The results were the same as those obtained with the nerve intact. Thus, the depletion is a direct result of the mutation on the presynaptic terminal, rather than the result of presynaptic activity. The above observations coincide with our previous physiological finding that the neuromuscular transmission of shi^{ts1} is reversibly blocked by high temperature (29° C). The physiological blockage of transmission may be attributable to the reversible depletion of synaptic vesicles.

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1449 A COMPARISON OF THE SHORT AND LONG TERM EFFECTS OF AXOTOMY ON THE FACIAL, VAGAL AND HYPOGLOSSAL NEURONS OF THE ADULT HAMSTER. S.K. Jacob and T.E. Durica. Dept. Anat., Rush Coll. Health Sci., Chicago, IL 60612.

The purpose of this study was to observe the cytology of the axon reaction of different cranial neurons in the adult hamster. In one group of animals the right facial and hypoglossal nerves were severed, while another group of animals underwent a right vagotomy. The left nerves remained intact to serve as a control. Animals were allowed to survive 5, 30 and 60 days postoperative (dpo). Following cardiac perfusion brain stems were double-embedded, serially sectioned, and stained with buffered thionin. Histological examination at all dpo times revealed cytological differences between the three neuronal groups.

Facial motor neurons showed classical signs of chromatolysis at 5 dpo. By 30 dpo the cells were hyperchromic due to heavy clumping of the Nissl substance, however, their overall somal and nuclear appearance resembled those of the normal side. Some of the nucleoli were seen to have a normal appearance at this time. Facial neurons were normal at 60 dpo though slightly hyperchromic.

Hypoglossal neurons exhibited central chromatolysis at 5 dpo. By 30 dpo a variety of responses could be found in the cells. Most neurons were hyperchromic though they had a normal configuration. Some cells were irregular in shape with a dark, finely granular cytoplasm, while others continued to show a central chromatolysis. No normal nucleolar pattern could be found in any of these cells. Only the first two types of responses were seen at 60 dpo, no chromatolytic cells remained.

Vagal neurons displayed a central type of chromatolysis at 5 dpo, however, their nucleoli were normal in appearance. By 30 dpo neurons continued to show a pale cytoplasm with a highly irregular outline, also the nucleolar appearance was not normal at this time. There was a marked neuronal cell loss by 60 dpo; the few remaining large neurons were swollen with a pale cytoplasm and an eccentricly placed nucleus.

While the short term response is similar in all 3 groups of neurons, the long term cytological changes are markedly different. The differences in the axon reaction seen in this study reflect the ability of the different cells to recover from the stress of injury. Variations in the cytological aspects of this response may indicate differences in the metabolic state of these neurons at the time of their injury.

1450 AN IMMUNOHISTOCHEMICAL METHOD TO DEMONSTRATE PIA-GLIAL BASAL LAMINA ON LIGHT MICROSCOPIC SECTIONS. Tim F. Kowalski, Earl R. Feringa and H. Lee Vahlsing*. Depts. Neuro. and Path., V.A. and Univ. of Mich. Med. Ctrs., Ann Arbor, MI 48105.

The basal lamina (BL) at the pia-glial interface is an ultramicroscopic structure which may be a guide or an impediment to axonal regeneration in the central nervous system (CNS). Existing staining methods for pia-glial BL, (P.A.S. and immunofluorescent antibody stains) are inappropriate for permanent light microscopic preparations. We have developed a staining technique for epithelial BL which is highly specific, extremely sensitive, permanent, relatively inexpensive and is suitable for light or electronmicroscopy (EM).

The basement membrane (BM) from CNS tissue of isogeneic female albino Wistar rats was isolated by the technique of Meezan(1975). Using this BM preparation as an antigenic source, we developed a hyperimmune serum. This serum was exhaustively absorbed on rat splenic pulp to remove undesirable antibodies to endothelial BL and collagen. Using the PAP indirect antibody staining technique, we tested the specificity of this splenic absorbed (SA) serum to various tissues. The following table lists the results of our staining specificity checks. These results indicate that this BL staining technique is specific for epithelial BL of the rat and of some other species.

PURPOSE	SERUM	TISSUE	BL STAINED
positive control	crude	rat spinal cord	endothelial mesodermal
			epithelial ectodermal
	SA	rat spinal cord	epithelial ectodermal
negative control	SA*	rat spinal cord	none
	SA**	rat spinal cord	none
specificity for epithelial BL	SA	rat spleen	none
	SA	rat lens capsule	epithelial ectodermal
specificity for ectodermal BL	SA	rat tongue	epithelial ectodermal
	SA	rat esophagus	epithelial entodermal
	SA	rat sciatic nerve	epithelial ectodermal
interspecies crossreactivity	SA	mouse, monkey, dog spinal cord	epithelial ectodermal
EM stain localization	SA	rat spinal cord	epithelial ectodermal

* goat anti-rabbit globulin step omitted during staining
** SA adsorbed on rat CNS BM antigen

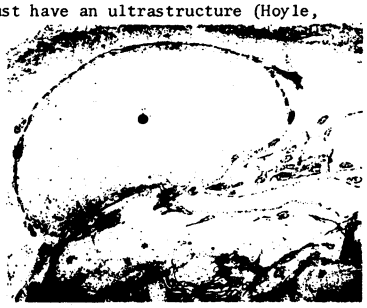
1451 ULTRASTRUCTURE OF THE SOMA OF AN IDENTIFIED DOPAMINE-CONTAINING NEURON OF THE SPINY LOBSTER CNS. Pinky Drosten Kushner and Eric Schabtach*. Dept. of Biology, U. of Oregon, Eugene OR 97403.

Each commissural ganglion of the spiny lobster contains a large (150 μm) neuron, readily identified by its characteristic somal position and primary neurite projection within the ganglion. This cell exhibits catecholamine histofluorescence (Kushner and Maynard, Br.Res.129 13, 1977), synthesizes dopamine from labeled tyrosine (Barker, Kushner and Hooper, Br.Res.161 99, 1979), and contains measurable amounts of dopamine (0.31 pmoles) (Kushner and Ono, Neurosci.Abs.4 610, 1978). We have examined its ultrastructure seeking possible unique morphological features.

The cell is characterized by dense cytoplasm with closely packed rough and smooth endoplasmic reticulum, in random array. There are numerous Golgi (~8 Golgi/10 μm^2), in close proximity to which are vesicles of varying sizes and densities, with many coated vesicles on the concave side. Multivesicular bodies are common with inclusions of small irregular vesicles of varying densities. Although the fixation we used was designed to emphasize densely staining vesicles associated with biogenic monoamines (Wood's fix as modified by Friend, Cell Tiss. Res. 175 369, 1976), we see few typical dense-cored vesicles, either Golgi associated or cytoplasmic.

Near parallel swirls of microtubules concentric to the nucleus funnel into the large primary neurite. The nucleus is small (25 μm) relative to the size of the soma and possesses a smooth unindented nuclear membrane.

This somal architecture is very different from that of the identified molluscan dopamine neuron (Anodonta: Zs.-Nagy in Neurobiology of Invertebrates, ed. Salanki, 1968; Planorbis: Berry in Biochemistry of Characterized Neurons, ed. Osborne, 1978) where granular vesicles are abundant. Likewise the octopamine neurons of locust have an ultrastructure (Hoyle, J. Neurobiol., in press) more similar to the molluscan dopamine neuron than to this lobster dopamine neuron. The paucity of densely precipitating vesicles is a feature shared with the presumed dopamine neuronal somata of the vertebrate substantia nigra. (Hokfelt and Ungerstedt, Br. Res. 60 269, 1973).



1452 POTASSIUM FERRICYANIDE: AN IMPROVEMENT IN NEURAL FIXATION Lauren A. Langford and Richard E. Coggeshall. Departments of Anatomy and of Physiology and Biophysics, The Marine Biomedical Institute, The University of Texas Medical Branch, Galveston, Texas, 77550.

After most electron microscopic fixation procedures, the myelin sheath appears frayed and the gyres of myelin often appear to be artificially separated in many places. This problem is particularly difficult for central myelin. In addition, numerous randomly distributed unmyelinated axons in peripheral nerves and spinal roots often show swellling or marked indentations. In our experience, the use of potassium ferrocyanide, as recommended by Karnovsky (1971, Abst. Am. Soc. Cell Biol., p. 146) helps alleviate these problems and in addition improves the contrast of membranes. The difficulty with the ferrocyanide procedure is that many cytoplasmic organelles such as ribosomes are not well preserved and the chromatin pattern of nuclei seems altered for the worse. Thus the ferrocyanide mixture is very useful for certain problems but probably cannot be used as a general procedure. Recently, however, we have found that the addition of potassium ferricyanide to the osmic acid postfixation solution has many of the virtues of the Karnovsky ferrocyanide procedure but seems to preserve the cytoplasm and nucleus like the regular fixation methods. If 1.5% potassium ferricyanide is placed into the buffered osmium tetroxide postfixation fluid, the number of frayed myelin sheaths and poorly fixed unmyelinated axons is reduced. There is usually a great improvement in the appearance of the tissue at the light microscopic level and a significantly decreased number of "bad" myelin sheaths, "swollen" axons, "blown" mitochondria, etc., and the contrast of the tissue is usually enhanced. It is to be emphasized that the most important varibles in the quality of the finished pictures are still such things as the success of the perfusion, but in our experience, the addition of potassium ferricyanide to the osmium tetroxide solution makes good fixation better and allows some data to be obtained from otherwise hopeless material. Pictures to show the differences between 1) regular, 2) ferrocyanide, and 3) ferricyanide fixation procedures will be presented. This work is supported by NIH grants NS 07377 and NS 10161.

1453 OBSERVATIONS ON THE FINE STRUCTURE AND CONNECTIONS OF THE AREA SUBPOSTREMA OF THE CAT. R. A. Leslie and D. G. Gwyn. Dept. Anat., Sch. Med., Dalhousie Univ., Halifax, N.S., Canada B3H 4H7.

The area subpostrema (ASP), corresponding to the parvocellular and gelatinous parts of the nucleus of the solitary tract, has been examined with the electron microscope. In some cases the nodose ganglion was removed unilaterally several days prior to microscopy. The neuronal complement of the ASP was seen to consist of a population of a single type of small cell bodies, about 10μm in diameter. Nuclear membranes were very deeply indented and were surrounded by an often attenuated perikaryon containing highly irregular arrays of rough endoplasmic reticulum with no regular parallel cisternae. Surrounding these somata were narrow flanges of astrocyte-like cells which often were linked to each other with gap junctions. The associated glial cell bodies were usually closely apposed to the neuronal somata. Many bundles of fine, unmyelinated axons coursed through the ASP usually in a rostro-caudal direction. Typical axo-dendritic and axo-somatic asymmetric synapses were seen to contain either round, clear vesicles or pleomorphic vesicles. Some round vesicle-containing terminals also contained a few large (80nm diameter) dense-cored vesicles. Dendo-dendritic and dendro-somatic synapses were occasionally seen and contained only pleomorphic vesicles. Very rarely an axo-axonal synapse was seen associated with a terminal containing round, clear vesicles. Degenerating terminals of vagal origin contained round, clear vesicles together with occasional large dense-cored vesicles. These terminals seemed to make up only a small percentage of the axo-dendritic synapses of the ASP and occurred singly throughout the substance of the area. Some of these were undoubtedly of gastric origin since the ASP has been recently shown to receive afferent fibres from the wall of the stomach (Gwyn, Leslie and Hopkins, 1979).

1454 AXOTOMY INDUCED LONG TERM NUCLEOLAR ALTERATIONS. L. Kirschen McLoon and A. LaVelle., Dept. Anat., Univ. Ill.-Med. Center, Chicago, IL 60612.

Changes in nucleolar structure, a primary neuronal response to axotomy indicating alteration in RNA and protein metabolism, have been especially well characterized in adult hamster facial neurons. An intranucleolar body (INB), composed of ribonucleoprotein, is present within the normal nucleolus of most of these neurons. The formation of the INB, the final step in nucleolar cytomaturation, occurs well after adult somal size and Nissl body configuration are present. Axotomy causes dispersion of the INB in the adult and prevents its normal formation in development. This study examined the long term effect of injury to developing neurons on their nucleolar morphology as seen with the light microscope.

INB formation was examined after either ligature/axotomy or crush performed at 15 days postnatal age before the INB had formed, at 20 days when the INB was just beginning to form, and at 25 days when the mature nucleolus was present. Animals were sacrificed at 4, 10, 15, 30, 50, 100 and 200 days after injury.

For all operative ages, crush injury resulted in complete INB return by 50 days. By 200 days INB return was still at control levels. With regeneration, the state of nucleolar maturation at the time of operation did not seem to be a decisive factor in eventual INB return after crush injury.

Although both types of injury extended the normal timetable for nucleolar maturation, lig./axotomy at 15 days postnatal age drastically delayed the formation of the INB. Even by postoperative day 200 the number of INBs were significantly depressed on the injured side. Thus, lig./axotomy, and the concomitant lack of reconnection, affected the long term capability of the youngest neurons to recover. This appeared dependent on the stage of nucleolar maturation at the time of injury. In contrast, after lig/axotomy at 20 and 24 days, the normal nucleolar configuration was attained by postoperative day 100 and maintained at 200 days. Therefore, once the final stage of nucleolar maturation, INB formation, was initiated, axotomy did not depress eventual full INB recovery.

In summary, injury was seen to result in long term alterations in nucleolar configuration. Full INB recovery was dependent on the level of nucleolar maturation at the time of injury only in the absence of functional nerve regeneration. These nucleolar changes correlate well with previous studies of the reactive metabolism of these neurons.

1455 PHYSICAL DEVELOPMENT: NEW APPLICATIONS TO SILVER STAINING OF NEURAL TISSUE. Bjorn H. Merker*. (SPON: A.M. Graybiel). Dept. Psychol., M.I.T., Cambridge MA 02139.

Gallyas has introduced a set of silver stains based on the principle of physical development (e.g. Acta Neuropath. 1970, 16: 35-43). In these stains a chemical pretreatment selectively enhances the argyrophilia of a given component of neural tissue. That component is then made visible by deposition of metallic silver through the "seeding" process of physical development, identical for all the stains. In comparison with conventional chemical reduction methods of silver staining, the generation of pigment by physical development is slow, allowing precise control over the degree of impregnation. Furthermore, pigmentation is not limited by the amount of ionic silver bound to histological elements in pretreatment steps, making high levels of contrast achievable. The application of physical development to silver impregnation of Nissl substance and to contrast enhancement of normal axons will be described.

If sections of formalin-fixed brain are exposed to physical development after oxidation in a highly acidic medium, Nissl substance stains black on a virtually colorless background. In addition to its excellent photographic reproducibility, this high contrast Nissl stain offers the advantage of outlining electrode and pipette tracks in black, greatly facilitating their identification in the setting of cytoarchitectonic boundaries.

In that physical development removes the limitation of tissue-bound silver from the staining process, it was postulated that methods from photographic chemistry for manipulating contrast on photographic plates might be adapted for use on silver stained neural tissue. The approach was successfully applied to the Fink-Schneider stain for normal axons (Science 1969, 163: 895-902, note 31), in which high levels of background pigmentation impede visibility, particularly in tissue with short post-fixation time. After completion of the Fink-Schneider procedure, background pigmentation is removed from sections by a modified Farmer's Reducer. Silver is then re-deposited by physical development in greater amounts than originally present. The "bleach-intensify" cycle can be repeated, each time resulting in a higher level of contrast between axons and background. Usually 2 cycles of progressive contrast enhancement suffice to produce stark black impregnation of even the finest fiber components on a virtually colorless background.

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1456 IMMUNOCYTOCHEMICAL LOCALIZATION OF THE GLIAL FIBRILLARY ACIDIC (GFA) PROTEIN IN THE MUELLER CELL OF THE HUMAN RETINA. D.K. O'Dowd* and L.F. Eng. Dept. Path., VA Med Cen and Stanford U. Sch. Med., Stanford, CA. 94305

Mueller cells have been classified as modified retinal astrocytes (Polyak 1941, Maghales and Coimbra 1971) that span the thickness of the retina. These purported glial cells, recognized by classical morphology, have been the subject of a number of recent studies, attributing them with what have been traditionally considered neuronal functions. Some of these include storage and synthesis of neurotransmitters (Riepe and Norenburg, 1977), uptake and degradation of certain neurotransmitters (Sarthey and Lam, 1978) and electrophysiological activity, as demonstrated by the b-wave of the electroretinogram (Miller and Dowling, 1970).

The immunocytochemical technique of Sternberger, the peroxidase-anti-peroxidase method, was employed to localize the GFA protein in formalin-fixed, paraffin-embedded 6 micron sections of the human eye. Rabbit antiserum to the GFA protein and seven human eyes obtained at autopsy were utilized. At the light microscopic level, the positive staining of the Mueller cells for the GFA protein clearly delineated the glial network of the retina. The most prominent area of staining revealed a laminated structure formed by horizontal glial processes in the nerve fiber layer of the entire retina. We have observed that GFA protein was present in the Mueller cells, throughout the thick radial processes, and in the fine villous projections. Since GFA protein has been localized specifically in astroglial elements (Eng and Rubinstein, J. Histochem. Cytochem. 27:513, 1978), this study confirms the classification of the Mueller cell as an astrocyte. With this technique, we were also able to partition the ora serrata into three distinct regions, representing alterations in the Mueller cell morphology, on the basis of GFA protein staining.

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1457 SEQUENTIAL CHANGE IN CYTOPLASMIC AREA AND LYTIC ENZYME DISTRIBUTION IN A REACTIVE GLIAL CELL POPULATION. Linda L. Phillips and James E. Turner, Dept. Anat., Bowman Gray Sch. Med., Wake Forest Univ., Winston-Salem, NC 27103.

The glial cell population of the newt optic nerve has been shown to respond to lesion over a time interval of the first 30 minutes after transection, involving a series of morphological changes, visible at the light and EM levels (Soc. for Neurosc. Abst. IV:399, '78). Portions of the response appear to involve cytoplasmic hypertrophy and lytic enzyme (Acid Phosphatase) distribution. The purpose of this study was to determine what pattern these two parameters follow during the 30-minute response period. Newts were anesthetized, orbital nerves cut and animals sacrificed at 5, 10, 15, 20 and 30 minutes post lesion (mpl). Nerves were removed, one group processed for electron microscopy, and a second for the EM localization of Acid Phosphatase. Random regions, both central and peripheral in the nerve cross section, were sampled and with a computer interfaced digitizer the areas of neuronal and glial compartments were calculated. A determination of Acid Phosphatase localization was made in comparable regions. Results showed decrease in the ratio of peripheral neuronal to glial area (N/G) from values of 3.13 ± .377 (5 mpl), to 2.55 ± .398 (15 mpl) and 2.76 ± .295 (20 mpl). Each of these values was significantly different ($p < .001$) from that of lesioned control nerves (N/G = 6.24 ± .495), whereas central N/G values were not significantly different. Preliminary histochemical results show no qualitative difference between experimental nerves 10 mpl and unlesioned controls, both presented random background distribution of reaction product. At 20 mpl Acid Phophatase localization was specific for the extracellular spaces between degenerating axons. Few vesicular localizations were presented, in contrast to that of 30 mpl, where vesicles associated with glial cytoplasm were labelled in addition to the extracellular enzyme pockets. These results indicate 1.) a sequential increase in glial compartment area which correlates with the change in both mitochondrial and Golgi density during the first 30 mpl (Anat. Rec. 193: 653, '79), and 2.) the possible differential distribution of a lysosomal enzyme (Acid Phosphatase) over the same time period.

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1458 SYNAPTIC VESICLE DEPLETION IN FROG SYMPATHETIC GANGLIA STIMULATED BRIEFLY IN VITRO: DEMONSTRATION BY RAPID-FREEZING AND ELECTRON MICROSCOPY. J.J. Pysh and Gery K. Florek*. Dept. Anat., Northwestern Univ., Med. and Dent. Schs.

The purpose of this study was to investigate the distribution and concentration of synaptic vesicles in nerve terminals of sympathetic ganglia following short periods of tetanic stimulation. The method of rapid-freezing and freeze-substitution was utilized in order to estimate reliably the distribution of synaptic vesicles in nerve terminals in the living state.

The ninth sympathetic ganglia of bullfrogs were used because most, if not all, preganglionic fibers enter through the trunk. Experiments utilized a specially designed stimulation - recording specimen carrier upon which ganglia were mounted, electrically stimulated and recorded, and finally lowered into a modified Van Harreveld apparatus for rapid freezing. Five ganglia served as unstimulated controls and 5 were stimulated supramaximally, by way of the preganglionic trunks, at 20-25 Hz for 5 minutes. Rectangular pulses, 0.25-1.0 msec, were used. Postganglionic recordings were obtained from ganglial rami. After freezing, ganglia were processed by freeze-substitution in 2% OsO_4 in acetone for 3 days at -80°C and then prepared conventionally for transmission electron microscopy. Several hundred preganglionic terminals near the surface of the ganglia were obtained for analysis.

Ultrastructural evaluation indicated synaptic vesicles in preganglionic nerve terminals had a more uniform distribution in rapidly frozen ganglia than those chemically fixed. Morphometric analysis of 40 control and 40 stimulated axosomatic synapses revealed that the concentration of clear-cored vesicles within 500Å of the plasma membrane of the active zone and in the entire terminal declined 35% and 37% respectively after 5 minutes of stimulation. This suggests that the concentrations of the two populations of vesicles are related. This decrease in synaptic vesicle concentration approximated the decline in amplitude of postganglionic potentials recorded in vitro and approximates declines in acetylcholine release, EPSP amplitudes and postganglionic potentials, after 5 minutes of electrical stimulation, reported by others in sympathetic ganglia.

These data provide further evidence that transmitter is secreted in sympathetic ganglia by the exocytosis of synaptic vesicles and that the rate of transmitter secretion is dependent, in part, upon the availability of vesicles for release.

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1459 INFANT BRAINSTEM: CORRELATIVE SCANNING ELECTRON MICROSCOPY AND GOLGI STUDY. James J. Quattrochi*, Leopold Liss, Nobuhisa Baba*, P. T. McBride*, and Darrell N. Simone*. Dept. Pathology, Sch. Med., Ohio State University, Columbus, Ohio 43210.

Reticular ultrastructure was studied in the magnocellular and parvocellular complexes of postmortem infant brainstem with the application of scanning electron microscopy (SEM). A correlative technique using SEM and the rapid Golgi method identified neurons and reticular processes in thirty-four infants ranging in age from 1 day to 8 months. In all cases the postmortem time did not exceed six hours. Suitable verification of apparent dendritic projections exhibiting spine-like extensions was established by examination of adjacent sections using light microscopy. These spine-like extensions were observed to be of the three morphological types described by Peters and Kaiserman-Abramof (1970). Stellate neuronal-like bodies were observed to be contained within the surrounding neuropil. In addition, neurites in close parallel apposition appeared to resemble a topographical synaptic arrangement. SEM analysis of the infant pons and medulla may provide new data concerning ultrastructural reticular correlates.

1460 MYELIN ULTRASTRUCTURE IN COPPER DEFICIENCY. Frank A. Rawlins, Victor Canestri*, Carmen López-Jiménez*. Dept. Biophysics. I.V.I.C. Apartado 1827. Caracas 101, Venezuela.

There are several biochemical and histological studies indicating that a deficiency of copper have severe consequences on the normal development of the nervous system in humans and other vertebrates. From these studies it seems that myelination is largely affected by copper deficiency although no ultrastructural analyses are available. The purpose of the present work is to provide information on the role played by copper in the maintenance of the myelin sheeth ultrastructure in optic nerve of adult rats. A group of 25 day old female rats was fed a copper-free diet and deionized double distilled water for 8 weeks. Control rats were fed the same diet and distilled water with 15 µg/g of copper added to it, during the same period. The optic nerves were processed for electron microscope examination and myelin was isolated from the whole brain. It was found that copper concentration in plasma and brain as well as quantity of myelin isolated from copper-deficient rats were significantly less than that from controls. Concentration of copper in myelin isolated from copper deficient brains was slightly lower than controls but the difference was not significant. The electron microscope analysis showed advanced edema at the innermost region of the myelin sheeth with marked axonal compression in 15% of the fibers analysed. Edema seems to be formed by swelling of the inner loop with expansion of the myelin sheeth which surrounds it. Edema is observed mainly in large caliber fibers. Small and medium caliber fibers showed only a slight swelling of the myelin inner loop. The results indicate that the innermost region of the myelin sheeth is highly sensitive to copper deficiency and suggest that copper distribution may not be homogeneous within the myelin sheeth. Supported partialy by CONICIT Grant 31.26.S1-0754.

1461 PUTATIVE TISSUE BASOPHIL/MAST CELL: A RESIDENT OF THE PIGEON OLFACTORY BULB. Garl K. Rieke, Daniel E. Bowers*, and Marvin S. Cannon*. Department of Anatomy, College of Medicine, Texas A&M University, Olin E. Teague Res. Center, College Station, Texas, 77843.

Small cells (8-10 µm) with a single nucleus and metachromatic granules have been observed in the various laminae of the olfactory bulbs of 16 pigeons (Columba livia). These cells have not been observed in the spinal cord, cerebellum, paleostriatal complex and the hyperstriatum. The cells were present in the bulbs of birds perfused through the heart (0.05 M phosphate and sucrose buffer, 3% glutaraldehyde, 1% paraformaldehyde, 600 mOsmol, pH 7.3) and in bulbs fixed by immersion. The presence of the putative tissue basophil or mast cell in the bulbs fixed by perfusion or immersion is important since forced diapedesis cannot account for their presence. The cells are rarely seen adjacent to blood vessels. Electron micrographs show these cells in the neuropil. Their cell surface possesses small pseudopodia, which suggest motility. By light microscopy utilizing blood smears and buffy coats, and Azure B staining, coarse granules within these cells stain metachromatically and average one micrometer in diameter. By electron microscopy, the granules appear membrane-bound and demonstrate a grid-like substructure. A second type of granule having a dense pleomorphic center surrounded by a pale zone, also is seen. The coarse granules may contain acid-mucopolysaccaride. In addition, histochemical staining suggests that histadine may be present in these granules. Cells in blood smears and buffy coats of the pigeon that are identified as basophils closely resemble the cells seen in the olfactory bulb. Further histochemical studies on frozen sections of the bulbs and ultrastructural observations of blood basophils should clarify whether the cell observed in the bulb is the same as that seen in the peripheral blood.

Basophils or mast cells have not been described in the neuropil of other avian species. Mast cells have been found in the brain of dogs and hedgehogs. The putative basophil or mast cell in the pigeon bulb may have functions similar to those proposed for this cell in connective tissue or as yet undisclosed functions.

1462 ABNORMALITIES OF AXOLEMMAL MEMBRANE SPECIALIZATIONS IN MYELIN DEFICIENT "SHIVERER" MICE. J. Rosenbluth, Departments of Physiology and Rehab. Medicine, New York University School of Medicine, New York, N. Y. 10016.

The mouse mutant known as "Shiverer" is characterized by a paucity of central nervous system myelin. Examination of thin sections of spinal cord, cerebellum and optic nerve reveals thin myelin sheaths with numerous irregularities. "Loops" of glial cytoplasm are apposed to axons in various locations not necessarily related to nodes of Ranvier. Although typical paranodal structures are uncommon, junctions containing "transverse bands" occur frequently between glial processes and axon plasma membranes. Peripheral nerves exhibit subtle irregularities consisting of myelin lamellae that terminate against the axolemma in internodal regions and greater numbers of loose lamellae containing cytoplasm. Peripheral myelin sheath thickness appears to be within normal limits, however. Analysis of freeze-fracture replicas of central nervous system fiber tracts shows few examples of typical paranodal membrane specializations in the E fracture face. However, distorted and bizarre junctional membrane specializations occur frequently in the form of isolated patches or strips of membrane revealing the characteristic paracrystalline pattern but oriented longitudinally or obliquely rather than nearly transversely. Occasionally several such strips, none of which encircles the axolemma, may occur side by side. The orientation of the strips may shift within a given region and the direction of the paracrystalline pattern within such a strip may also change abruptly. A few examples of typical E face nodal particle accumulations have been encountered adjacent to more or less normal paranodal or hemiparanodal regions. However, isolated paracrystalline patches are usually not associated with particle accumulations in the immediately adjacent membrane, except in instances where "lakes" of membrane are entirely surrounded by the paracrystalline membrane. The results indicate that even though myelin formation is grossly deficient in quantity and bizarre in structure, glial cells and axons in the central nervous system of Shiverer mice are still capable of forming the unique junctions normally found in the paranodal region. The lack of consistent association of E face particle accumulations with isolated paracrystalline patches is compatible with the hypothesis that complete spiral collars of paracrystalline membrane are required for effective restriction of the movement of intramembranous particles resulting in their local concentration.

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1463 TISSUE CULTURE STUDIES OF SCHWANN CELL PROLIFERATION DURING DEVELOPMENT AND DEGENERATION. J. Salzer*, L. Glaser*, and R.P. Bunge. Dept. Anat. & Neurobiology, Washington Univ. Sch. Med., St. Louis, Mo.

Periods of substantial Schwann cell proliferation occur during embryonic development and following peripheral nerve transection or crush (Wallerian degeneration). The wave of proliferation observed during development (Asbury,1967) may be stimulated by an axonal mitogen (Wood and Bunge, 1975). By contrast, the increase in the number of Schwann cells in the distal transected nerve (Abercrombie and Johnson,1946) may result from a breakdown product of the axon (Abercrombie and Santler,1957) or the myelin sheath, or be engendered by the space vacated by the degenerating nerve fibers (Joseph,1950). These suggestions would explain their observation that proliferation is greatest in degenerating myelinated nerves and least in degenerating unmyelinated nerves

When rat dorsal root ganglia (DRG's), treated with antimetabolites to suppress fibroblast outgrowth (Wood,1976), are placed in tissue culture neurites elongate and become ensheathed by Schwann cells. Initially Schwann cells proliferate rapidly, but as each neurite is fully ensheathed division slows considerably and myelination commences. Using autoradiography to follow the incorporation of tritiated thymidine, we studied the effect of DRG excision (e.g. axotomy) on Schwann cell proliferation. In the young explant (two weeks in vitro), as in the developing peripheral nervous system, Schwann cell proliferation is very high (labeling indices of 30% or more). In these cultures if the neuronal somas, which are confined in the ganglion, are mechanically excised proliferation rapidly decays to less than 1% only 48 hrs. later. In more mature cultures (six weeks in vitro), most cells were quiescent at the time of excision and remained quiescent indefinitely post-axotomy. However the myelin forming Schwann cells (which could be recognized specifically by their intracellular myelin debris) did divide in response to axotomy. The peak labeling period occurred at four days post-excision at which time approximately 35% of the cells which had been myelin related, incorporated thymidine. These results suggest that the mitogenic signals during development and degeneration are distinct. Breakdown of myelin debris or turnover of Schwann cell membranes may be the signal operative in Wallerian degeneration.

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1464 APPLICATION OF SCANNING ELECTRON MICROSCOPY TO THE CENTRAL NERVOUS SYSTEM. A. B. Scheibel, L. Paul*, and I. Fried*. Departments of Anatomy, Psychiatry and Physiological Psychology, University of California, Los Angeles, CA 90024.

Scanning electron microscopy (S.E.M.) has been used for several years in the delineation of surface topography of biological systems, i.e., villous linings of the respiratory and female reproductive tracts, ependymal surfaces of ventricles, dissociated elements from tissue culture, etc. We now report on the use of S.E.M. methodology in the study of structure and interrelationships within the central nervous systems of several species, including man. Neuronal, dendritic, axonal, glial and vascular tissue surfaces are made available to scanning methods by blunt dissection and "creative" tearing techniques which follow natural cleavage planes along the surfaces of these elements.

Following perfusion (where possible) with buffered paraformaldehyde solutions, further fixation for 24-48 hr of small selected tissue block and careful "tearing" of tissues across areas which were to be examined, the resulting blocks were post-fixed in 1/3 of 1% osmium tetroxide solution, then critical point freeze-dried, sputter-coated with palladium-gold in an argon-filled medium, and examined in an ETEC scanning electron microscope at magnifications of up to 20,000 x.

As indicated by the series of accompanying drawings and photographs, sensitive 3-dimensional delineations of neuronal-glial interrelations, synaptic topography and neuropil-vascular configurations can be examined. Characteristic topographies of specific brain areas are gradually becoming clear, particularly in cerebellum, hippocampus and spinal cord. We are in process of developing labeling techniques to enable identification and tracing of individual cell-membrane molecular species.

(Supported in part by USPHS grant NS 13871-01A1.)

1465 ISOLATED RAT BRAIN CAPILLARIES; FREEZE-FRACTURE CONFIRMATION OF INTER-ENDOTHELIAL JUNCTION INTEGRITY. Richard R. Shivers and Gary W. Goldstein. Department of Zoology, University of Western Ontario, London, Ontario, and Department of Neurology,University of California Medical Center, San Francisco, California.

Fractions of rat brain tissue containing high concentrations of intact brain capillaries have been proposed as useful models for in vitro studies of blood-brain barrier permeability (Goldstein et al, J. Neurochem., 25, 1975). Preliminary studies of these preparations have suggested that the zonulae occludentes of the isolated segments of capillaries retain the impermeability to the protein tracer horseradish peroxidase exhibited by them in vivo. These junctions are therefore assumed to be functionally "tight" in vitro. In order to determine the precise structural organization of these occluding junctions, including an estimate of their tightness, and to demonstrate a method of simple but precise assessment of junctional integrity, pellets of isolated rat brain capillaries were freeze-fractured and then replicated with platinum and carbon. The freeze-fracture images of inter-endothelial zonulae occludentes reveal complex intramembrane arrays of ridges and grooves characteristic of tight junctions. Longitudinal fractures of the cellular lining of capillaries expose vast expanses of inter-endothelial plasma membrane interfaces and the junctional complexes situated between the cells. From such images, the complex and elaborate architecture of the zonulae occludentes can be readily appreciated. Situated on PF fracture faces are 6-8 parallel ridges which display a high degree of anastomosing between adjacent strands. The EF fracture face contains grooves complementary to the PF face ridges. The zonulae occludentes of these capillary endothelial cells are similar in complexity to those reported for reptilian brain capillaries (Shivers, Brain Res., 1979) and can be presumed therefore "very tight". This study demonstrates that freeze-fracture of pellets of brain capillaries alleviates sampling problems inherent in whole tissue preparations and, in addition, demonstrates the usefulness of freeze-fracture as a tool to monitor junction structure during in vitro investigations of the blood-brain barrier. (Supported by the National Research Council of Canada, National Foundation-March of Dimes, and USPHS).

1466 LYSOSOMES AS SPECIFIC STORAGE DEPOTS FOR NEUROTRANSMITTER. Ludmila J. Shkolnik*, Daniel J. Goldberg, and James H. Schwartz. Div. of Neurobiol. & Behav. & Depts. of Physiol. and Pharm., Columbia U., New York, N.Y. 10032. U.S.A.

Large lysosomes containing wear-and-tear pigment are characteristic of many nerve cells, both vertebrate and invertebrate. In *Aplysia* attention has been drawn to these particles because they were shown to be an intracellular Ca^{++} sink and can release Ca^{++} in response to light. They are typically situated in a dense perinuclear layer together with the Golgi apparatus, smooth endoplasmic reticulum and vesicles in cell bodies of pigmented *Aplysia* neurons, which make chemical synapses with other neurons and with peripheral organs. We now have an indication that these lysosomes, in addition to being degradative in function, may also serve as a specific depot for transmitter substance in the cell body.

We have been studying the localization of ^{3}H-serotonin injected directly into the cell body of the giant cerebral neuron (GCN), the identified serotonergic cell in the *Aplysia* cerebral ganglion, and have found a striking association of the labeled transmitter with large lysosomes. This localization is specific: ^{3}H-serotonin was not found in similar lysosomes in the perikaryon of R2, an identified *Aplysia* cholinergic neuron. ^{3}H-dopamine, ^{3}H-histamine, and ^{3}H-N-acetylgalactosamine injected into the serotonergic neuron were not localized in lysosomal bodies. These organelles are indeed lysosomal: we showed that they contain acid phosphatase by electron microscope cytochemistry. Moreover, examination of isolated GCN cell bodies by fluorescence microscopy revealed the presence of abundant autofluorescent yellow pigment characteristic of lipofuscin.

It is our working hypothesis that ^{3}H-serotonin labels organelles containing membranes that once were components of the serotonergic vesicle. Lysosomes might accumulate the transmitter with a high degree of specificity because they contain membranes, perhaps recycled from the neuron's terminals, that retain their ability to concentrate and bind serotonin. Alternatively the lysosomes may not take up serotonin directly from the cytoplasm, but may engulf new serotonergic vesicles already charged with transmitter. Thus the lysosomes would regulate the supply of vesicles as well as the supply of the transmitter. Whatever the mechanism, lysosomal binding of exogenously introduced transmitter may be a useful technique for identifying the transmitter type of a neuron.

1467 FURTHER NOTES ON THE SEARCH FOR "DOUBLE" RETROGRADE AXONAL LABELING TECHNIQUES: "EVERYTHING BUT THE KITCHEN SINK." Dennis A. Steindler. Dept. of Anat., Michigan State University, East Lansing, MI 48824

Several macromolecules have been tested as potential "double-labels" to be used in combination with horseradish peroxidase (HRP) for determining the degree of axonal branching within particular regions of the central nervous system. Previous investigators have reported retrograde axonal transport and subsequent autoradiographic, fluorescent and histochemical demonstrations of 3H-adenosine, 3H-HRP, 3H-apo-HRP, 125I-tetanus toxin, 125I-wheat germ agglutinin(WGA), bovine serum albumin, certain fluorescent substances (i.e.Evans Blue), and iron dextran complex (Schubert and Kreutzberg,'75; Geisert,'76; Hayes and Rustioni,'78; Price et.al.,'77; Schwab et.al.,'77,'78; Kristensson and Olsson,'71; Steward and Scoville,'76; Kuypers et.al.,'77; Olsson and Kristensson,'78).

This investigator has injected various dyes, iron dextran complex, tritiated RNA precursors (i.e.orotic acid), alkaline phosphatase, and labeled WGA in different regions of mouse cerebrum, cerebellum, basal ganglia and related brainstem nuclei. Preliminary observations are encouraging using lectins such as WGA that are either tritium-labeled (New England Nuclear, S.A. 4.37 Ci/mmol) or covalently linked to enzymes (i.e.alkaline phosphatase) or biotin (E.Y.Laboratories, San Mateo, Ca.). The mechanisms of uptake and transport are not known, but the sequence most likely begins with WGA binding to specific glycoproteins and glycolipids located on the cell surface (Schwab et. al.,'78). In addition to autoradiography, there are also several alternatives for histochemically demonstrating the lectin, all of which utilize lectin or substrate conjugates. For example WGA (M.W.35,000) bound to alkaline phosphatase (M.W.140,000) will hydrolyze the phosphate group of 5-bromo-4-chloro-3-indolyl-phosphate producing an insoluble blue indigoid dye (Chu,pers. com.; Tsou et.al.,'67) that is compatible with a brown reaction product using 3,3' diaminobenzidine tetrahydrochloride in HRP histochemistry. WGA bound to biotin (M.W.244) may afford better uptake and transport because of the considerably smaller molecular weight of biotin compared to AP, and sections can be reacted with avidin (which displays an extremely high binding affinity for biotin) bound to either AP or ferritin for light and electron microscopic detection of the transported lectin.

Analysis of degrees of divergence in the collateralized projections within the motor system will ensue following technical refinements of the aforementioned procedures.

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1468 COMPLEMENTARY REPLICAS OF FREEZE-FRACTURED NODES OF RANVIER IN AMPHIBIAN PERIPHERAL NERVE. J.H. Tao-Cheng* and J. Rosenbluth. (SPON: K. Rubinson). Departments of Physiology and Rehab. Med., New York University School of Medicine, New York, NY 10016.

Sciatic nerves of frog tadpoles were fixed and freeze-fractured, and complementary replicas of nodes of Ranvier examined. The results confirm a previous study of amphibian myelinated fibers in the central nervous system (J.Neurocytol.5:731) in showing equally high concentrations of intramembranous particles in the E and P fracture faces of the nodal axolemma but low concentrations of E face particles in the internodal region relative to the internodal P face particle concentration. By comparing complementary replicas it is possible to demonstrate unequivocally that a large proportion of the nodal E face particles are large in diameter and cast long shadows, while in the P fracture face the proportion of large particles is distinctively lower and many more small particles and irregularly-shaped structures are present. Tracings of E and P fracture faces of the nodal and paranodal axolemma and of the junctional Schwann cell membranes were prepared and superimposed face to face in order to determine whether the particles in the respective tracings overlap. Almost none do, indicating that the particles in the respective fracture faces are distinct from each other and do not represent fragments of the same units. In the paranodal axolemma the helical groove (E face) or ridge (P face) that faces the extracellular space between terminating Schwann cell "loops" contains particles similar in size and shape to those in the nodal axolemma, and here too the particles in the complementary faces do not overlap. In addition, in this study replicas were consistently viewed shadowed side up, and examination of the junctional region of the paranodal axolemma showed that the orientation of the diagonal pattern is invariably the same. The angle formed by the diagonal pattern and the ridges or grooves is always positive (~+30°) in the E face and negative (~−30°) in the P face. Moreover, this angle is rather consistent with respect to the ridges or grooves regardless of the angle between them and the long axis of the fiber. The Schwann cell membrane forming the paranodal junction exhibits regularly spaced (30 nM) rows of particles in both fracture faces. There are more particles (~70%) in the P face than in the E face (~30%) and the particles in the two faces do not overlap. Since the particles in the two faces of this specialized Schwann cell membrane fall into approximately the same size range and are aligned with each other without overlapping, they may be members of the same population, most of which which remain attached to the P face in the cleaving process.

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1469 NORADRENERGIC NERVES AND MAST CELLS AFTER ELECTRICAL STIMULATION IN THE PRESENCE OF PHENTOLAMINE. Å. Thureson-Klein, R. L. Klein and L. Stjärne.* Dept. Pharmacol. Univ. Miss. Med. Ctr. Jackson, MS 39216 and Karolinska Inst. Stockholm, Sweden.

Biopsy specimens of omental veins were obtained during surgery under general anesthesia. These were normotensive female patients who had not been exposed to any medication known to release histamine. The excised veins were divided into segments, mounted in a bath and subjected to electrical field stimulation at 1 Hz for 60 sec. with or without the α-receptor blocking agent, phentolamine (7.5×10^{-7}M) present. Control segments were superfused for the length of time of the experiment. Small tissue blocks from several areas of all segments were prepared for electron microscopy. Noradrenergic nerve terminals with typical large and small dense-cored vesicles and mast cells with large characteristic scroll-containing granules were well preserved and easily identified in control veins. The distance between mast cells and noradrenergic axons and terminals was frequently in the same range as that between nerve terminals or mast cells and smooth muscle cells, i.e., 1 µm or less. After electrical stimulation without phentolamine present there was an increase of presumptive exocytotic profiles along the terminal membrane and an increase in the total number of small vesicles by 30% when compared with unstimulated controls. There was no obvious change in the mast cell ultrastructure. However, after stimulation in the presence of phentolamine, both nerve terminals and mast cells showed morphological changes. In the nerve terminals there was a significant increase of both small and large vesicles that were in immediate contact with the terminal membrane. The total number of large and small vesicles had declined when compared to superfused controls but there was an increase of small clear vesicles. In the mast cells, many of the intracellular granules had fused and showed decreased electron density. Granules and released scrolls were present extracellularly. Anastomosing cavities filled with amorphous material were prevalent in other mast cells. It is possible that the degranulation of mast cells following electrical stimulation in the presence of phentolamine reflects a direct releasing effect of a compound which is structurally reminiscent of histamine. It may also be due to an indirect effect involving noradrenaline and ATP overflow after blockade of the prejunctional α-adrenergic receptors.

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1470 A NETWORK OF SUBEPENDYMAL POLYANIONIC ELECTROLYTES IN THE LATERAL VENTRICLE OF THE RAT. Richard M. Torack and Lois Grawe*. Dept. Pathol., Washington Univ. Sch. Med., St. Louis, MO 63110

A system of acidic mucopolysaccharides in in the brain of young adult rats has been demonstrated by means of histochemical and electron microscopical techniques. This extracellular material appears to be present in the lateral wall and angle of the lateral ventricle. In contrast to other extracellular mucopolysaccharide which can be identified in cryostat sections, this network is not extracted by chloroform/methanol (2:1) or xylene. Cationic aldehyde fuschin is bound selectively at pH 1.5. The system has an affinity for alcian blue at pH 1.0 and in a medium containing 0.3M-0.5M Mg_2Cl_2. Staining with alcian blue at pH 1.0 will block subsequent treatment with aldehyde fuschin. Metachromasia is demonstrable with toluidine blue at pH 1.5. The network binds cationized iron (Hale's colloidal iron) at pH 1.5.

Electron microscopic study of rat brain fixed by perfusion and immersion in 2% glutaraldehyde and 5% paraformaldehyde reveals an expanded extracellular space (ECS) measuring up to 1000 Å in width at the lateral angle and underlying the lateral wall of the lateral ventricle. An ependymal-like cell is present in the lateral angle which is partially bounded by this expanded E.C.S. space. At the lateral wall, the expanded E.C.S. is confined to the immediate subependymal area. Tissue chopper sections reacted with cationized iron reveal a localization of electron dense material in the dilated E.C.S. beneath the ependymal lining.

These findings are considered to be consistent with a network of polyanionic electrolytes in the periventricular extracellular space at these sites, which should be important in trans-ependymal ionic movement.

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1471 EFFECTS OF CASTRATION ON DOPAMINE METABOLISM IN RAT STRIATUM AND LIMBIC FOREBRAIN. Lloyd M. Alderson*, Matthew S. Starr*, and Michael J. Baum. Laboratory of Neuroendocrine Regulation, M.I.T., Cambridge, MA 02139.

Psychopharmacological studies have suggested that increased activity in dopaminergic pathways facilitates the expression of sexual behavior in the male rat. Castration of the male rat leads invariably to the disappearance of masculine sexual behavior. Experiments were carried out to determine whether this castration-dependent reduction in copulation is correlated with changes in catecholamine synthesis or in dopamine (DA) metabolism in brain regions rich in DA-containing nerve terminals.

In an initial experiment catecholamine synthesis was estimated in castrated and sham-operated adult male rats (Long-Evans) by measuring the accumulation of dihydrophenylalanine (DOPA) by radioenzymatic assay 30 min after injection of NDS-1015 to inhibit aromatic L-amino acid decarboxylase activity. Males were killed at night either 15 or 30 days after castration or sham-operation. The levels of DOPA measured in striatal slices and in limbic forebrain (Nucleus Accumbens plus lateral septal region) were identical in castrated and sham-operated males at both postoperative times. Thus we were unable to confirm the report (Engel et al., 1979, Pharm. Biochem. Behav. 10:149) of increased DOPA accumulation in each of these neural tissues after castration.

Dopamine metabolism was studied in striatum and limbic forebrain by estimating the levels of 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) using high pressure liquid chromatography. Males were again killed 15 or 30 days after castration or sham-operation. In striatum the levels of DOPAC were significantly lower in castrated males 30 days post-operatively. Striatal HVA also tended to be lower in castrates than in sham-operated males at both times, although this difference did not reach statistical significance. In limbic forebrain no significant differences between castrated and sham-operated males were detected in DOPAC or HVA, although DOPA levels tended to be lower in castrates both 15 and 30 days postoperatively. The results are consistent with the notion that testicular steroids, or their neural metabolites, normally facilitate the release of dopamine in the male rat striatum and thereby contribute to the activation of sexual behavior.

1472 CHARACTERISTICS OF CATECHOLAMINERGIC NERVES IN THE NEUROINTERMEDIATE LOBE OF THE RAT PITUITARY. R.H. Alper, K.T. Demarest and K.E. Moore. Dept. Pharmacol. & Toxicol., Michigan State Univ., East Lansing, MI 48824.

Histochemical fluorescent techniques have revealed that the neurointermediate lobe (NIL) of the rat pituitary contains catecholaminergic nerves (Björklund, Z. Zellforsch. 89: 573, 1968). Dopamine (DA) nerves terminating in the NIL originate in the arcuate nucleus, forming the tuberohypophyseal system; there is some controversy concerning the origin of the norepinephrine (NE) nerves. Using a radioenzymatic assay it was determined that the rat NIL contains approximately 7 ng DA/mg protein and 2 ng NE/mg protein. Superior cervical ganglionectomy reduced the content of NE by one-third but did not alter the content of DA. This suggests that all of the DA and two-thirds of the NE in NIL is contained within nerves of central origin. The activity of the catecholamine nerves was estimated by determining the rate of turnover of DA and NE in the NIL. The concentrations of both monoamines declined exponentially following the administration of α-methyltyrosine (250 mg/kg, i.p.). Synthesis rates calculated from these data revealed that the synthesis of NE (0.3 ng/mg protein/hr) is much less than that of DA (2.6 ng/mg protein/hr).

The activity of nigrostriatal DA nerves has been estimated by quantifying the accumulation of DOPA in the striatum after the inhibition of DOPA decarboxylase. Since the synthesis of DA (estimated from decline of this amine after α-methyltyrosine) represents 90% of the total catecholamine synthesis in the NIL, the accumulation of DOPA in NIL should be appropriate for estimating the activity of tuberohypophyseal DA nerves. In the following experiments the concentration of DOPA in various brain regions containing DA nerve terminals was measured 30 minutes after administration of 3-hydroxybenzylhydrazine (NSD 1015, 100 mg/kg, i.p.). DOPA accumulation increased in the NIL, but not in the median eminence or striatum, after 48 hr of water deprivation or the addition of 2% NaCl to the drinking water. DOPA accumulation was also selectively increased in the NIL 60 and 90 min following the administration of α-melanocyte stimulating hormone (100 µg/kg, s.c.). These results suggest that DA nerves of the tuberohypophyseal system are regulated, in part, by hormones released from NIL. (Supported by USPHS grants NS09174, Fellowship NS06026 and Training Grant GM07392.)

1473 SUPPRESSION OF LORDOSIS BEHAVIOR IN THE FEMALE RAT DURING MESENCEPHALIC ELECTRICAL STIMULATION. Gary W. Arendash* and Roger A. Gorski. Dept. Anat., Sch. Med., UCLA, Los Angeles, CA 90024.

It has been shown that serotonin (5HT) may play an inhibitory role in the control of sexual receptivity in the rat. Since all known serotoninergic neurons have their cell bodies within the brainstem raphe nuclei, the effects of biphasic electrical stimulation of the mesencephalic raphe nuclei and adjacent periaqueductal grey (PAG) on lordosis behavior were investigated, using four different sets of stimulation parameters. Ovariectomized, estrogen-primed animals that had been previously implanted with concentric bipolar electrodes were tested between 4-8 hrs after a progesterone injection (0.5 mg) which normally produces high levels of receptivity during this period. A 25 mount control period was obtained prior to each stimulation period, which also lasted for 25 mounts. Most rats were tested with 3 or often all 4 sets of stimulation parameters. During stimulation within the dorsal raphe nucleus (DRN) at frequencies of 10/sec (0.5 msec pulse duration) or 2/sec (2 msec pulse dur.), lordosis behavior induced by male rat mounting remained at high control levels. However, DRN stimulation at frequencies of 100/sec (0.5 msec pulse dur.) or 10/sec (2 msec pulse dur.) caused a marked and immediate suppression (53% and 56%, respectively) in receptivity ($p<0.001$). This suppression may not be due to activation of serotoninergic neurons originating in the DRN since DL-p-chlorophenylalanine (320 mg/kg, an inhibitor of 5HT synthesis), given 71 h prior to stimulation, did not modify the suppression. In contrast to the effects induced by DRN stimulation, activation of the median raphe nucleus had no significant effect on lordosis. However, stimulation within the PAG adjacent to the DRN at a frequency of 10/sec (0.5 or 2 msec pulse dur.) produced an immediate, dramatic decrease (81% and 80%, respectively) in receptivity ($p<0.001$), although PAG stimulation at frequencies of 100/sec (0.5 msec pulse dur.) or 2/sec (2 msec pulse dur.) was ineffective. All currents used were below threshold for eliciting noticeable behavioral effects, and never exceeded 200 uA. These results indicate that electrical stimulation of the mesencephalic DRN-PAG region in highly receptive estrogen-progesterone treated rats can markedly suppress lordosis behavior. This suppression could be due to either 1) activation of a midbrain ascending neuronal pathway(s), such as the dorsal longitudinal fasciculus in the PAG; or 2) activation of a descending inhibitory pathway(s) originating in or passing through the DRN-PAG region which may inhibit the lordosis reflex arc at the level of the medulla or the spinal cord. Since the DRN-PAG region contains significant numbers of estradiol-concentrating cells, estrogen may act to decrease the efficacy of a DRN-PAG inhibitory pathway, thus removing a tonic inhibitory influence on the reflex arc for lordosis. (Supported by HD-01182 and F32 HD-05671).

1474 HORMONE ACCUMULATION IN THE BRAIN OF THE ZEBRA FINCH AFTER INJECTION OF VARIOUS STEROIDS AND STEROID COMPETITORS. Arthur P. Arnold. Dept. Psychol. and Brain Res. Inst., UCLA, Los Angeles, CA 90024.

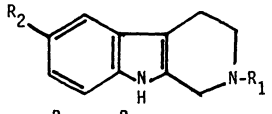
In castrated male zebra finches (Poephila guttata), the autoradiographic method was used to determine the distribution of hormone accumulating cells in selected brain regions after injection of tritiated testosterone (T), 5α dihydrotestosterone (DHT), or estradiol (E). The brain regions selected for analysis all show cellular accumulation of hormone after T injection. They were: the magnocellular nucleus of the anterior neostriatum (MAN), caudal nucleus of the hyperstriatum ventrale (HVc), robust nucleus of the archistriatum (RA), nucleus intercollicularis (ICo), tracheosyringeal portion of the hypoglossal motor nucleus (nXIIts), periventricular magnocellular nucleus (PVM), and infundibular region (INF). The first five of these brain areas are thought to be involved in the control of song and other vocalizations, and the last two are hypothalamic regions.

Labelled cells are found in MAN, HVc, RA, and nXIIts after injection of T and DHT but not after E; labelled cells are found in ICo after injection of any of these; in PVM and INF, labelling is greatest after E and least after DHT.

Other males were injected with one of a number of non-radioactive steroids before receiving an injection of tritiated T to determine which steroids would prevent cellular accumulation of radioactivity, presumably by competing for receptor sites. Non-radioactive steroids used as pre-injected competitors were T, DHT, E, cyproterone acetate (Cyp A), androstenedione (AE), and androstanedione (AA), and these were injected at 1000 or 100 times the dose of tritiated T. Non-radioactive T was effective in eliminating cellular accumulation in all brain regions. The other steroids, when injected at the 1000 times dose, reduced accumulation in all brain regions to some extent. However, DHT, Cyp A, and AA were relatively more effective in their competition in MAN, HVc, and nXIIts, and less effective in PVM and INF. E and AE were more effective in PVM and INF, and relatively less effective in MAN, HVc, and nXIIts. These results, when taken together, suggest that T or its non-aromatized metabolites are predominantly accumulated in cells in MAN, HVc, RA, and nXIIts, whereas T or its aromatized metabolites are predominantly accumulated in PVM and INF.
Supported by NSF grant BNS 77-05973.

1475 THE IDENTIFICATION AND QUANTIFICATION OF 1,2,3,4-TETRAHYDRO-β-CARBOLINE AND 6-METHOXY-1,2,3,4-TETRAHYDRO-β-CARBOLINE IN RAT BRAIN AND ADRENAL GLAND. S. A. Barker* and R. E. Harrison* (SPON: J. A. Monti), Neurosciences Program, University of Alabama in Birmingham, B'ham, AL 35294.

GC/MS analyses of rat brain and adrenal gland extracts have led to the identification of 1,2,3,4-tetrahydro-β-carboline (THBC) and 6-methoxy-THBC (6-MeO-THBC) as naturally occurring constituents of these tissues. Trace amounts of 2-methyl-THBC (2-MTHBC) were also identified.



R_1	R_2		
H	H	=	THBC
H	OCH_3	=	6-MeO-THBC
CH_3	H	=	2-MTHBC

Rat brains and adrenal glands from individually housed animals were homogenized and spiked with deuterated standards for 6-MeO-THBC and THBC. The samples were extracted and derivatized to form the corresponding heptafluorobutyryl compounds (Barker et al., Biochem. Biophys. Res. Commun. 87:146-154, 1979). Analyses were conducted by selected ion monitoring, comparing ion mass ratios and retention times of the added deuterated standards with peaks for the endogenous β-carbolines.

	THBC	6-MeO-THBC	2-MTHBC
BRAIN	17.5 ± 4.86 ng/g	35.6 ± 16.6 ng/g	TRACE
ADRENAL	325 ± 45 ng/g	1113.7 ± 300 ng/g	TRACE

The implications of these findings, in view of the known pharmacological effects of these compounds on amine uptake and monoamine oxidase activity, will be discussed.

1476 DECREASED SODIUM CONSUMPTION AND IMPAIRED NATRIURESIS FOLLOWING ELECTROLYTIC ABLATION OF ANTEROVENTRAL THIRD VENTRICLE (AV3V) PERIVENTRICULAR TISSUE IN RATS. S. L. Bealer, M. J. Brody*, J. R. Haywood*, G. D. Fink*, K. A. Gruber*, V. M. Buckalew*, and A. K. Johnson. Cardiovascular Center, Univ. Iowa, Iowa City, IA 52242 and Bowman Gray School of Med., Winston-Salem, NC 27103.

Electrolytic ablation of anteroventral third ventricle (AV3V) periventricular tissue results in temporary adipsia, chronic hypernatremia and expanded blood volume, suggesting that mechanisms of sodium regulation are altered. The present experiments were designed to determine if AV3V lesions change sodium consumption under ad lib conditions and the natriuresis typically seen following isotonic blood volume expansion.

Rats were maintained on sodium deficient chow with continuous access to 2.0% NaCl solution and water. Animals were equated for sodium solution intake and received either electrolytic lesions in the AV3V region or sham lesions. NaCl solution and water intakes were recorded for 16 days postsurgery and following the hyponatremia induced by s.c. Formalin. Separate groups of animals underwent AV3V ablation or sham lesions. These animals were implanted with venous, arterial and bladder catheters, and isotonic NaCl solution was infused intravenously at .51 ml/min until 10% of body weight was given. Urine and sodium excretion was measured during the infusion period. In addition, blood samples were taken, pooled and bioassayed for natriuretic hormone according to the procedures of Gruber and Buckalew (Proc. Soc. Exp. Biol. Med., 159:463-467, 1978).

Animals with lesions surrounding the AV3V consumed significantly less sodium solution and showed lower sodium preference following surgery than sham lesioned animals. However, both groups increased sodium solution consumption following s.c. Formalin. These data indicate that AV3V lesions attenuate sodium intake but do not render animals insensitive to their sodium levels.

In addition, AV3V lesioned animals showed markedly attenuated urine and sodium excretion compared to control animals during volume expansion. Bioassay for natriuretic hormone revealed high levels in control animals, while no activity was seen in blood samples from animals with AV3V lesions.

These data demonstrate that rats with AV3V periventricular tissue lesions ingest less sodium than controls while on sodium deficient chow, but remain sensitive to body sodium concentrations. In addition, rats with AV3V lesions have a low capacity for natriuresis when volume expanded. This could be the result of impaired release of natriuretic hormone. These studies suggest that the integrity of the AV3V periventricular tissue is essential for normal sodium regulation.

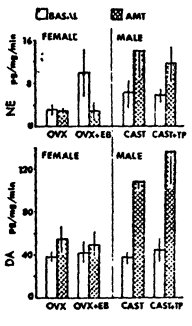
1477 A SEX DIFFERENCE IN THE ENDOGENOUS RELEASE AND IN THE EFFECT OF TEMPERATURE ON THE RELEASE OF PROLACTIN AND LH FROM THE ANTERIOR PITUITARY OF RATS IN PERIFUSION. Deborah Beaudry* and V.D. Ramirez (SPON: R. Gillette). Dept. of Physiol. and Biophys. and Neural and Behavioral Biology Program, Univ. of Illinois, Urbana, IL 61801.

Holtzman rats were decapitated at the same time each afternoon, the anterior pituitary gland removed and immediately placed in ice cold Krebs Ringer phosphate buffer glucose-BSA medium at pH 7.4. Pituitary glands were quartered and placed into individual perifusion chambers bubbled with a mixture of 95% O_2-5% CO_2, and maintained in a 37°C water bath. The medium was pumped into the chambers at a constant rate of 1 ml/4 min. After a 1 hour stabilization period perifusate was collected in 4 min intervals on ice. The samples were stored at -20°C until the RIA's were performed. The average release rate of prolactin from female glands was, diestrus: 4.00±.28; proestrus: 3.98±.27 and estrus: 4.62±.30. The average rate of prolactin release from male glands was 2.13±.10. The release rate of LH in females was, diestrus: 2.70±.12; proestrus: 4.89±.47; and estrus: 5.40±.38 while in males was 16.5±1.5. The effect of temperature on this system was analyzed as part of the validation of the technique. As expected the basal release of prolactin decreased for both males and estrous females when the temperature was lowered from 37°C to 0°C. In two estrous female glands the rate of prolactin release decreased from 3.6 and 2.8 ng/mg/min to 0.46 and 0.81 ng/mg/min, respectively. Prolactin secretion from male glands (n=6) went from 2.06±.20 at 37°C to 0.67±.05 at 0°C. Pituitary LH release showed quite a different response. In the male, decreasing the temperature resulted in a marked stimulation of this release from 18.0± 2.7 to 61.1±4.6 (n=6). In two estrus female rats the anterior pituitary glands did not respond to the challenge of temperature: the release rates were 4.10 and 5.70 ng/mg/min at 37°C and 5.10 and 5.40 ng/mg/min at 0°C, respectively. In summary, pituitary glands perifused in vitro show a clear sexual difference in the endogenous release rates of both prolactin and LH. In addition, 0°C temperatures reduced the secretion rates of prolactin in both male and female pituitaries. In marked contrast, LH secretion from the male gland increased with decreasing temperature. Curiously, the temperature change produced no observable effect on LH release from the pituitaries of estrous females.

1478 SEX DIFFERENCES IN THE AMPHETAMINE STIMULATED RELEASE OF ENDOGENOUS CATECHOLAMINES IN VITRO. Jill Becker and V.D. Ramirez. Dept. of Physiology and Biophysics and Neural and Behavioral Biology Program, Univ. of Illinois, Urbana, IL 61801.

Employing a perifusion system from which the release of endogenous norepinephrine (NE) and dopamine (DA) can be measured (Neurosci. Abst. 4, 267, 1978), we have investigated the effects of gonadal steroids on basal and 10^{-3}M d-amphetamine sulfate (AMT; initial conc. in chamber) stimulated release of catecholamines (CA) from brain tissue fragments.

Small pieces of tissue were taken from the medial basal hypothalamus (MBH) and striatum of intact males, castrated males + 500 µg testosterone propionate (CAST + TP) and castrated males + oil (CAST); as well as from ovariectomized females + 5 µg estradiol benzoate (OVX + EB) and OVX + oil. Tissue was taken 11 days after surgery; TP, EB or oil admin. last 4 days. Six animals were used for each of these perifusions. Striatal tissue was also taken from intact females on each of the 4 days of the estrous cycle, but only 1 animal was used for these experiments.



Release of NE and DA from striatal fragments of male and female rats by AMT: effect of castration and hormone replacement (n=3)

In the male groups, a pulse of AMT stimulated a dramatic release of both NE and DA from the striatum and MBH. Neither CAST nor CAST + TP had any effect on the AMT stimulated release of the CA's. In females, AMT stimulation of the striatum in OVX and OVX + EB did not result in release of either CA. In contrast, MBH taken from OVX + EB but not OVX + oil responded to AMT with release of both NE and DA. During the estrous cycle, there was a fluctuation in the responsiveness of striatal tissue to AMT. NE release was observed only on diestrus-2. However, release of DA was seen on all days of the cycle except proestrus. In addition, basal release of DA and NE was highest during proestrus, a time when tissue levels of DA are also their highest (Crowley et al., Brain Res. 147, 315, 1978).

In conclusion, a clear sex difference in the in vitro amphetamine-stimulated release of CA's from these two areas of the brain has been demonstrated. This AMT-stimulated release is dependent on the in vivo presence of gonadal steroids in females but not in males. (RIAS Grant NSF-SER 76-18255)

1479 HORMONE ACCUMULATION IN MOTONEURONS INNERVATING PENILE STRIATED MUSCLES IN THE RAT. S.Marc Breedlove and Arthur P. Arnold. Dept.Psychol. and Brain Res.Inst., UCLA, Los Angeles, CA 90024.

Male rats with a mid-thoracic spinal transection display clusters of penile flips in response to restraint of the preputial sheath (B.Hart, Science 155, 1283, 1967). In castrated males these spinal reflexes are maintained by daily injections of testosterone (T). These penile reflexes can also be maintained by the implantation of T in the spinal cord, without causing an increase in accessory sex gland weight (Hart & Haugen, Phys. & Bhr.3, 735, 1968). This latter finding, in conjunction with the report of Sar & Stumpf (Science 197, 77, 1977) that spinal motoneurons accumulate 5α-dihydrotestosterone (dhT) or its metabolites, suggests that the T may facilitate these reflexes by direct action on the spinal cord. We now report the use of horseradish peroxidase (HRP) to localize motoneurons innervating penile muscle that may be involved in these spinal reflexes. We also report autoradiographic studies showing that the motoneurons of this particular spinal region are labeled after injection of tritiated T or dhT, but not estradiol (E).

The muscle bulbocavernosus of male rats was injected with 15-30 µl of 30% HRP. After 24 hours the rats were sacrificed and the lumbar through coccygeal spinal cord removed, sectioned, and the HRP stained by a modification of the de Olmos method. Labeled motoneurons were consistently found in the medial ventral horn, adjacent to the ventral funiculus and 200-400 µ ventral to the central canal, from L4 to S1, especially L5 and L6.

For the autoradiographic study, radioactively labeled T, dhT, or E was injected i.v. into previously castrated and adrenalectomized male rats (N=3 per hormone). The rats were sacrificed 1 hour later, the spinal cord (L1 to Co3) frozen, sectioned, and processed for autoradiography. Following injection of labeled T or dhT, neurons that accumulated radioactivity (i.e. had 5 times the background level) included motoneurons in the area labeled following injection of HRP into the bulbocavernosus muscle. The hormones were also accumulated by other motoneurons of the ventral horn, lamina X of Rexed, the sacral and lumbar intermediolateral nucleus, and a few cells of the dorsal horn. These results agree with those of Sar & Stumpf (1977). Estradiol, or its metabolites, was not taken up by the motoneurons, but was taken up by neurons of lamina X and cells of the dorsal horn, especially laminae I and II, confirming the results of Pfaff & Keiner (J.Comp.Neurol. 151, 121, 1973) and Keefer et.al. (Proc.Soc.Exp.Biol.Med. 143, 414, 1973).

Supported by NSF Grant BNS 77-05973 to A.P.A.

1480 DOPAMINE ANTAGONIST ACTIVITY OF TETRAHYDROISOQUINOLINES. D. R. Britton, C. Rivier*, T. Shier*, F. Bloom and W. Vale. Peptide Biology Lab. and Alcohol Research Center, The Salk Institute, La Jolla, CA 92037.

Tetrahydroisoquinolines (TIQ's) have been tested for their ability to act as opiate and dopamine receptor agonists or antagonists in several systems. The TIQ's were tested for their in vivo and in vitro capacity to modulate prolactin (PRL) secretion from the pituitary and for their ability to antagonize the binding of ^{3}H-Naloxone and ^{3}H-Spiroperidol to membrane preparations from rat hypothalamus and from bovine anterior pituitary. Receptor binding studies demonstrate that some TIQ's could be classified as having higher affinity for opiate receptors (tetrahydropapaverine, papaverine, 6-methyl-salsolinol, i-carboxysalsolinol and 3,4-deoxynorlandanasoline carboxylic acid), others having higher dopamine receptor affinity (salsolinol and 7-methyl-salsolinol) or approximately equal affinity for the two binding sites (6,7-dimethylsalsolinol and tetrahydropapaveroline). Freely moving male Sprague-Dawley rats were prepared with indwelling venous catheters for administration of various TIQ's and blood sampling for assessment of PRL immunoreactivity. Tetrahydropapaveroline (THP) which was equipotent in binding to dopamine and opiate receptors produced a several-fold increase in plasma PRL. This finding might result from either an agonist effect at extrapituitary opiate receptors or a dopamine antagonist effect at the level of the pituitary. This latter hypothesis is supported by the observation that concomitant administration of the opiate antagonist, naloxone, did not block the stimulatory effect of THP on PRL secretion while dopamine significantly reduced THP-induced PRL secretion.

Incubation of rat anterior pituitary cells with TIQ's produced no changes in basal secretion of PRL. However, several TIQ's shown to compete with ^{3}H-Spiroperidol for binding sites were effective in reversing the inhibition of PRL secretion by dopamine. The potency of the various TIQ's in this system correlates well with their ability to displace ^{3}H-Spiroperidol from binding sites. The data derived from these three systems support the hypothesis that THP and other TIQ's stimulate PRL secretion by their ability to act as dopamine antagonists.

1481 CATECHOLAMINE SECRETION BY ELECTRICALLY STIMULATED SLICES OF BOVINE ADRENAL MEDULLA. J.C. Brooks, D.H. Burke* and S. Treml*. Dept. Basic Sci., Sch. Dent., Marquette Univ., Milwaukee, WI 53233.

Chemical stimulation has been used in numerous studies of catecholamine secretion from slices of adrenal medulla. However, chemical stimulation cannot simultaneously excite all of the cells in the slice because of the time required for diffusion of the secretagogue to reactive sites in the interior of the slice. In contrast, electrical stimulation should simultaneously excite all of the chromaffin cells in the tissue slice. Therefore, we felt that electrical stimulation might provide the basis for kinetic experiments involving stimulus-induced secretory processes, including ion movements, protein synthesis and catecholamine synthesis and release.

The studies reported here have been designed to determine if electrically-induced catecholamine secretion is real, and not a consequence of cell damage or secretion secondary to stimulation of cholinergic terminals remaining in the tissue. Thin slices of bovine adrenal medulla were stimulated in air with teflon mesh-covered platinum electrodes applied to two surfaces. Catecholamine secretion for 24 mm^2 slices was linearly related to voltage from 20-60 volts using the following stimulus parameters: square monophasic pulses of 0.8 m sec. duration, 50/sec for 10 sec. Secretion was not enhanced by increasing voltage over the range 60-100 volts. At 60 volts electrically-induced secretion is about 2.5 times that of unstimulated control slices, a value comparable to that for carbachol-induced secretion. Lactic dehydrogenase (LDH) release after stimulation was used to determine if apparent catecholamine secretion was due to electrically-induced cell damage. For both unstimulated and electrically stimulated slices, the amount of LDH release was about 7% of the total tissue content of the enzyme, indicating that secretion is not due to cell damage.

Electrically-induced secretion was not affected by the presence of the muscarinic antagonist atropine (0.01 mM) while 0.01 mM hexamethonium, alone or in mixtures with atropine, reduced secretion by 27%. Thus, approximately 75% of the electrically-induced secretion is due to direct stimulation of the chromaffin cells, the remainder being secondary to acetylcholine release by cholinergic terminals present in the tissue slice.

Acetylcholine-induced secretion from chromaffin cells is dependent upon extracellular calcium concentration. With electrical stimulation in calcium-free medium, secretion was reduced by only 19% compared to stimulation in complete or calcium-enriched media. Thus it appears that sufficient calcium for a nearly normal secretory response is available from internal cellular compartments.

Based upon these results, electrical stimulation is a useful means of evoking secretion. (Supported N.I.H. 1508 RR 09076-01).

1482 SERUM PROLACTIN IN HUMANS PARALLELS NEUROLEPTIC INDUCED CHANGES IN DOPAMINE RECEPTOR SENSITIVITY. Walter Armin Brown, Thomas P. Laughren# and Phillip H. Robzyk#. Neuroendocrine Res. Lab. VA Medical Center and Brown University, Providence, RI 02908

Studies of nigrostriatal and mesolimbic dopamine (D) activity in rats have consistently shown an increase in D receptor sensitivity and receptors following withdrawal from neuroleptic (N). We and others have also shown enhanced D receptor sensitivity in tuberoinfundibular D system following N treatment as indicated by changes in both baseline and apomorphine induced serum prolactin (pro) concentration. We now report that patients withdrawn from chronic N treatment, maintained drug-free for several months and then treated acutely with N, show systematic predictable shifts in serum pro which parallel the changes in central D activity observed in rat studies.

Subjects were 20 male schizophrenics who had been taking N for 2 to 15 years. They were maintained on their usual dose of N for 2 weeks, had N gradually withdrawn over 2 weeks, and were then maintained drug-free indefinitely or until symptoms warranted resumption of N. Blood samples were drawn between 8 and 9 a.m. once weekly following an overnight fast at which time patients also underwent clinical assessment. Serum pro was measured by radioimmunoassay.

Serum pro showed systematic changes over time during and following N withdrawal ($F=2.74$ $p<.025$). Mean pro fell rapidly during N withdrawal reaching its lowest point (2.8 ± 0.9 ng/ml) one week after complete withdrawal. After this first drug-free week, pro rose to a stable baseline level (3.9 ± 1.5 ng/ml) which showed little variation over the ensuing drug-free weeks and months. In all patients pro was lower after the first drug-free week than in subsequent drug-free weeks. The mean pro after return to N treatment was considerably higher (mean=86%) than that during chronic treatment. In all instances, pro was highest during the first week of acute N treatment showing a slight but consistent fall over subsequent weeks. Patients with tardive dyskinesia had a more rapid rate of fall in pro following N withdrawal than those without tardive dyskinesia, and also showed the greatest change in pro between chronic and acute N treatment.

Our findings of transient enhanced inhibition of pro secretion following N withdrawal and both early and delayed tolerance to the pro elevating effects of N parallel the known N-induced changes in receptor sensitivity and D turnover. These data suggest that assessment of serum pro under suitably controlled conditions may provide a method for identifying patients who have disorders involving alterations in central D systems.

1483 EFFECTS OF ADIPSIA-PRODUCING LESIONS OF THE ANTEROVENTRAL THIRD VENTRICLE (AV3V) ON FINE STRUCTURE OF THE SUPRAOPTIC NUCLEI AND NEURAL LOBE IN THE RAT. J.R. Carithers*, H.-D. Dellmann*, S.L. Bealer, M.J. Brody and A.K. Johnson (Spon: D.G. Emery). Dept. Vet. Anat. ISU, Ames, IA 50011 and Dept. Pharm. Sch. Med., SUI, Iowa City, IA 52240.

Small lesions of the AV3V in rats cause adipsia, but there is no compensatory antidiuresis, and plasma levels of antidiuretic hormone (ADH) are lower than control values. Therefore, the ultrastructure of the supraoptic nucleus (SON), the main site of synthesis of ADH, and the neural lobe of the hypophysis (NL), where ADH is stored and released, were examined in rats which had received adipsia-producing lesions in the AV3V three days earlier. Controls were rats which had received sham lesions three days earlier and intact rats deprived of water for three days. The fine structure of the SON and NL of sham lesioned rats resembles that of intact animals. Neuronal somas in SONs of water-deprived rats show signs of stimulated secretory activity. The sizes of the cells, their nuclei and especially nucleoli are increased, endoplasmic reticulum is hypertrophied and more often dilated and a striking accumulation of neurosecretory granulated vesicles (NGV), especially dense immature forms, occurs. A considerable decrease in the number of NGV is observed in many axons of the NL, and numerous axon terminals are devoid of NGV and contain only electron lucent vesicles of varying sizes; frequently an extensive smooth axonal endoplasmic reticulum and large vacuoles occur within these axons.

In rats rendered adipsic by AV3V lesions, most neuronal somas in the SON resemble those of sham lesioned animals, although occasional cells contain accumulations of NGV. Degenerating fibers are common, and nerve terminals undergoing degeneration are present in axodendritic synapses and synapses on neurosecretory somas. In the NL of AV3V-lesioned rats many axons and axon terminals are enlarged and contain an increased number of NGV, usually densely packed; abundant dense bodies and multilamellar bodies are evidence of increased crinophagic activity. Occasional axons are degenerating and being phagocytosed by pituicytes.

Responses to osmotic stimuli and to angiotensin are abolished or severely attenuated in rats with AV3V lesions. It appears likely that degenerating axons and terminals present in SONs of lesioned rats are processes of osmoreceptors and angiotensin receptors, the somas or fibers of which are damaged by effective lesions. Consequently, in this study the ADH-producing neurons were not stimulated by the severe dehydration to release their NGV. Their accumulation in the NL indicates that synthesis and transport of NGV is not impaired within this time period.

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1484 TEMPORAL EFFECTS OF MELATONIN: MORNING INJECTIONS OF MELATONIN ABOLISH THE REPRODUCTIVE INHIBITORY EFFECTS OF AFTERNOON INJECTIONS OF MELATONIN IN THE FEMALE SYRIAN HAMSTER. H. J. Chen*, G. C. Brainard*, and R. J. Reiter, Dept. of Anatomy, UTHSC at San Antonio, Texas 78284.

In male or female Syrian hamsters kept under a light:dark cycle of 14:10, melatonin (Mel) exerts inhibitory effects on gonadal function only if injected in the late afternoon or shortly before the onset of darkness. In male hamsters, such an effect can be blocked by the subcutaneous implantation of Mel pellets (Reiter et al., Endocr. Res. Commun. 4:35, 1977). The purpose of the present experiment was aimed at determining if the inhibitory effects of afternoon (p.m.) administration of Mel on gonadal function could be blocked by a large dose of Mel given in the morning (a.m.) or by 5-methoxytryptophol (5-Mtol) given 1 hour preceeding the p.m. injection of Mel. Female Syrian hamsters, 3-4 months of age, were kept under LD 14:10 (lights were on from 0600 to 2000 hours) and were given either vehicle (Veh) at both 11 a.m. and 5 p.m. (controls); 1 mg Mel at 11 a.m. and Veh at 5 p.m.; 1 mg Mel at 11 p.m. and 25 µg Mel at 5 p.m.; 100 µg 5-Mtol at 4 p.m. and 25 µg Mel at 5 p.m.; or Veh at 4 p.m. and 25 µg Mel at 5 p.m. Daily vaginal discharges were examined to determine the stage of the estrous cycle and, at the end of the experiment, reproductive organ weights were recorded. By the eighth week of treatment, 80 to 100% of animals in groups treated with 25 µg Mel at 5 p.m. and Veh in the morning, or 5-Mtol or Veh at 4 p.m. stopped cycling. Their uteri were infantile in appearance and uterine weights were significantly ($P<0.001$) depressed. Controls or animals injected with 1 mg Mel at 11 a.m. and Veh or 25 µg Mel at 5 p.m. demonstrated normal 4-day estrous cycles throughout the experiments. Uterine weights were 3 times larger than those of the animals treated with p.m. Mel without a.m. Mel. These results indicate that a single injection of 1 mg of Mel in the morning can render the neuroendocrine-reproductive axis of the female Syrian hamster completely refractory to the inhibitory effect of the late afternoon injections of Mel. It is postulated that the relatively large dose of Mel given in the morning had so saturated the Mel receptors of the target tissues that the p.m. injection of Mel could no longer exert its inhibitory effects. Presumably the Mel receptors are down regulated by the administration of the indoleamine itself. (Supported by NSF Grant No. PCM 77-05734).

1485 THE RESPONSE OF HIPPOCAMPAL CA1 PYRAMIDS TO GONADAL STEROIDS: In Vivo Effects of Estradiol in Male Rats. Nicolas L. Chiaia, Richard M. Vardaris, and Timothy J. Teyler. Kent State Univ., Kent, OH 44242 and Northeastern Ohio Universities College of Medicine, Rootstown, OH 44266.

In a prior experiment we observed gender-related changes in the excitability of CA1 pyramidal cells when gonadal steroids were administered to hippocampal slice preparations (Vardaris & Teyler, 1978, Neurosci. Abst., no. 1145). Enhanced amplitudes of CA1 field potentials were obtained from male slices 10 and 20 minutes after exposure to 100 pM 17-beta-estradiol in the superfusate. The present study was designed to determine whether similar effects occur in the intact preparation.

Male rats were curarized and respirated with room air and metafane gas during stereotaxic surgery. Wound margins and pressure points were procainized. Final placement of the recording micropipette tip in the cell body layer of CA1 and the tip of the steel stimulating electrode in the Schaffer collateral system was verified electrophysiologically through laminar analysis. A presteroid input/output function was recorded 40 minutes after withdrawal of general anesthesia. Stimulus voltages were selected to permit recordings of extracellular EPSPs as well as population spikes. The presence of paired-pulse facilitation and recurrent inhibition served as criteria for appropriate physiological status of the preparations.

One hundred and 200 microgram doses of 17-beta-estradiol benzoate were administered by intraperitoneal cannulation. Input/output functions were obtained 20, 40, and 60 minutes after infusion of the steroid. The same series of stimulus intensities was used in all input/output functions for a given preparation. Analysis of the recorded potentials revealed that both dosages increased the amplitudes of monosynaptic EPSPs and population spikes in the CA1 subfield. This effect was reflected in steepened slopes of the post-steroid input/output functions with no significant change in thresholds.

These results are consistant with our findings using in vitro slices from male hippocampus. It thus appears that the changes in excitability produced by estradiol can be obtained from intact preparations in the presence of normal metabolism.

1486 BIOCHEMICAL CHARACTERIZATION OF LHRH ACTIVITY IN HYPOTHALAMUS, PITUITARY PORTAL PLASMA AND SYSTEMIC PLASMA OF RAT. Melvin Ching, Dept. Anat., Med. Col. of Virginia, Richmond, Va. 23298.

Current studies focused on the separation, by electrophoresis followed by solvent run on TLC plates, of LHRH immunoreactive elements in methanol or acetic acid-ethanol extracts of systemic plasma, portal plasma and hypothalami. The specificity of our radioimmunoassay was re-examined by quantitating the displacement of radiolabelled LHRH from the Sorrentino E22 and F3 antibodies by LHRH analogues relative to equal weights of synthetic LHRH. RIA of eluates of silica gel scrapes from all TLC plates to which extracts were applied revealed LHRH activity confined to a radius not exceeding 2 cm from where synthetic LHRH spots. This region comprised less than 3% of the plate's surface area. Patterns of peak immunoreactive LHRH distribution of both extracts of systemic plasma were similar and fell within the zone of 3H-LHRH migration. LHRH extracted from portal plasma was more evenly dispersed over a wider area encompassing zones of radiolabelled and non-labelled synthetic LHRH migration. Hypothalamic LHRH also displayed a dispersed profile, similar to that of portal plasma, but with more components that exhibited lower and higher electrophoretic mobility. Only portal extracts exhibited a sizeable component (25%) having slightly lesser Rf values. Progressive declines in displacement of radiolabelled LHRH from the E22 antibody occurred with D ala^6 (67%), D phe^2, D ala^6 (51%), Des gly^{10} ethylamide (21%), and D ala^6, Des gly^{10} ethylamide (4%) LHRH analogues when compared to synthetic LHRH (100%). However, amino acid substitutions at the 6 or 2 and 6 positions resulted in marked enhancement of binding affinity to the F antiserum (522% and 576%, respectively) whereas removal of glycine at the C-terminal with or without alanine substitution at position 6 caused precipitous declines in relative activity (1% and 0%, respectively). Moreover, 11 TLC zones were simultaneously tested for LHRH activity with E and F antisera and in 5 of these the F antiserum measured more than 10 times the quantity of LHRH as the E. Precipitous declines in exogenous LHRH activity in heated (30min at 60 deg. C) as well as unheated plasma occurred during the first 10min followed by smaller decrements or a plateauing of hormone decline over the remaining 30min to a final concentration of 100-300pg/ml. Thus, these studies suggest that the LHRH immunoreactive elements in hypothalamus, portal plasma and systemic plasma are biochemically similar with perhaps only minor modifications in molecular structure or configuration. Moreover, they point to the possible existence of a heat resistant enzyme whose action appears directed at the C-terminal. The possibility remains that more than 70% of the radioimmunoassayable LHRH in the peripheral circulation may exist as the Des gly^{10} analogue. These studies reiterate the importance of characterizing the antiserum employed in the radioimmunoassay. (Supported by USPHS Grant HD 12276).

1487 EFFECTS OF SEPTAL LESIONS WITH KAINIC ACID IN PREPUBERAL FEMALE RATS ON THE RELEASE OF LUTEINIZING HORMONE AND THE ONSET OF PUBERTY. Richard W. Clough* and Jorge F. Rodriguez-Sierra. Dept. Anat., Univ. Nebraska Med. Ctr., Omaha, NE 68105

Estradiol benzoate (EB, 5 µg/rat, sc in oil) injected into 25 day old Sprague-Dawley female rats results in a pronounced increase of plasma luteinizing hormone (LH) released from the pituitary gland during the afternoon two days later. Kainic acid (KA, 2.5 µg/2 µl), an excitotoxic analogue of glutamic acid, or saline were injected into the septal region prior to administration of EB. Plasma samples were obtained by jugular puncture at 1000 and 1600 hours two days after EB. Determination of plasma LH was made by RIA. Intraseptal administration of saline had no effect on the EB induced LH surge in plasma, in contrast to the administration of KA which markedly inhibited the response:

		Mean Plasma LH (ng/ml)	
Groups	(n)	1000 h	1600 h
Controls	6	15.8 ± 3.3	2918.3 ± 800.3
Saline infusion	6	22.5 ± 5.7	2521.2 ± 677.2
KA infusion	5	32.4 ± 10.5	130.2 ± 27.3

In order to test whether the septal area was involved in the normal onset of puberty in the female rat, a different group of animals received septal KA or sham lesions at 25 days of age and were monitored daily for vaginal opening (VO) and vaginal smear cyclicity. KA lesioned animals showed a delayed onset of VO in contrast to that of control rats. Sham-lesioned rats had a slight delay in the onset of their VO, probably due to non-specific damage by the vehicle saline infused into the septal area. Vaginal cyclicity was normal in all animals after VO. All rats reached puberty at approximately the same body weight, pointing to a delay in somatic growth in the KA and sham lesioned groups. Since KA destroys cell bodies without disrupting fibers of passage, our results suggest that intrinsic septal neurons are involved in the ontogenesis of the positive feedback of estrogen on LH release. In addition, our results point to a role of the septal area in the normal somatic development of the female rat and the development of events that lead to the onset of adult female reproductive ability. Supported by NIH grant HD-11011 and NSF RIAS-SER77-06922.

1488 CHANGES IN PITUITARY AND PLASMA LH LEVELS IN OVARIECTOMIZED RATS: EFFECTS OF NALOXONE, ACTH, PHOTOPERIOD AND ADRENALECTOMY. Ilene R. Cohen* and David R. Mann* (SPON: Jane Bos Downer). Dept. Biol. Sci., SUNY, Binghamton, NY 13901, and Dept. Physiol., School of Medicine, Morehouse College, 223 Chestnut St., SW, Atlanta, GA 30314.

The ability of naloxone to inhibit the actions of ACTH on LH secretion was examined in ovariectomized rats maintained in either a 14h light:10h dark (14:10) cycle or an 8h light:16h dark (8:16) schedule. Adult rats were ovariectomized (OVX) or ovariectomized and adrenalectomized (OVX/ADX) and implanted sc with a silastic capsule of naloxone. Beginning the following day and continuing for seven days rats received daily injections of ACTH (4IU/100g BW) at 1000h. Animals were sacrificed by decapitation at 1200h on day 7. Regardless of treatment, OVX animals housed in 8:16 had higher pituitary LH concentrations than those housed in a 14:10 schedule (43.9±2.5 vs 34.5±1.7µg/mg, $p<.002$). This effect of photoperiod was enhanced by naloxone; in 8:16 pituitary LH values were elevated by naloxone (47.5±4.2 vs 40.2±2.3µg/mg for sham implanted rats), and in 14:10 they were reduced (32.4±2.6 vs 36.4±2.1µg/mg for sham implanted rats). ACTH treatment did not affect pituitary LH levels in OVX animals. Plasma LH levels in OVX rats were not significantly altered by photoperiod or any treatment. Very different results were obtained with OVX/ADX rats. No effect of photoperiod on pituitary or plasma LH was seen in these animals. ACTH treatment increased pituitary LH (29.3±2.1 vs 23.8±1.5µg/mg for saline injected rats, $p<.03$), but had no effect on plasma LH. Naloxone significantly lowered plasma LH in OVX/ADX animals regardless of treatment or photoperiod (187.3±15.5 vs 283.7±25.4 ng/ml for sham implanted rats, $p<.002$), while having no effect on pituitary LH levels. Our data demonstrate that: (1) the action of naloxone on pituitary LH is influenced by the length of the photoperiod and the presence of the adrenal glands is necessary for this effect, and (2) only in the absence of the adrenal glands did ACTH elevate pituitary stores of LH. (Supported by NSF Grant no. PCM 79-06684)

1489 LOCAL INCREASE IN THE UPTAKE OF [^{14}C] 2-DEOXYGLUCOSE AFTER ELECTROCHEMICAL DEPOSITION OF IRON IN THE BRAIN. J.A. Colombo and S. Saporta, Department of Anatomy, College of Medicine, University of South Florida, Tampa, Florida 33612.

Since the original description by Everett and Radford (1961), electrochemical stimulation technique has been widely used in the field of neuroendocrinology. It has been assumed that delivering anodic direct current through a stainless steel electrode increases local neuronal activity, and that this increase in activity is mediated through the release of iron ions into the surrounding brain tissue. One of us has previously shown that multiunit activity recorded within the area of iron deposition undergoes a significant increase (Colombo et al, 1974; 1975). We now report evidence that electrochemical stimulation will increase the uptake of [^{14}C] 2-deoxyglucose within the area of iron deposition in the brain. Adult male rats were anesthetized with pentobarbital and an indwelling catheter placed in one femoral vein. A pair of electrodes were lowered stereotaxically into the preoptic suprachiasmatic region. The electrode pair consisted of an insulated 00 stainless steel insect pin and a 125µm teflon insulated platinum wire. The electrodes were allowed to stabilize for 10 minutes before the onset of 30µA ADC stimulation delivered for 30 sec sequentially through each electrode. Three to four minutes after stimulation, a bolus of 40µCi/100g body weight of [^{14}C] 2-deoxyglucose was injected in 0.5 ml of saline. The injection was completed in 1 minute. Thirty minutes later, the animal was decapitated, the brain removed and frozen. Brain sections (20µm) were taken alternately for either autoradiography or for identification of the area of iron deposition with Gamori's iron stain. The sections were exposed to x-ray film for 6 days. Autoradiographs show a clear increase in the concentration of the radiolabeled 2-deoxyglucose which overlaps with the area of iron deposition. A comparatively reduced concentration of labeled 2-deoxyglucose is visible immediately surrounding the lesioned area at the tip of the platinum electrode. These results indicate that iron deposition produced by electrochemical means results in increased local metabolic activity of the exposed neuronal population. Experiments with [^{3}H] 2-deoxyglucose are currently under way in order to analyze this effect in a more quantitative manner.

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1490 EFFECTS OF GONADAL STEROIDS ON THE DEVELOPMENT OF THE HAMSTER CIRCADIAN PACEMAKER. Fred C. Davis*, Janet D. Alvis* and M. Menaker. Institute of Reproductive Biology, University of Texas, Austin, TX 78712.

Sexual dimorphism of the circadian pacemaker that underlies locomotor activity of hamsters has been previously reported (Alvis et. al., 1978, The Physiologist 21:). This dimorphism, measured as a difference in the ability of males and females to entrain to a light/dark cycle with a period greater than 24 hours (24.75), persists following castration, demonstrating that the dimorphism does not depend on circulating gonadal steroids or on estrus cyclicity. This suggests that sexual differentiation of the pacemaker occurs during development. The present study was carried out to determine if neonatal (within 24 hours of birth) castration or neonatal treatment with testosterone propionate affects the differentiation of the circadian pacemaker underlying locomotor activity in hamsters.

Three treatment groups were studied:

1) Neonatally sham castrated males and oil injected females
2) Neonatally castrated males and females
3) Neonatally injected (TP, 600µg,SC) males (simultaneously castrated) and females (castrated at 21 days of age).

Entrainment behavior of these groups was observed under a light/dark cycle identical to that used by Alvis et. al. The results are summarized below.

1) As previously reported, intact males showed a greater ability to entrain to this cycle than did females.
2) There were no differences among the three groups of males.
3) Both castrated and TP injected females were masculinized relative to intact females.

These results indicate that neonatal castration does not influence the male circadian pacemaker as tested in this entrainment paradigm. The female pacemaker, however, was dramatically affected by castration. Any effect of TP on the females is obscured by the prepubertal castration of these females. Confirmation of the importance of the ovaries will depend on additional studies that include prepubertal castration, neonatal estrogen treatment, and long term adult castration. Because the mechanisms underlying ovarian cyclicity include a circadian pacemaker, the present findings suggest that differentiation of the pacemaker may be involved in the differentiation of reproductive control mechanisms.

Additional evidence that gonadal steroids influence the mechanisms of circadian rhythmicity was seen in the effects of TP on the day to day variation of activity onset in males. The single TP injection restored stability that would otherwise be lost as a result of neonatal castration. Supported by NIH grant HD-03803

1491 RAT PARS INTERMEDIA ACTION POTENTIALS AND THEIR RESPONSE TO VARIOUS AGENTS.

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Action potentials have recently been recorded from a number of endocrine glands and their tumors. It appears that this electrical activity is related to cell secretion. Prolactin, from the pars distalis, and MSH, from the pars intermedia, have several phenomona in common. They both respond to similar releasing or inhibiting factors of hormone release, both spontaneously secrete in the absence of hypothalamic control, and action potentials can be recorded from either one. Thus, data obtained from prolactin secretion seems to fit well with MSH secretion and vice versa. There is difficulty in obtaining information on electrical activity from the pars distalis because of its heterogeneous population of cells. One has a hard time defining exactly which cell is being recorded from and consequently must usually rely on tumors. The pars intermedia lobe has the advantage of having relatively few cell types and presents a better target for electrophysiological studies.

Following the decapitation of 250 gram rats (SD), neurointermediate lobes, containing the pars intermedia and pars nervosa complex, were carefully separated from the rest of the pituitary and placed in a container with Kreb's Ringer. The preparation was then gassed in a 95% O_2-5%CO_2 mixture. Glass microelectrodes, filled with 4M NaCl, were lowered into the tissue to record extracellular potentials. When a cell with spontaneous electrical activity was found, various agents were added to the surrounding media and any changes observed. At a 10^{-6}M concentration of isoproterenol, a rapid increase in spike frequency was found, lasting several minutes. High potassium (10X) and norepinephrine (10^{-5}M), on the other hand, caused a decrease in spike frequency and, in some cases, eliminated the activity all together. The latency between the application of experimental solutions and the onset of any change was about 30 seconds. Therefore, agents which enhance or reduce the release of MSH are correlated with an increse or decrease in the frequency of action potentials in the pars intermedia.

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1492 AGGRESSIVE BEHAVIOR IS DEPENDENT ON GONADAL STEROIDS IN MALE BUT NOT FEMALE RATS.

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Intense and frequent male aggression is seen when a strange male rat is placed into a small colony containing resident male and female rats. Under these circumstances, males and females differ markedly in their level of aggression. However, when confronting a strange female, the pattern of male-female differences reverses. The present study compared the aggressive behavior of male and female colony residents in response to male and female intruders and the importance of hormonal condition for attack.

Ten small colonies were established each with two Long-Evans male rats and two females. After each colony began producing litters they were tested for response to a male intruder. Within a few weeks one of the two males in each colony reliably attacked male intruders. This alpha male and one of the two females were then tested together biweekly for response to male and female intruders. Male residents intensely attacked intact, castrated and testosterone propionate (TP) treated male intruders; less male aggressive behavior was seen toward castrated intruders treated with estradiol benzoate (EB) or EB plus progesterone (P). Male residents rarely attacked or mounted female intruders independent of the hormonal condition of the females. Female residents, on the other hand, intensely attacked all female intruders, except for lactating intruders. Females also attacked castrated male intruders which had been treated with EB or EB + P. Less female attack was directed at intact and castrated TP males. Female aggressive behavior was not correlated with the female residents' reproductive condition (e.g. pregnancy, lactation). In a second series of experiments the importance of resident endocrine condition was examined. Half of the resident males and females were gonadectomized and then tested for aggressive behavior for 7 weeks. The intensity and probability of male attack toward male intruders declined slowly after castration. The rate of decline in male aggressive behavior by castrates was similar to the decline of their mounting of receptive females seen in parallel tests for sexual behavior. Ovariectomy was not followed by any decrease in female aggression toward female intruders. In fact, ovariectomized females were somewhat more aggressive than gonadally intact females.

These experiments demonstrate that both male and female rats can be highly aggressive but that this behavior is sexually dimorphic, both in terms of the characteristics of the most effective stimuli and in the gonadal control of the behavior. The hormonal determinants of male and female aggressive behavior are obviously quite different.

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493 EFFECTS OF INTRAVENOUS DOPAMINE ON HUMAN SERUM PROLACTIN ON AND OFF NEUROLEPTICS.

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The role of central and peripheral dopamine in the regulation of prolactin (PRL) secretion has been studied extensively. Alterations in dopaminergic function (which have been implicated in the pathogenesis of schizophrenia) are reflected in changes of circulating levels of serum PRL. We report here the effects of sustained intravenous dopamine administration on PRL secretion in normal unmedicated male volunteers (N = 6), and in male schizophrenic patients (N = 6) during and following discontinuation of treatment with a dopamine receptor blocker (pimozide). Dopamine was infused at a rate of 4 ug/Kg/min. for three hours. Blood samples were collected every 15 min. one hour prior to, during and two hours postinfusion. Six patients were infused on chronic pimozide and the procedure was repeated twice, one and four weeks following treatment discontinuation. In normal subjects circulating PRL levels were significantly suppressed during the infusion; following cessation of the procedure PRL rebounded to levels significantly above baseline. Baseline serum PRL was significantly higher in patients on pimozide compared either to controls or to the same patients off pimozide. No significant differences in baseline serum PRL were detected between one and four weeks off pimozide in patients. Prolactin secretion was suppressed by dopamine under all three experimental conditions. However, percent suppression of serum PRL was greater when patients were on or one week off compared to four weeks off pimozide. In addition, rebound of serum PRL above baseline levels occurred only when patients were off pimozide four weeks. The implications of these findings regarding (a) the effects of neuroleptic withdrawal on dopaminergic function and (b) the regulation of PRL secretion by peripheral and central dopamine will be discussed.

1494 TUBEROINFUNDIBULAR DOPAMINERGIC NERVE ACTIVITY ESTIMATED FROM THE ACCUMULATION OF DOPA IN THE MEDIAN EMINENCE.

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The rate of accumulation of DOPA in the striatum (ST) following the administration of a decarboxylase inhibitor has been used to estimate the activity of nigrostriatal dopaminergic nerves (Carlsson et al., Pharmacol. Rev. 24: 371, 1972). The present study was undertaken to determine if DOPA accumulation in the median eminence (ME) could be used to estimate the activity of tuberoinfundibular dopaminergic nerves. The concentration of DOPA in ME and ST of the rat was determined 30 min after the administration of 3-hydroxybenzylhydrazine (NSD 1015; 100 mg/kg, i.p.) by a radioenzymatic assay sensitive to 50-125 pg of DOPA. In this assay tissue extracts containing DOPA are incubated with catechol-O-methyltransferase and ^{3}H-S-adenosylmethionine and the radioactive product, 3-methoxytyrosine, separated by ion-exchange chromatography and charcoal adsorption. Since the ME, unlike the ST, contains appreciable amounts of norepinephrine (NE), experiments were designed to estimate the relative contributions of the synthesis of DA and NE to the observed accumulation of DOPA. The accumulation of DOPA in ME was essentially the same in control rats and in rats treated with intraventricular injections of 6-hydroxydopamine in order to selectively deplete NE. This result is consistent with the observation that the rate of synthesis of DA greatly exceeds that of NE in the ME as calculated from the rate of decline of these amines following the administration of α-methyltyrosine (αMT). These results suggest that DOPA accumulation in the ME can be used as an index of tuberoinfundibular DA nerve activity. To confirm this proposal the accumulation of DOPA was analyzed after pharmacological manipulations which have been shown previously (using the αMT-induced decline of DA) to selectively alter DA neuronal activity in the ST and/or ME. Ninety minutes after the administration of the DA agonist, piribedil (30 mg/kg, s.c.), DOPA accumulation was reduced in the ST but not in the ME. The DA antagonist, haloperidol (2.5 mg/kg, s.c.), increased DOPA accumulation in the ST at 2 and 8 hr after injection and in the ME at 16 hr after injection. Three daily injections of estradiol benzoate (25 µg/kg, s.c.) increased the accumulation of DOPA in ME, but not ST; this estrogen-induced increase in DOPA accumulation in ME was not observed in hypophysectomized rats. These results are consistent with previous findings utilizing the decline of DA after αMT to estimate DA turnover in ME and ST, and they indicate that DOPA accumulation after NSD 1015 provides a reliable means of estimating the activity of tuberoinfundibular DA nerves. (Supported by USPHS Grant NS 9174 and Fellowship NS 6026.)

1495 EFFECTS OF ESTROGEN TREATMENT ON NORMAL AND SENSITIZED RAT STRIATAL DOPAMINE (DA) RECEPTORS AND DA-SENSITIVE ADENYLYL CYCLASE. T. Di Paolo*, F. Labrie*, A. Dupont*, N. Barden* and P. Langelier* (SPON: S. Radouco-Thomas). Laboratory of Molecular Endocrinology, CHUL, Québec, G1V 4G2, Canada.

Estrogen treatment has recently been found to exert potent antidopaminergic activity at the pituitary and striatal levels. Such treatment decreases the apomorphine-induced acetylcholine accumulation in rat striatum. Since behavioral supersensitivity to DA drugs has been associated with increased striatal DA receptor levels, we have examined the effect of estrogen treatment on this parameter. Estrogen treatment (17ß-estradiol, 10 µg twice a day for 7 days) of adult ovariectomized rats led to a small increase (20%, $p < 0.05$) of [^{3}H] spiroperidol binding in rat striatum, nucleus accumbens + olfactive tubercle and frontal cortex. The increased binding is due to a corresponding increase in the number of binding sites with no change of affinity. Specificity of binding remained unchanged after estrogen treatment. In order to dissociate possible pre- and post-synaptic effects of estrogens, the binding of [^{3}H] apomorphine and [^{3}H] -spiroperidol was studied in animals bearing an unilateral lesion of the substantia nigra or injected at the same site with 6-OH-DA. The striatal [^{3}H] apomorphine and [^{3}H] spiroperidol binding sites were 30-50% increased on the lesioned side with no change of apparent dissociation constants. The stimulatory effect of estrogen treatment was of similar magnitude on the intact and lesioned sides with no change of affinity for the two labeled ligands. Lesion of the substantia nigra reduced basal adenylate cyclase by 50% but increased the sensitivity and maximal response to DA. Chronic treatment with estradiol failed to modify striatal adenylate cyclase of normal or supersensitized (following lesion) striatal tissue. The present data suggest that the potent desensitizing effect of estrogen treatment on DA action at the striatal level is exerted at a step subsequent to binding to the DA receptor and activation of adenylate cyclase. Moreover, they indicate that the estradiol-induced hyposensitivity and lesion-induced supersensitivity are caused by different mechanisms.

1496 SEXUAL BEHAVIOR IN MALE RATS FOLLOWING INTRACEREBRAL ESTROGEN Gary P. Dohanich* and Ingeborg L. Ward* (SPON: O. B. Ward, Jr.) Dept. Psychol., Villanova Univ., Villanova, PA 19085.

Sexual behavior and reproductive morphology were examined in gonadally-intact male rats following implantation of estradiol benzoate (EB) or cholesterol into the medial preoptic area (MPOA). Three groups of males with varying potentials for lordotic and ejaculatory behavior were chosen. These were normal copulators, normal noncopulators and prenatally stressed noncopulators. Previous studies have indicated prenatally stressed males to have a high potential for lordotic behavior (Ward, Science 175:82, 1972). Every male implanted with EB (N=17 into the MPOA showed lordotic behavior within 7 days of chronic treatment. There were no significant group differences. Highest levels of responding were obtained after 13-16 days (LQ=70-82). Cholesterol (N=15) was not effective. The study provides the first unequivocal demonstration that, like females, male rats are capable of high levels of lordotic behavior following application of EB to behaviorally sensitive CNS sites.

Central EB failed to activate male copulatory behavior in both the control and prenatally stressed noncopulator group. Ejaculatory behavior was impaired in some males that had copulated prior to central EB treatment. Animals treated with EB for 19 days also showed a significant depression in the weight of the testes and epididymis suggesting that EB applied to the MPOA reduces androgen secretion in intact males by acting on the CNS-pituitary-gonadal feedback system.

1497 HYPERPROLACTINEMIA AND MATING BEHAVIOR IN THE MALE RAT: EFFECTS OF PREVIOUS SEXUAL EXPERIENCE AND BROMOCRIPTINE TREATMENT. P.C. Doherty, Jr.*, A. Bartke*, and M.S. Smith*. (SPON: N. Hagino) Depts. of Anat. and Ob/Gyn, University of Texas Health Science Center, San Antonio, TX 78284 and Dept. of Physiol., University of Massachusetts Medical School, Worcester, MA 01605

We have recently reported that hyperprolactinemia induced by grafting four pituitaries under the kidney capsules suppresses mating behavior in male rats and mice (Svare et al., Biol. Reprod., in press). We have also noted that sexual experience after induction of hyperprolactinemia can attenuate the behavioral deficits produced by grafting. To determine whether sexual experience prior to grafting can alter the effects of hyperprolactinemia, the effects of pituitary grafts on sexual behavior were compared in experienced and naive male rats. Sexual experience was provided by housing males with cycling females until spermatozoa were found in the vaginal smears (taken daily), and by testing with an ovariectomized receptive female. Three weeks after pituitary transplantation or sham surgery, evaluation of copulatory behavior was begun. Males were placed in observation chambers with ovariectomized females made receptive by injections of estrogen and progesterone and were scored for mount latency, number of mounts, intromission latency, number of intromissions, ejaculation latency and number of ejaculations. Each test lasted for a period of one hour. Pituitary grafts had a significant suppressive effect on mating behavior in both groups of animals. Experienced animals showed deficits in all parameters studied except the number of mounts, while the naive animals differed from their respective controls only in latency to mount, latency to intromission and number of intromissions.

Treatment with bromocriptine (CB-154, an inhibitor of prolactin release) at 1 mg/day for 14 days, injected 12 hrs. prior to testing, led to increases in sexual activity in both the shams and the grafted animals. Although there were no significant differences in individual measures of behavior in CB-154 treated and control rats, the frequency of measures showing improvement was significantly greater in the CB-154 treated animals.

These results indicate that 1) hyperprolactinemia induced by pituitary grafts can cause deficits in mating behavior in male rats despite previous sexual experience, and 2) that the inhibition of prolactin secretion by bromocriptine may not be the sole mechanism by which CB-154 improves sexual behavior in hyperprolactinemic animals.

1498 CORTICOSTERONE AND RNA METABOLISM IN THE RAT HIPPOCAMPUS. Linda A. Dokas* (Spon: H. J. Waller), Department of Biochemistry, Medical College of Ohio, Toledo, OH 43699

Recent reports have provided evidence that corticosteroids may have direct biochemical effects upon the hippocampus. Corticosterone, the functional glucocorticoid in the rat, enhances the synthesis of a particular hippocampal protein of 54,000 daltons (Etgen, Lee and Lynch, Brain Res. 165:37). Adrenalectomy and glucocorticoid replacement have effects on a high affinity uptake system for GABA in hippocampal synaptosomes (Miller, et al., Psychoneuroendocrinology 3:155). Since autoradiographic evidence suggests that effects of corticosterone in the hippocampus are mediated by nuclear localization of the steroid with subsequent incorporation of [^{3}H]-uridine into RNA, the present study was initiated to characterize hippocampal RNA metabolism in the rat in response to corticosterone.

The hippocampus was removed from anaesthesized adult male Sprague-Dawley rats before 11 a.m. to minimize the endogenous levels of corticosterone in the animals. Each hippocampus was halved bilaterally and divided between Hepes-salts-glucose media with and without 1.8×10^{-8}M corticosterone. Following incubation of the tissue from 4 animals for 1.5 hrs ± steroid, 100µCi of [^{3}H]uridine was added to each incubation for an additional 2.5 hrs. Incorporation of the [^{3}H]uridine into RNA was measured as total TCA-precipitable radioactivity/mg protein, 85% of which was RNase-sensitive. The uptake of [^{3}H]uridine into hippocampal tissue was determined as TCA-soluble radioactivity/mg protein. Incubation with corticosterone decreased the labeling of the hippocampal TCA-soluble fraction by -17.3 ± 1.5% (n=6), relative to controls. In spite of this reduction in the labeled RNA precursor pool, corticosterone-treated hippocampal tissue showed incorporation of [^{3}H] uridine into total RNA equal to or greater than that seen in control incubations. The TCA-precipitable radioactivity to total radioactivity ratio for corticosterone incubations showed an increase of +24.8 ± 7.3% (n=6) as compared to control incubations. When cytoplasmic and nuclear fractions were prepared, increased incorporation of [^{3}H]uridine into RNA in response to corticosterone was found in the nuclear fraction only, where a +32.0 ± 10.4% (n=3) increase was seen, while there was no measureable increase in cytoplasmic RNA labeling. This work indicates that corticosterone induces alterations in both hippocampal RNA and RNA precursor metabolism. The increased labeling of nuclear RNA in response to corticosterone may represent synthesis of hippocampal hn nuclear RNA species which serve as precursors for particular mRNA fractions whose translation mediates the final neurochemical events which are a consequence of the binding of corticosterone to hippocampal receptors. Supported by NIH Endocrinology Training Grant 94120.

1499 ANALYSIS OF THE INFLUENCE OF TESTOSTERONE AND TRIAMCINALONE ON DIFFERENT SKELETAL MUSCLE GROUPS by Debra DuBois*, Joseph E. Tropea*, Joan Warrenski*, and Richard R. Almon, from the Division of Cell and Molecular Biology, Department of Biological Sciences, State University of New York at Buffalo, Buffalo, NY 14260, supported by a grant from the Muscular Dystrophy Association of America.

Skeletal Muscle has two primary functions within the organismal context: movement and storage of metabolic substrates. Both of these functions are controlled and balanced by chemical messengers from the nervous and endocrine systems. Among these are Acetylcholine, Insulin, Epinephrine, Androgens, and Corticosteroid. In the present series of experiments we have examined the effect of Testosterone and Triaminalone on several groups of skeletal muscles in the rat. Normal and castrated male rats were treated for 12 days with either Testosterone or Triamcinalone or both steroids simultaneously. The extensor digitorum longus, soleus, levator ani, tibialis, and gastrocnemius muscles were removed for analysis following the treatment.

Comparisons were made among treatment groups as well as with untreated and sham injected control groups. The analyses includes: gross body weight; the weights of the 5 muscle groups; weights of the liver, kidney, prostate and adrenals; the interaction of ^{125}I-α-Bungarotoxin, ^{125}I-Insulin and ^{125}I-Hydroxybenzylpindolol with membrane fractions from the various skeletal muscles; the interaction of five androgens (Testosterone, 5-α dihydro testosterone, Androstanediol, Androstenediol, R1881) and 5 corticosteroids (cortisol, corticosterone, dexamethazone, triamcinalone, aldosterone) with cytesol from the several muscle groups; and glycogen content of the several muscles. It has previously been suggested that the anabolic actions of androgens may antagonize the catabolic influence of glucocorticoids. The results clearly demonstrate this hypothesis to be incorrect. The results suggest that androgens may modify the glucocorticoid effects and that glucocorticoids may modify the androgen effect but that a clear antagonism between these two steroids is not indicated. Furthermore, although each muscle group examined responds uniquely, slow fibers appear to be much less sensitive to both Testosterone and Triamcinalone.

1500 EFFECT OF ELECTRICAL STIMULATION ON PLASMA CORTICOSTERONE LEVELS IN URETHANE ANESTHETIZED FEMALE RATS. Jon D. Dunn, Dept. Anat., Sch. Med., Oral Roberts Univ. Tulsa, OK 74171.

Although it is commonly stated that the hippocampus inhibits pituitary-adrenal function, data obtained in several laboratories indicate that the hippocampus may also provide stimulation. To pursue the question of a defferential hippocampal influence on pitutiary-adrenal function, plasma levels of corticosterone (CpdB) obtained prior to and following sham or electrical stimulation were determined fluorometrically for adult female rats which had been anesthetized with urethane (1.35g/kg). All rats were tracheotomized and connected to a respirator, placed on a heating pad and subsequently positioned in a stereotaxic apparatus. Cortical EEG, ECG, Heart rate (HR), blood pressure (BP) and respiration were routinely monitored; timed blood samples (0.3ml) were obtained from a catherized femoral artery. In sham stimulated rats HR, BP and Cpd B were obtained every 30 min over a 2.5 hr. period. The HR (Bts/min), BP (mm of Hg) and Cpd B (μg/100ml) for 7 sham stimulated rats averaged over 6 sampling periods were 385± 19, 95± 6 and 79.3± 5.8 respectively. In electrically stimulated rats, timed blood samples were taken 30 min before, just prior to stimulation (biphasic square waves, 100μA, 50HZ, 1msec, 10 sec on/10 sec off for 30 min) and 5, 10, 15, and 30 min after initiation of stimulation. Whereas stimulation of the dorsal hippocampus produced an increase in plasma Cps B at 15 min (17%) and 30 min (21%), stimulation of the ventral hippocampus resulted in decreased plasma Cpd B levels at similar sampling times (18% and 27%). No change in Cpd B levels was observed following stimulation of the corticomedial amygdala, corpus callosum, fornix or cerebral cortex. Of interest, the largest increase in Cpd B levels was observed following stimulation of the caudate nucleus and globus pallidus (30 min, 36%). These data indicate that the hippocampus differentially influences pituitary-adrenal function, the dorsal hippocampus being facilitory whereas the ventral hippocampus is inhibitory, and suggests that electrical stimulation of the urethane anesthetized rat may provide information regarding sites inhibitory to pituitary-adrenal activity.

1501 CHANGES IN LOCOMOTOR ACTIVITY ASSOCIATED WITH THE PHOTOPERIODIC RESPONSE OF THE TESTES IN MALE GOLDEN HAMSTERS. Gary B. Ellis* and Fred W. Turek (SPON: C. Enroth-Cugell). Dept. of Biological Sciences, Northwestern University, Evanston, IL 60201.

The light-dark environment is known to be a major environmental signal entraining circadian rhythms of locomotor activity. Recent evidence indicates that steroid hormones also influence vertebrate activity rhythms. The precise contributions of such external (e.g., photoperiod) and internal (e.g., hormones) environmental factors in regulating activity are not well defined. In order to assess the effects of the light-dark cycle and changes in the reproductive system upon locomotor activity, male golden hamsters were exposed to either 14 h of light per 24 h (LD 14:10) or to LD 6:18 for 210 days. Wheel-running activity was recorded continuously, and testicular size and serum testosterone were measured periodically throughout the study.

The short photoperiod induced a decrease in testicular width, serum testosterone, and the number of wheel revolutions per 24 h period, when compared to the levels of these variables exhibited by LD 14:10 hamsters. Prolonged exposure to LD 6:18 resulted in a spontaneous increase in these three parameters to levels indistinguishable from those observed in LD 14:10 animals.

Coincident with LD 6:18-induced testicular regression was an increased lability of the time of daily activity onset. A return to stability in the day-to-day time of activity onset was coincident with spontaneous testicular regrowth in LD 6:18 hamsters.

LD 6:18 exposure induced an expansion of the daily activity time. This increased duration of the daily active phase persisted even after the spontaneous increase in testicular width, serum testosterone, and number of wheel revolutions per day.

The onset of activity in all hamsters exposed to LD 14:10 occurred between 0-1 h after lights-off and did not vary appreciably for individual animals during the 210 days of LD 14:10. The time required for stable reentrainment following a shift from LD 14:10 to LD 6:18 varied between 30-120 days among individual hamsters, and the phase relationship of activity onset to lights-off after 200 days of LD 6:18 ranged between -1 and -6 h. The phase relationship of activity onset to lights-off was not affected by an increase in serum testosterone during spontaneous testicular recrudescence.

These results indicate that the number of wheel revolutions per day and the lability of daily activity onset are correlated with photoperiod-induced changes in the hamster reproductive system. In contrast, the duration of the daily active phase and the phase relationship between activity onset and lights-off are relatively independent of changes in the reproductive system and are a function of the entraining light-dark cycle. (NIH HD-09885)

1502 IN VITRO INTERACTIONS OF ESTRADIOL AND TWO ANTIESTROGENS WITH CYTOPLASMIC ESTROGEN RECEPTORS FROM FEMALE RAT HYPOTHALAMUS. Anne M. Etgen and Richard E. Whalen. Department of Psychobiology, University of California, Irvine, CA 92717.

Several synthetic antiestrogens, including nafoxidine and tamoxifen, are capable of antagonizing estrogen induction of female sexual behavior (lordosis) and have therefore been used as a tool to examine potential molecular mechanisms of estrogen activation of behavior. The ability of such compounds to prevent cytoplasmic estrogen-receptor complex formation in neural and peripheral target tissues led to the proposal that antiestrogens operate by direct competition for the estrogen binding site on the receptor protein, i.e., by competitive inhibition. The present experiments investigated this possibility by comparing the ability of estradiol (E_2), which is presumably a competitive inhibitor of its own binding, and the antagonists nafoxidine and tamoxifen to compete for hypothalamic estrogen receptor binding in vitro when the concentrations of both the agonist (^{3}H-E_2) and competitor are varied over a wide range.

Unlabeled E_2 produced a linear, dose-dependent inhibition of ^{3}H-E_2-receptor binding in hypothalamic cytosols isolated from ovariectomized adult female Sprague-Dawley rats; the shape of the competition curves indicated that, as predicted for a competitive inhibitor, the estrogen appeared to reduce the affinity of ^{3}H-E_2 for its receptor. In contrast, neither nafoxidine nor tamoxifen generated simple, linear competition curves. Unlike E_2, these antagonists reduced ^{3}H-E_2 binding at extremely low competitor concentrations. A comparison of the relatively linear portion of the antiestrogen competition curves with the same portion of the E_2 curves further suggested that the former compounds produced a significantly sharper decrease in ^{3}H-E_2 binding than did E_2 itself at high competitor concentrations. These results are inconsistent with a competitive inhibition model and indicate that the synthetic antiestrogens may antagonize estrogen action via allosteric (conformational) interactions with the hypothalamic receptor. It is proposed that the synthetic antagonists bind with high affinity to a non-estrogen binding site on the receptor, thereby inducing conformational changes in the receptor which reduce its affinity for E_2 and alter its functional competence to interact with the nuclear components involved in estrogen activation of lordosis.

1503 EFFECT OF ELECTROLYTIC LESION OF THE MEDIAL RAPHE NUCLEUS ON 5-HTP + LU10-171-INDUCED INCREASES IN SERUM PROLACTIN LEVELS IN RATS. Richard G. Fessler and Herbert Y. Meltzer. University of Chicago Pritzker Schl. Medicine, Chicago, Ill. 60637.

Previous studies have demonstrated serotonin (5-HT) can stimulate prolactin secretion in the rat. In vitro studies have shown that 5-HT does not induce prolactin release by a direct effect on the pituitary. Several studies have suggested the median or dorsal raphe may be the locus of a 5-HT-induced release of a peptidergic prolactin releasing factor (PRF). To examine this question further, the effect of low dose 5-hydroxytryptophan (5-HTP) (30 mg/kg ip) plus 5-HT reuptake blockade with either Lilly 110140 (10 mg/kg ip) or Lu10-171 (5 mg/kg ip) was examined in rats which received either electrolytic lesion of the medial raphe nucleus (MR) or sham lesion. MR lesion one week prior to study significantly antagonized the serum prolactin increases in response to this drug regimen in four separate replications. In these experiments, it was further demonstrated that: 1) neither 5-HTP, at this dose, nor administration of a 5-HT reuptake blocker alone, induced significant increases in serum prolactin in either lesioned or sham animals, 2) the increased serum prolactin levels following 5-HTP + reuptake blocker were positively correlated with 5-HT concentrations in the median eminence, 3) the increased serum prolactin concentrations were not dependent upon decreased concentrations of dopamine (DA) in the median eminence, and 4) total destruction of the MR was necessary to demonstrate total blockade of the 5-HTP + reuptake blockade-induced increases in serum prolactin. These results suggest that presynaptic 5-HT terminals located in the median eminence and arising from cell bodies located in the MR, are necessary for serotonergically mediated effects on prolactin secretion induced by 5-HTP.

1504 EFFECTS OF DIFFERENTIAL FORNIX ABLATIONS ON THE CIRCADIAN RHYTHMICITY OF ADRENAL CORTICOSTEROIDS AND LOCOMOTOR ACTIVITY - A 48 HOUR STUDY. Christine T. Fischette, Henry M. Edinger, and Allan Siegel. Depts. of Physiology and Neuroscience, College of Medicine & Dentistry of New Jersey, Newark, NJ 07103.

In a previous study we showed that lateral fornix ablation in adult male rats disrupts the circadian rhythmicity of adrenal corticosteroids that is normally attuned to light-dark cycles, while medial fornix ablation and neocortical ablations do not. We hypothesized that the anteroventral subiculum, the source of the medial corticohypothalamic tract (mcht), is responsible for modulating this periodicity by nature of its input to the suprachiasmatic nucleus and the medial basal hypothalamus. In the present study we confirm and extend our previous findings by, 1) lengthening the period of observation to 48 hours, and 2) by examining the effect of differential fornix ablations on other rhythmic parameters, i.e., locomotor activity, body temperature, and food and water intake. Seven to ten days after medial or lateral fornix ablation, animals were placed on activity platforms. Blood samples were withdrawn at 4h intervals over a period of 48h by tail vein venipuncture in order that corticosteroid determinations could be obtained simultaneously with locomotor activity measurements. In addition, rectal body temperature, food and water intake were recorded.

Spectral analysis revealed that in 7 out of 10 lateral fornix ablated animals, the dominant period of the adrenal corticosteroid rhythm (r) appeared outside the circadian range ($20h \leq r \geq 28h$). One of the animals exhibited a significant period at 30h, while the others demonstrated significant periods in the ultradian range ($0 \leq r \leq 20h$). In contrast, intacts exhibited dominant periods within the circadian range with hradly any significant ultradian components. With regard to locomotor activity, all animals displayed dominant circadian rhythms regardless of corticosteroid pattern. In half of the lateral fornix ablated group we obtained a clear dissociation between dominant periods of locomotor activity and dominant periods of adrenal corticosteroids. The circadian rhythm of rectal body temperature was also disrupted in the lateral fornix ablated group. Our study supports the hypothesis of separate oscillators controlling circadian functions, and suggests that the anteroventral subiculum, via the mcht, is an important modulator of some, but not all, circadian parameters.

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1505 RESPONSES OF SERUM CORTICOSTERONE, FREE FATTY ACIDS, AND GLUCOSE TO FOOTSHOCK AND CONDITIONING STIMULI IN RATS. Michael J. Frey* and Gary D. Coover, Dept. Psych., Northern Illinois Univ., DeKalb, IL 60115.

Three experiments involving a session of intense electric footshocks delivered for 2 sec every 2 min were performed with male hooded rats. In the first two experiments elevations of serum corticosterone, free fatty acids (FFA), and glucose concentration were found within 10 min of the onset of the footshock session except under two conditions. That is, FFA did not increase significantly when the blood samples were obtained from the jugular vein or decapitated trunk of ether-anesthetized rats (Exp. 1), but did increase significantly when the blood sample was obtained from the decapitated trunk of unanesthetized rats (Exp. 2). Also, the glucose elevation was not significant in nondeprived rats using unanesthetized decapitation, but was in 24-hr food-deprived rats (Exp. 2) and in both deprived and nondeprived rats using prior anesthetization (Exp. 1). Of course, 24-hr deprivation elevated baseline (presession) FFA and lowered baseline glucose concentrations. With longer shock session durations, corticosterone elevated further by 40 min but no higher by 80 min regardless of food deprivation or sampling condition, but food deprivation did cause greater elevations. Glucose elevated further by 40 min in 24-hr deprived rats, but then declined with 80 min of footshock. The response of FFA was nonmonotonic, decreasing to baseline levels with 40-min shock sessions, and then rising to the highest concentration of all by 80 min. This explains, in part, apparent contradications in the literature regarding short-latency responses of FFA to stress in rats.

In Experiment 3, nondeprived rats were tested for responses to placement in the chamber where they had been shocked, or not (simply placed in the chamber), for 1 hr, 48 hr previously. The former, fear conditioned, rats exhibited significantly greater corticosterone, but not FFA or glucose, elevations than the latter, never-shocked, controls. No glucose elevations occurred during the testing session, in previously shocked or unshocked groups by 40 or 80 min of testing. However, even the unshocked rats exhibited a corticosterone elevation, and an overall FFA increase occurred with testing, indicating that mild stress does not necessarily produce a nonmonotonic FFA response. The corticosterone elevations of the fear conditioned rats were smaller if the wall color and lighting of the chamber were altered on the test day from what they had been during fear conditioning. This stimulus generalization decrement indicates that conditioned fear, and not solely sensitization, mediates the increased corticosterone response in previously shocked rats. The results indicate the comparative value of corticosterone as an emotional response measure.

1506 DEVELOPMENT OF NORMAL FETAL SUPRAOPTIC NEURONS GRAFTED INTO ADULT HOSTS WITH A CONGENITAL LACK OF VASOPRESSIN PRODUCING NEURONS. Don Gash*, John R. Sladek, Jr. and Celia Sladek. (SPON: D.E. Scott). Depts. of Anatomy and Neurology, Univ. of Rochester, Rochester, NY 14642

The region of the hypothalamus containing the supraoptic nucleus was dissected out of normal 17-day post coitus Wistar/Lewis rat embryos and stereotaxically transplanted into the median eminence of adult female Brattleboro rats (which congenitally lack vasopressin-producing neurons). The adult hosts were sacrificed either 20- or 40-days following transplantation. Their median eminences were dissected out and either processed for light microscopic fluorescence and immunohistochemistry or homogenized for radioimmunoassay. In 4 out of 6 animals examined, the transplants were found growing in the 3rd ventricle, juxtaposed to and receiving a blood supply from the median eminence. The transplants ranged up to 2mm long in a rostral-caudal direction and 1 x 1.5mm in width and height. Hypothalamic homogenates from 3 of 8 animals receiving supraoptic transplants contained radioimmunoassayable vasopressin ranging from 580 to 870pg/animal. Sham operated animals or animal receiving a comparably sized transplant of hippocampus did not have detectable levels of vasopressin. Vasopressin-containing neurons, with axons coursing to the host's median eminence, were identified in the transplants by immunohistochemistry. By fluorescence microscopy, catecholamine-containing neurons were also found in the transplants. Some catecholamine-containing fibers from the host seemed to traverse the interface between the host and the transplant. Studies are in progress to determine the ability of the transplant to release vasopressin into the host's circulation and thus enhance the host's ability to conserve water.

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1507 CHRONIC DEXAMETHASONE ALTERS CONTENT OF IMMUNOREACTIVE ACTH-LIKE MATERIAL IN RAT ARCUATE NUCLEUS. M.J. Gibson, D.T. Krieger, A.S. Liotta‡ M.J. Brownstein‡ and B.S. McEwen. Mt. Sinai Sch. Med., Dept.Med., Div. Endocrinol., New York,NY 10029; Lab. Clin. Sci., NIMH, Bethesda, MD 20014 and Rockefeller Univ. New York,NY 10021.

Immunoreactive ACTH-like material has been reported in the hypothalamus of several species. Cell bodies containing such material are localized to the arcuate nucleus area. In view of previous studies on differential effects of corticosterone and dexamethasone on pituitary suppressibility, the present study was designed to study the effect of these steroids on concentrations of immunoreactive ACTH-like material in specific rat hypothalamic nuclei, pituitary, and plasma.

Solid pellets were implanted subcutaneously in male Sprague-Dawley rats (200-250 gm). Pellets were either 100% cholesterol (CHOL), 100% corticosterone (B), 50% cholesterol and 50% dexamethasone (DEX 50), or 75% cholesterol and 25% dexamethasone (DEX 25). One week later, animals were decapitated within 20 seconds of original contact. Trunk blood was collected for subsequent ACTH immunoassay and corticosterone (CBG) assay. Anterior pituitaries were removed and frozen in 0.1 N HCl. Brains were immediately frozen on dry ice. Median eminence, arcuate nucleus, ventromedial hypothalamus, and dorsomedial hypothalamus were removed by microdissection and for each area pools of three specimens were homogenized in 0.2 M HCl for determination of immunoreactive ACTH-like material. Adrenals were removed and weighed.

Group	Plasma B ug%	Plasma ACTH pg/ml	Ant. Pit. ACTH[a] µg/0.5ml	Arc.Nucleus ACTH[a] pg/100µg	Adrenal Wts. mg
CHOL	3.1±2.9	137.3±22.1	1.33±0.09	41.0±7.1	420.1±29.4
(n)	(9)	(3)	(9)	(3)[e]	(9)
B	24.5±5.9[c]	37.0±8.5	1.24±0.17	65.4±26.1	234.6±13.7[d]
(n)	(9)	(3)	(9)	(3)[e]	(9)
DEX 50	≤2.5±0	<10, <50	0.67±0.05[d]	126.5±38.1	213.6± 9.4[d]
(n)	(9)	(2)	(9)	(3)[e]	(9)
DEX 25	≤2.5±0	<10, <15	0.87±0.17[b]	82.5±4.2[c]	225.7±17.3[d]
(n)	(9)	(2)	(9)	(3)[e]	(9)

[a]West "midportion" antibody; [b]p<0.05; [c]p<0.01; [d]p<0.001; [e]n = 3 pools of 3

Significant depression of adrenal weight was seen in all steroid treated groups, while pituitary ACTH content was significantly lower only in the dexamethasone treated group. Dexamethasone treatment however was associated with a significant increase in arcuate nucleus immunoreactive ACTH-like concentration; while (data not presented) ACTH-like content of the other brain regions studied was not affected. Studies are in progress to determine whether differential effects can be seen in other brain areas.

1508 INFLUENCE OF ESTROGEN ON GLUTAMIC ACID DECARBOXYLASE (GAD) ACTIVITY: TIME COURSE AND LOCALIZATION. John H. Gordon, Marilyn Y. McGinnis* and Roger A. Gorski. Dept. of Anat. Sch. Med. UCLA, Los Angeles, CA 90024.

Ovariectomized female rats treated systemically with estradiol benzoate (EB: 2 µg/day for 3 days) are known to show decreased GAD activity in the substantia nigra (SN) and ventral tegmental region (VTR) relative to oil treated controls. The aim of the present study was to characterize the effect of estrogen on GAD activity in spayed female rats by examining both the response to local intracranial estrogen implants and the time course of the response following systemic estrogen treatment. In the first experiment, estradiol-17β (E_2) or cholesterol was implanted unilaterally into one of five different brain areas. After three days of steroid exposure, animals were sacrificed, and GAD activity was measured in right and left halves of the SN and the VTR. When E_2 was implanted in the preoptic area (POA) or ventral medial nucleus of hypothalamus (VMN), two regions heavily labeled with tritiated E_2, no significant GAD response to EB occurred. However, GAD activity was decreased in the SN when E_2 was implanted in the caudate nucleus (CPU) or amygdala (AMY), and in the VTR when implanted in the nucleus accumbens septi (ACB). Thus changes in GAD activity were found in brain regions rich in dopamine terminals and containing presumed sites of origin of gabaminergic fibers having their terminals in the SN and VTR. No response to cholesterol occurred regardless of implant site. In addition, there was localization with respect to the side of brain in which the response was found in that GAD activity was decreased only in the side of the brain in which the E_2 was placed. Lastly, there was localization with regard to the SN and VTR: ie, E_2 implanted in the CPU or AMY decreased GAD activity only in the SN while E_2 implanted in the ACB decreased GAD activity only in the VTR. In the second experiment, the time course of changes in GAD activity was measured in ovariectomized rats given a single systemic injection of either 8 µg EB or oil. Rats were sacrificed at 0, 12, 29 or 53 h post injection. It was found that GAD activity in the SN was maximally suppressed at 29 h after EB, whereas decreased GAD activity in the VTR was apparent 12 h after EB but had returned to normal by 29 h. Oil injections had no significant effect on GAD activity. These results suggest that there may be two separate and distinct gabaminergic pathways which are differentially responsive to estrogen; a striato-nigral system projecting to the SN and a mesolimbic-VTR system possibly having cells of origin in the ACB.

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1509 INHIBITORY ACTION OF OPIOID PEPTIDES ON THE RELEASE OF DOPAMINE INTO HYPOPHYSIAL PORTAL BLOOD. G. A. Gudelsky and J. C. Porter, Depts. Ob/Gyn. & Physiol., Cecil H. and Ida Green Ctr. for Reprod. Biol. Sci., Univ. of Texas, Southwestern Med. Sch., Dallas, TX 75235.

Opioid peptides are present in the arcuate-median eminence region of the hypothalamus (Bloom et al., 1978; Sar et al., 1978; and others), suggestive of a role for these compounds in the regulation of pituitary function. Indeed, administration of opiate-like peptides results in an elevation of serum prolactin concentrations (Cusan et al., 1977; and many others). Since prolactin secretion is believed to be under the tonic inhibitory control of tuberoinfundibular dopaminergic neurons, we have addressed the issue of whether the effect of opioid peptides on prolactin secretion is the result of an inhibition of the release into hypophysial portal blood of dopamine from these hypothalamic neurons. The concentrations of dopamine in pituitary stalk blood of rats were measured after the intraventricular administration of 10µl of a solution containing β-endorphin (10µg), the enkephalin analog, [D-Ala^2]met-enkephalinamide (100µg), or the solvent vehicle (0.15M NaCl). Pituitary stalk blood was collected for 1 hr from pentobarbital-anesthetized rats. Dopamine was analyzed in acidified plasma extracts employing a sensitive radioenzymatic procedure. The concentration of dopamine in pituitary stalk plasma of vehicle-treated, ovariectomized rats was 1.2 ± 0.3 ng/ml (mean ± SE). Compared to these vehicle-treated control animals, the dopamine concentrations were significantly lower in ovariectomized rats which had received either β-endorphin or the enkephalin analog. In these two groups of animals the mean concentration of dopamine was approximately 0.2 ng/ml. The concentration of dopamine in stalk plasma of ovariectomized rats injected with estradiol benzoate (25 µg/kg, sc) for 3 days was 4.0 ± 0.8 ng/ml. In estrogen-primed, ovariectomized rats treated with the enkephalin analog, the mean concentration of dopamine in stalk plasma was 0.3 ng/ml which was less than 10% of the dopamine concentration in the vehicle-treated control animals. When ovariectomized, estrogen-primed rats were pretreated with the opiate antagonist, naloxone (5 mg/kg, sc), the enkephalin analog was no longer effective in reducing the concentration of dopamine in pituitary stalk plasma. These findings are supportive of the view that the inhibitory effect of opioid peptides on the release of dopamine into pituitary stalk blood is one mechanism by which these peptides stimulate prolactin secretion. In addition, these results are suggestive that endogenous opioid peptides may regulate the release of dopamine from tuberoinfundibular neurons.

1510 A CELLULAR ANALYSIS OF ANDROGEN- AND ESTROGEN-INDUCED SEXUAL DIFFERENTIATION IN THE ZEBRA FINCH SONG SYSTEM. Mark. E. Gurney and Mark Konishi. Div. Biology, Caltech, Pasadena CA 91125.

Male zebra finches sing a brief song phrase to the female during courtship. Castration of an adult male reduces the bird's frequency of singing; testosterone replacement reinstates the behavior. Testosterone treatment of female zebra finches does not activate song nor induce other elements of courtship behavior.

We find that correlative changes of brain and behavior in zebra finches are organized by sex hormones during development. Newly hatched zebra finch chicks were subcutaneously implanted with silastic pellets containing either 50 µg of dihydrotestosterone or 50 µg of 17 β-estradiol. Testosterone treatment activates song in adult females which were implanted with estradiol when chicks, but fails to activate song in those females which had received dihydrotestosterone. The singing females approach a sexual partner with pivoting movements, straighten to an erect posture, fluff their throat feathers, and rapidly repeat their song phrase in a behavioral sequence which closely resembles that of the male.

In zebra finches, brain nuclei of the efferent pathway for control of song show dramatic sex differences in their volume (Nottebohm and Arnold; Science 194 [1976] 211-213). We find that 17 β-estradiol treatment of genetically female chicks organizes male-like cytoarchitectonic differentiation of the telencephalic song nuclei RA, HVc, MAN, and X. Dihydrotestosterone induces masculinization of the brain stem song nuclei nXII and DM. Dendritic field spread, soma size, and the consequent volume of the brain nucleus is larger in males than females at all levels of the song system. The exposure of female chicks to either androgen or estrogen supports growth of the hormone's respective target neurons in either the brain stem or telencephalic song nuclei. These neurons reach a size identical with that of the equivalent cell type in a normal male. Our anatomical comparison of normal adult male and female song systems reveals that all cell types and all identified connections are present in both sexes. Thus, the specification of cellular identity - i.e., position, number, dendritic morphology and efferent synaptic projection - is expressed independently of the hormonal environment. Rather than selecting pathways of anatomical differentiation, androgens and estrogens exert a similar pleiotrophic effect on their respective target neurons. Although in the female song system we identify all the cell types and connections of the male, testosterone does not activate song. Thus, 17 β-estradiol may also exert a specific inductive effect on the telencephalic song neurons which renders them physiologically competent to respond to testosterone in the adult. (Supported by NIH Grant No. HD10501)

1511 OXYTOCIN AND VASOPRESSIN RELEASE BY SUBSTANCE P INJECTED INTO THE CEREBRAL VENTRICLES OF RATS. Jaya Haldar*, Donald L. Hoffman*, Gajanan Nilaver* and Earl A. Zimmerman. Departments of Pharmacology and Neurology, College of P&S, Columbia University, New York, N.Y. 10032.

Substance P (SP) has been found by immunocytochemical methods in fibers innervating a number of hypothalamic nuclei. These may be sites where this peptide acts to regulate the secretion of anterior pituitary hormones such as gonadotrophins, growth hormone and prolactin. Since we have recently demonstrated relatively dense projections of SP fibers to magnocellular nuclei of the hypothalamus, particularly the paraventricular nucleus, central effects of substance P on the release of neurohypophysial hormones produced in these regions was studied.

Via chronic indwelling cannulae into a lateral cerebral ventricle (LCV), SP was administered to urethane anesthetized lactating rats and milk-ejection pressure was recorded as an indication of oxytocin release. Antidiuretic responses were followed after LCV injection of SP in alcohol-anesthetized water-loaded rats. Injection of 1-4 μg (1-4 μl) produced a sustained increase of milk-ejection pressure in lactating rats and 0.2-0.4 μg produced long lasting antidiuresis in water loaded rats. 1 μg LCV β-endorphin blocked the milk-ejection response to SP, while the same dose caused profound potentiation of the antidiuresis induced by SP. Both the inhibitory and the potentiating effects of β-endorphin lasted at least 90 min.

Although vasopressin is known to have an intrinsic milk-ejecting activity it is unlikely that its release accounts for this response in these experiments since β-endorphin had opposite effects on the antidiuretic and milk-ejecting responses to substance P. If vasopressin accounted for the milk-ejection response then β-endorphin should have enhanced rather than inhibited the SP effect. The results also suggest that β-endorphin may participate in the regulation of differential secretion of oxytocin and vasopressin. (Supported by NIH #AM-01940 and AM-20337).

1512 EFFECTS OF INDIVIDUAL HOUSING ON DIMETHYLTRYPTAMINE AND TRYPTAMINE IN RAT BRAIN AND ADRENAL GLANDS. Robert Harrison* and S. T. Christian, Neurosciences Program, University of Alabama in Birmingham, Birmingham, AL 35294.

Sensitive GC/MS assays have been developed for quantification of picogram amounts of tryptamine (TA) and N,N-dimethyltryptamine (DMT). Using this method, TA and DMT were measured in single brains and adrenal glands taken from 120 day old male Sprague-Dawley rats. Known amounts of α, α, β, β-tetradeuterated TA (DTA) and α, α, β, β-tetradeuterated DMT (DDMT) were added to tissue samples prior to deproteinization and delipidization under acid conditions. The amines were extracted from the basified mixture with methylene chloride and derivatized with heptafluorobutyryl imidazole according to Benington, Christian and Morin (J. Chromatog. 106:435, 1975). The derivatized products of this reaction were taken up in 1 ml methylene chloride and washed with distilled water. Microliter portions of this mixture were found suitable for analysis of the heptafluorobutyryl derivatives of TA, DTA, DMT and DDMT. Determinations were carried out on a Hewlett-Packard 5985 GC/MS equipped with a Supelco 4' glass column containing 2% SP-2250 on 100-120 mesh Chromosorb W-HP. Quantification was obtained by a comparison of ratios of ion densities of fragments specific for the heptafluorobutyryl derivatives of TA, DTA, DMT and DDMT from both pure standards and tissue extract.

These data indicate that DMT and TA are elevated in both brain and adrenals from animals that were individually housed for 28 days. If the animals are individually housed for 60 days, there is a decrease in the level of DMT from brain and adrenal while TA in these tissues remains elevated. This effect is enhanced considerably in younger animals. Further aspects of these phenomena are currently being investigated.

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1513 ELEVATIONS IN PLASMA CORTICOSTERONE IN RATS IN RESPONSE TO CONSUMPTION OF CONCENTRATED SUCROSE SOLUTIONS. Robert P. Hart*, Gary D. Coover, Allan Shnerson*, Dept. of Psychology, Northern Illinois Univ., DeKalb, IL 60115, and William P. Smotherman, Dept. of Psychology, Oregon State Univ., Corvallis, OR 97331.

In Exp. 1, male hooded rats were given a 23% w/W sucrose solution or a choice between this sucrose solution and tap water after 36 hr of water deprivation. Sucrose consumption was completed within the first 15 min of the 120-min session, and was associated with a marked elevation in plasma corticosterone level (to 50 μg/100 ml within 60 min) that remained elevated for the 120 min. The sucrose-water choice group consumed water after consuming sucrose, and exhibited a more rapid decline in corticosterone concentration during the interval between 60 and 120 min after the initiation of sucrose consumption. These data suggest that ingestion of concentrated sucrose solution facilitates pituitary-adrenal activity through dehydrational consequences that continue after sucrose consumption is terminated unless subsequent water intake rehydrates the animal. As a test of this dehydration hypothesis rats in Exp. 2 were deprived of water for 2, 24 or 48 hr and then consumed a sucrose solution (23% w/W). The three groups consumed the same amount of sucrose solution, and thus varied in degree of ultimate dehydration in accordance with hours of prior water deprivation. While all groups showed elevations in plasma levels of corticosterone, higher and more prolonged elevations occurred for the 24 than the 2, and for the 48 than the 24, hr deprived rats. As a further test of the dehydration hypothesis, rats in Exp. 3 consumed one of six concentrations of sucrose solution (ranging from .120 to 1.166 moles/liter) or one of two concentrations of glucose solution (.470 or 1.166 moles/liter) after 36 hr water deprivation. Increasing the concentration of sucrose resulted in a decrease in amount consumed and an increase in plasma corticosterone concentration. Glucose consumption produced similar effects, though more weakly than equimolar sucrose solution consumption. These data suggest that dehydration from consumption of highly concentrated sugar solutions inhibits further drinking and produces corticosterone elevations. The effect of rapid, acute dehydration on corticosterone levels is surprising considering the minimal effects of water deprivation per se on corticosterone levels and the interpretation of pituitary-adrenal activation as a sign of psychological stress.

1514 A POSSIBLE NEUROSECRETORY PATHWAY UTILIZING SUBSTANCE P (SP) IN THE HYPOTHALAMUS OF THE NORTH AMERICAN OPOSSUM. Raymond H. Ho and Carol Laxson*, Dept. Anat., Sch. Med., The Ohio State University, Columbus, O., 43210.

A very dense plexus of SP immunoreactive (SPI) fibers and terminals is present in the external layer of the median eminence (ME) in the human, monkey, domestic fowl and opossum, but not in the rat, guinea pig, mouse or frog. The location of these SPI terminals is suggestive of a regulatory role for this peptide in anterior pituitary function. Antibodies raised in rabbits against synthetic SP were used in the indirect antibody PAP method to localize SPI on 10μm coronal and sagittal sections of opossum brains that were fixed by intracardiac perfusion with Bouin's fixative. A SPI fiber pathway was observed that projected to the ME. This fiber bundle begins to form in the medial preoptic area and courses caudally and ventrally adjacent to the III ventricle toward the ME. SPI perikarya were observed scattered among the beaded SPI fibers in the medial preoptic region. These observations suggest that the SPI perikarya in the preoptic area are the cells of origin of the SPI projection. In addition, SPI cell bodies were also seen in the arcuate nucleus. These cells may also contribute to the SPI plexus observed in the ME. The specificity of immunostaining was established in control experiments in which anti-SP serum pretreated with an excess of SP failed to demonstrate the aforementioned structures on adjacent sections. We conclude that the SPI fibers and terminals in the ME of the opossum can be traced to the preoptic and the arcuate nucleus regions where SPI containing perikarya were observed. (Supported by the Snyder Fund and The Graduate School of The Ohio State University.)

1515 IMMUNOCYTOCHEMICAL IDENTIFICATION OF MELATONIN BINDING MATERIAL IN THE PINEAL GLAND OF THE RAT. W. R. Holloway*, L. J. Grota, and G. M. Brown. Dept. Psych., Sch. Med., U. Roch., Roch., N. Y. 14642, and Dept. Neurosci., McMaster U. Hamilton, Ont., Canada.

Using an immunohistochemical technique (Cell Tiss. Res., 1975, 162:141; Endocr., 1978, 102:63) we have localized a saturable binding material for melatonin (Mel) in the pineal gland (PG) of the rat. A quantitative double antibody procedure with anti-Mel (A-Mel) as the first antibody and FITC-labeled IgG as the marker was used. Fluorescence Intensity (FI) was measured with a Cadmium Sulfide photosensor.

PG from adult male rats (n=6) sacrificed 1.5 hr after lights-on showed successive increases in FI when 0, 10 ng, and 1000 ng Mel/ ml were added to A-Mel before incubation. Adding 5 mg Mel/ml failed to increase FI above this level, showing saturation of the binding material. An estimate of the proportion of the binding material occupied by endogenous Mel, 0.30, was determined by dividing the FI for 0 Mel by the maximum FI from the saturation curve. Using this method, a diurnal change in the proportion of receptors occupied by Mel was found; the degree of binding increased progressively through the lighted portion of the day, reaching a maximum value of 1.0 shortly after lights-out. For this sampling point, the highest FI occurred when 0 Mel was added to the antibody. FI decreased when Mel was added. Thus, a daily rhythm of Mel binding in the PG exists,evidenced by changes in the proportion of the receptor population occupied by endogenous Mel.

Initial studies to determine the specificity of the binding element show that it binds both Mel and N-acetyl-serotonin (NAS). Sections of PG from rats (n=4) killed 1.5 hr after lights-on were incubated with antibody containing either Mel or NAS (1000 ng/ ml). When an antibody which binds both Mel and NAS (A-NAI) was used, each indoleamine caused an increase in FI. When A-Mel, which binds only Mel, was used as first antibody, only Mel increased FI.

In conclusion, this procedure provides a means of visualizing and quantifying Mel receptors in the PG. The application of this method to the study of Mel binding in other tissues will be discussed.

This work was supported in part by NIMH grant MH-14650.

1516 THE EFFECT OF ANTEROVENTRAL 3RD VENTRICLE REGION LESIONS ON OSMOTIC STIMULATION OF VASOPRESSIN RELEASE BY THE ORGAN CULTURED RAT HYPOTHALAMO-NEUROHYPOPHYSEAL SYSTEM. Alan Kim Johnson and Celia D. Sladek. (SPON: Fred W. Mis). Dept. of Psychology, Univ. of Iowa, Iowa City, IA 52242, and Depts. of Neurology and Anatomy, Univ. of Rochester Sch. of Medicine, Rochester, NY 14642.

Rats with electrolytic lesions of the anteroventral 3rd ventricular (AV3V) region of the hypothalamus exhibit hypernatremia and chronic drinking deficits in response to hypertonic NaCl. These findings are suggestive of impaired osmoreception. The organ-cultured rat hypothalamo-neurohypophyseal system (HNS) previously has been shown to release vasopressin (VP) in response to osmotic stimuli. The ventral portion of the region damaged by AV3V lesions is included in the HNS explant. Thus, these studies were initiated to evaluate the osmotic response of HNS explants which were obtained from rats previously prepared with AV3V lesions.

Following electrolytic ablation of the AV3V region or sham lesioning and a 2 wk recovery period, HNS explants were removed from 5 AV3V lesioned rats and 6 sham lesioned rats. The explants were maintained in organ culture for 4 days. On the third day in culture, increasing the osmolality of the culture medium from 295 to 315 mOsm/kg H_2O by the addition of NaCl resulted in a 2.5 fold increase in VP release from the sham lesioned explants, but did not significantly alter VP release from the AV3V lesioned explants. On the subsequent day in culture, acetylcholine (10^{-5}M) stimulated VP release from 4 of the 5 AV3V lesioned explants as well as the sham lesioned explants. These data suggest that the osmoreceptors which are involved in controlling VP release from the organ cultured HNS may be located in the region anterior to the ventral 3rd ventricle.

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1517 ACTIVITY OF TUBEROINFUNDIBULAR DOPAMINERGIC NERVES DURING SURGES OF PROLACTIN AND LUTEINIZING HORMONE. C.A. Johnston, K.T. Demarest and K.E. Moore. Dept. of Pharmacol. & Toxicol., Michigan State Univ., East Lansing, MI 48824.

The purpose of the present study was to estimate the activity of tuberoinfundibular dopamine (DA) nerves during and after the elevation of serum concentrations of prolactin and luteinizing hormone (LH) which occur in normal female rats on the afternoon of proestrus, and which can be induced in ovariectomized rats by injections of estrogen. The activity of different DA neuronal systems was estimated by measuring the accumulation of DOPA 30 min after the injection of a DOPA decarboxylase inhibitor, 3-hydroxybenzylhydrazine (NSD 1015, 100 mg/kg, i.p.). The DOPA content was determined using a radioenzymatic assay in the median eminence (ME), striatum (ST) and neurointermediate lobe of the pituitary (NIL), regions containing terminals of the tuberoinfundibular, nigrostriatal and tuberohypophyseal DA neuronal systems, respectively.

Female rats which had at least 2 consecutive 4 or 5 day estrous cycles prior to the experiment were sacrificed by decapitation at appropriate times during the cycle. DOPA accumulation did not change in any brain region on the morning of diestrus, metestrus, or proestrus. DOPA accumulation in the ME significantly increased in the late evening of proestrus and on the morning of estrus. Much smaller but temporally related changes were also noted in ST and NIL.

Ovariectomized rats were injected with estradiol benzoate (5 µg/kg, s.c.) for 4 days. On the fifth day a surge of prolactin and LH was induced in half of these animals by an injection of 0.5 mg/kg estradiol benzoate at 1000 hr; the control group received injections of corn oil vehicle. DOPA accumulation was determined at 800, 1200, 1800, 2400 hr on day 6 and at 800 hr on day 7. There were no changes in DOPA accumulation in ME, ST, or NIL of the control group. Although the serum concentrations of prolactin and LH in the experimental group increased on the afternoon of day 6, DOPA accumulation was significantly increased selectively in ME only on the morning of day 7. Thus, the increased accumulation of DOPA in ME occurs 6-16 hr after the peak of the serum concentration of the hormones. This delayed activation of tuberoinfundibular DA neurons is consistent with previous studies in which prolactin concentrations in serum or CSF were elevated by pharmacological means. These results suggest that the normal and simulated surges of prolactin and LH are not the result of a reduction in the activity of tuberoinfundibular DA neurons, but that the elevation of serum concentrations of prolactin, and possibly LH, appear to cause a delayed increase in the activity of these nerves. (Supported by USPHS grant NS 9174.)

1518 IN VITRO EVIDENCE FOR MULTIPLE OSCILLATORS IN THE CHICK PINEAL GLAND. Charles A. Kasal*, Wim du Bois*, Michael Menaker, J. Regino Perez-Polo. Dept. Zool., Univ. Texas, Austin, TX 78712 and Dept. Human Biological Chemistry and Genetics, Univ. of Texas Medical Branch, Galveston TX 77550.

In Vitro studies done by us and others on chick (Gallus domesticus) pineal glands demonstrate that N-acetyltransferase, the enzyme involved in melatonin synthesis is under the control of a self sustained circadian oscillator located in the pineal gland. Cultured chick pineal glands maintained in constant darkness (DD) exhibit a persistant rhythm of NAT activity with peaks occuring approximately 24 hours apart and a phase which closely approximates that seen in vivo. These findings clearly suggest that the pineal gland contains one or more oscillators that can function independent of innervation and external time cues.

In the present study we asked whether the organ integrity is essential for maintenance of this rhythm in culture. Chicks raised from one day of age in a light-dark cycle of 12 hours of light (LD 12:12) were sacrificed at three weeks of age and pineals were removed and placed into culture. Glands were bisected along the sagittal plane and each of the halves were placed into separate culture flasks. Respective halves were retrieved simultaneously during sampling. Examination of the first and second day in culture in DD revealed rhythm in NAT activity similar to that seen in whole-gland preparations with high levels occuring during the projected night of the birds prior light-dark cycle. Maintaining the organ culture in a continued LD cycle for two days and then releasing into DD again revealed a persistant rhythmicity in NAT during the third and fourth day in culture.

The persistance of rhythmicity in each section of the pineal gland demonstrated that the whole gland is not necessary for the production of a circadian rhythm and that the pineal gland contains at least two, if not more, self sustained oscillators which may function independently to produce a circadian rhythm. The issue of possible multiplicity of oscillators will be addressed in experiments entailing cultures of dissociated pineal cells.

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1519 ANDROGEN CONCENTRATING CNS REGIONS OF THE SOUTH AFRICAN CLAWED FROG, XENOPUS LAEVIS: AUTORADIOGRAPHY WITH DIHYDROTESTOSTERONE. Darcy B. Kelley, Dept. Psychology, Princeton University, Princeton, N.J. 08544

Androgen concentrating regions in the CNS of X. laevis were mapped autoradiographically with the non-aromatizable androgen, dihydrotestosterone (DHT). One week after castration, five male frogs were injected with 200 uCi (0.0003 mg) of ^{3}H-DHT (1,2,4,5,6, 7,16,17-^{3}H-DHT, 200 Ci/mM). Frogs were sacrificed two hours later and the horizontally sectioned brains processed for steroid autoradiography. Control and experimental slides were exposed for 4, 6, or 8 weeks, developed, lightly stained with cresyl violet acetate and scanned for labelled cells (criterion: 5x background). After DHT injection the following brain regions contain labelled cells: anterior pituitary, posterior thalamus (TH), laminar nucleus of the torus semicircularis (TOR), dorsal tegmental area of the medulla (DTAM), principal nucleus of cranial nerve V, motor neurons of cranial nerve IX-X and large and small neurons in the ventral columns of the rostral spinal cord. These DHT results are compared to previously reported (Kelley et al., J. Comp. Neur., 164: 47, 1975; Morrell et al., J. Comp. Neur., 164: 63, 1975) labelling patterns following testosterone (T) or estradiol (E) injection into males below.

	TEL	APOA	VIN	TH	TOR	DTAM	IX-X
E	X	X	X	X	X	-	-
T	-	X	X	-	-	X	X
DHT	-	-	-	X	X	X	X

The limbic telencephalon (ventral striatum, ventral septum, rostral amygdala) contains labelled cells after E but not after T or DHT injection. The anterior preoptic area and ventral infundibular nucleus contain labelled cells after T or E injection but not after DHT. The TH and TOR contain labelled cells after DHT or E but not after T injection. Preliminary studies indicate that long term T treatment of castrated males will prevent labelling with ^{3}H-E in APOA, VIN, TH and TOR, but not in TEL. These results suggest that the TEL cannot aromatize T to E and thus differs from other E concentrating nuclei. The DTAM and IX-X nuclei contain labelled cells after T and DHT, but not after E. Many hormone concentrating brain nuclei have been implicated in control of reproductive behaviors. Both T and DHT are effective in restoring sex behavior to castrated males; E is not. The hormone differences in labelling patterns described here may in part explain the different effects of androgens and estrogen on male sex behavior.

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1520 SEROTONERGIC REGULATION OF RHYTHMIC CORTICOSTERONE SECRETION IN THE RAT. T. Kepic*, R. Morgan*, J. P. Allen and C. F. Rowlands. Peoria Sch. Med., Peoria, IL 61605 and Southwest Foundation for Research and Education, San Antonio, TX

Serotonin is known to affect ACTH secretion in mammals. The suprachiasmatic nucleus (SCN) of the hypothalamus is rich in serotonin and presumably modulates the rhythmic secretion of hormones including corticosterone (B). We studied the effect of SCN ablation or the administration of 5,7-dihydroxytryptamine (5,7-DHT), a serotonin neurotoxin, on diurnal B secretion. This was accomplished by either destroying the SCN bilaterally using a radio frequency lesion generator or injecting 150 µg of 5,7-DHT in 30 µl of an ascorbic acid-saline vehicle intraventricularly at diestrous in groups of 160 gm female Sprague Dawley rats. Animals given 5,7-DHT were pretreated with protriptyline (20 mg/kg, ip) to prevent uptake in norepinephrine neuron terminals. Data from the two experimental groups were compared to those obtained from sham-operated controls or following the intraventricular injection of the ascorbic acid-saline vehicle in protriptyline treated controls. Three weeks following neurosurgery, the rats were decapitated and trunk blood collected at 0700 hr (AM) or 1600 (PM) and B measured by radioimmunoassay. There was no significant difference in the mean plasma B concentration between those with SCN lesions compared with the 5,7-DHT treated group at either the AM or PM periods. There was no significant difference between the AM and PM mean plasma B levels in each experimental group. The actual AM and PM levels in both experimental groups were significantly higher ($P < 0.05$) and lower ($P < 0.01$) respectively, than in the two control groups which had the normally expected diurnal difference in plasma B levels. We conclude that the effect of either SCN destruction or administration of a serotonin neurotoxin had a similar effect. These observations support the hypothesis that a serotonergic influence from the SCN is involved in the regulation of the circadian secretion of corticosterone in the rat.

1521 ONTOGENY OF CATECHOLAMINE-NEUROPHYSIN INTERACTIONS IN THE RAT HYPOTHALAMUS. H. Khachaturian* and J.R. Sladek Jr. (SPON: J.P.J. Maurissen). Dept. Anat., Sch. of Med. & Dent., Univ. of Roch., Rochester, N.Y. 14642.

The development of the magnocellular, hypothalamo-neurohypophysial system of the rat was investigated with a combined fluorescence-immunocytochemical approach for the demonstration of monoamines and neuropeptides (McNeill T.H. & Sladek J.R., Jr., 1978, Science 200:72-74). This technique is particularly useful for the study of ontogenetic events, because due to possible timing heterogeneities among littermates, precise correlation of different neuron systems is difficult unless they are simultaneously examined in each animal.

Norepinephrine (NE) is known to influence vasopressin secretion. This may indicate an important functional relationship between NE terminals, as seen with fluorescence microscopy and peptidergic neurons of the supraoptic (SON) and paraventricular (PVN) nuclei. Furthermore, the appearance during the late gestational period of neurosecretory material and catecholamine (CA) varicosities in SON and PVN raises questions regarding the time of establishment as well as the nature of functional relationships during development.

Brains from embryonic and early postnatal rats, ages 16, 17, 18, 20, 21 and 22 days postcoitus (dpc) and 1, 4, 7, 14, 21, 28 and 35 days postnatal (dpn) were processed for formaldehyde-induced fluorescence of CA. Alternate sections to those taken for fluorescence were stained immunocytochemically, using anti-rat neurophysin (RNP, provided by Dr. A. Robinson). RNP was visualized in the neuronal perikarya of SON and PVN on 17 and 18 dpc, respectively; the majority of immunoreactive cells stained very lightly, with a few cells staining darker. The numbers of darker staining perikarya, axons and dendrites increased with age in both nuclei, up to 28 dpn and remained essentially the same through 35 dpn. A few CA varicosities were also observed in the SON and PVN on 17 and 18 dpc, respectively. The density of CA varicosities in SON and PVN increasd up to 14 dpn, beyond which there were only subtle differences among the remaining ages studied. The distribution of CA varicosities in SON and PVN began to resemble the adult pattern as early as 4 dpn, became more apparent on 7 dpn and was well established by 14 dpn with CA varicosities frequently encountered in close juxtaposition to the magnocellular neurosecretory neurons. The appearance of neurosecretory material in SON and PVN much earlier than the establishment of varicosity-cell contacts might indicate a trophic influence of the magnocellular neurons upon the ingrowing NE axons.

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1522 THE PARTIAL PURIFICATION OF PROTHORACICOTROPIC HORMONE (PTTH) FROM THE TOBACCO HORNWORM, *MANDUCA SEXTA*. Timothy G. Kingan* and R. W. Newburgh.* (Spon: Duncan P. Taylor) Dept. of Biochem. & Biophys., Oregon State Univ., Corvallis, OR 97331.

The insect protein hormone prothoracicotropic hormone (PTTH) has previously been partially purified from a Lepidopteran, *Bombyx mori* (Ishizaki and Ishikawa, Biol. Bull. 1967, 133: 355-368; Yamazaki and Kobayashi, J. Insect Physiol. 1969, 15: 1981-1990). We report here the partial purification of this hormone from the tobacco hornworm, *Manduca sexta* (Lepidoptera: Sphingadae). Hormone activity has been followed throughout purification with a larval bioassay (Gibbs and Riddiford, J. Exp. Biol. 1977, 66: 255-266). Material for purification was obtained from the powdered heads of wandering stage (5th instar) larvae. The 2% NaCl/0.1 mM phenylthiourea extract was heat coagulated, with the activity remaining in the supernatant. Extracts were subsequently fractionated with ammonium sulfate, fractionally precipitated with acetone, and then chromatographed on Sephadex G-100. Active fractions were pooled for ion-exchange chromatography on DEAE-Sephadex in 0.1 M Tris-HCl, pH 7.90, with linear gradients of NaCl. Active fractions were pooled, dialyzed, lyophilized, and then subjected to flat-bed isoelectric focusing in a granulated gel. Hormone activity co-eluted with ovalbumin (45,000 daltons) from a Sephadex G-75 column. Material was quantitatively absorbed by DEAE-Sephadex with greater than 90% of the activity recovered by elution with buffer containing 0.1 M NaCl. Isoelectric focusing indicated that activity is recoverable from at least two slightly acidic pI's.

623 IMMUNOCYTOCHEMISTRY OF RAT PINEAL GLAND USING ANTISERA GENERATED AGAINST ANTIOVULATORY ANALOGS OF LRF. Karl M. Knigge, Diane T. Piekut*, and Shirley A. Joseph*. (SPON: Mary Ann Romagnano). Dept. Anat., Univ. of Rochester Sch. Med., Rochester, NY 14642.

Ongoing studies indicate that the pineal gland secretes (both in vivo and in vitro) a non-indolic substance which inhibits LRF-induced release of pituitary LH. This pineal anti-LRF factor exhibits immunological characteristics which suggest that its structure may provide antigenic determinants similar to those of LRF itself; partially purified preparations of this factor cross-react with certain LRF antisera and both pinealocyte cell bodies and/or processes stain immunocytochemically with these antisera. A considerable number of analogs of LRF have been synthesized which are effective antiovulatory compounds; 2 of the most potent analogs are [D-Phe^2, D-Trp^3, D-Phe^6]-LRF ("B-2") and [Ac-Pro^1, D-Phe^2, D-Trp^3, D-Trp^6]-LRF ("K-43"). Antisera were generated against these analogs using a bisdiazotized benzidene conjugation to bovine serum albumin. Cross-reactivity with LRF was examined by RIA procedures and immunocytochemical characteristics were studied by comparing the staining of median eminence and pineal gland. Antisera to analog K-43 recognized LRF and stained cells in the pineal gland; antisera to analog B-2 also stained cells in the pineal but did not recognize LRF. These results focus attention upon the N-terminal portion of the pineal anti-LRF substance with particular reference to positions 1 and 2.

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625 3α-Androstanediol as an Inhibitor of Reproductive Function in the Immature Female Rat. Ilze Kraulis*, Sally J. Naish* and K.B. Ruf (SPON: F. Ervin), Dept. Ob. Gyn., McGill University, Montreal, Canada.

Immature female rats treated with 10 µg estradiol benzoate (EB) at 1200 h on d 23 and stimulated with 1 mg progesterone (P) 72 h later respond with a gonadotropin (GT) surge (Caligaris et al., Endocrinol., 55: 97, 1972) which may lead to ovulation. Aromatizable, but not ring A-reduced androgens, can effectively be substituted for EB in this system (Kraulis et al., Endocrinol. 103: 1822, 1978). As shown in the Table below, the ring A-reduced androgen, 5α-androstane-3α, 17β-diol (3α), 6 mg/100 g, can completely eliminate the estrogen-induced GT surge, if given 30 min before EB.

	EB only	EB/P	3α/EB/P	3β/EB/P
LH /ng/ml)	58±29	1089±215	21±16	600±93
FSH /ng/ml)	393±78	2178±489	349±107	1597±309
(4-5 animals per group)				

Implantation on d 21 of silastic capsules of the 3α-, but not of the 3β-diol, led to signs of hypopituitary hypogonadism in 51-d-old rats as indicated by small-for-weight pituitaries as compared to blank implanted controls (4.63±0.28 vs 7.56±0.75 mg), ovaries (21.02±9.96 vs 39.88±2.60 mg), and uteri (145±32 vs 248±21 mg). This inhibitory effect on the reproductive system may be reversible, since upon removal of the capsules (d 51) 4 out of the 6 animals were successfully impregnated. These studies suggest that the 3α-diol, a prominent product of the immature rat ovary (Eckstein et al., Endocrinol., 94: 224, 1974) may serve as a natural inhibitor of sexual maturation during the prepubertal period. (Supported by MRC grant MA-6235).

1524 CONNECTIONS OF HYPOTHALAMIC NEUROSECRETORY NUCLEI WITH VISCERAL SENSORY STRUCTURES IN THE BRAINSTEM OF THE RAT. Edward Tongju Koh* and Juarez A. Ricardo* (SPON: W.J.H. Nauta). Dept. of Psychology, Massachusetts Institute of Technology, Cambridge, Mass. 02139 and Dept. of Anatomy, Harvard Medical School, Boston, Mass. 02115.

Recent reports have shown that the paraventricular nucleus of the hypothalamus (pa) receives direct input from both the caudal portion of the nucleus of the solitary tract (nts; Brain Res. 153:1) and the parabrachial region (pb), and projects directly back to these structures. In view of pa's role in releasing antidiuretic hormone (ADH) and oxytocin (OXY), such relations of pa may represent mechanisms by which information from vagal and glossopharyngeal receptors modulates neurohypophysial effects on the body's fluid status and triggers adenohypophysial responses to some types of stress. In the present study, the anterograde autoradiographic and retrograde horseradish peroxidase (HRP) tracing techniques were used to further examine this neural system.

HRP injected into either the caudal portion of the nts or pb labeled neurons in pa. In both series, the population of labeled neurons lies within parvocellular portions of pa, avoiding the magnocellular regions which occupy its lateral borders (Brain Res. 108:187). At the caudal pole of the nucleus the population extends laterally away from the third ventricle and establishes continuity with a group of labeled neurons within the posterior fornical nucleus (pfo; J. comp. Neur. 128:181), an accessory neurosecretory cell group which lies dorsal to the fornix.

Tritiated leucine and proline injected into either the caudal part of the nts or pb labeled a continuous region including both pa and pfo. Within pa, tracer substance is again heavily concentrated within parvocellular territories, with little labeling above background levels in magnocellular regions.

All of the projections described are bilateral, with uncrossed components which are heavier than their crossed counterparts.

The present findings suggest that pa's interactions with visceral sensory structures are mediated by its parvocellular regions and perhaps also by pfo, with which it shares both afferent and efferent visceroceptive connections. In the light of evidence that the hypophysial projections of pa arise chiefly from its magnocellular portions (Brain Res. 88:403), the axons it sends to nts and pb are probably not collaterals of the hypothalamo-hypophysial tract. However, pfo also projects into this tract, meaning that its projections to visceroceptive brainstem nuclei may well arise from neurons which also send their axons to the pituitary. This notion is strengthened by reports that antibodies to ADH and OXY bind within pathways to nts and pb (Anat. Rec. 190:349; SNS 4:1316). (Supported by USPHS grants NS 06542, MH 25515 and 5 T05 GM 02220, and by Fellowship Award 04-75/0167 to J. A. R. from the Fundação de Amparo à Pesquisa do Estado de São Paulo, Brazil.)

1526 THE ANTERIOR PITUITARY DOPAMINE RECEPTOR; A MULTIPLE SUB-SITES COMPLEX. F. Labrie*, T. Di Paolo*, B. Gagné*, J.P. Raynaud* and J.R. Boissier* (SPON: J.F. Estable-Puig). MRC Group in Molecular Endocrinology, CHUL, Québec, G1V 4G2, Canada, and Centre de Recherches Roussel-UCLAF, Romainville, France.

Since it is well documented for a large series of hormones (and also some neurotransmitters) that the response of a target tissue is largely dependent upon the level of specific tissue receptors, it becomes important to gain a detailed understanding of the characteristics of binding to the dopamine (DA) receptor. In order to avoid interference by possibly different properties of pre- and post-synaptic receptors in the central nervous system, our studies were performed using a tissue containing exclusively post-synaptic DA receptors, the anterior pituitary gland. In agreement with previous observations in brain and pituitary, DA agonists were more potent than antagonists to displace [^{3}H] apomorphine binding from bovine anterior pituitary membranes while the opposite was true for [^{3}H] haloperidol and [^{3}H] spiroperidol binding, such data being compatible with two states of the DA receptors. However, when the potent DA agonist [^{3}H] RU24213 (N-n-propyl-N-phenylethyl-β-(3-hydroxyphenyl) ethylamide hydrochloride) was used as ligand, the pattern of displacement was RU24213 > haloperidol > (+) butaclamol > spiroperidol = (-) butaclamol while dihydroergocryptine, apomorphine and DA were inactive up to 10^{-4}M, this specificity being highly antagonistic. Moreover, the specificity of binding of another potent DA agonist, [^{3}H] dihydroergocryptine was closer to that of antagonists than agonists. The presence of multiple subsites was also clearly indicated by the ligand-specific potency of each unlabeled drug, the presence of biphasic curves, the extension of many displacement curves over many orders of magnitude and the variable maximal levels of displacement obtained. The present data show that the specificity of binding to a probably pure population of postsynaptic dopaminergic receptors does not correlate necessarily with the pharmacological agonistic and antagonistic properties of the ligand used. Such findings clearly indicate that the DA receptor exists as a multiple subsites complex and that some of these subsites (at least five, specific for each dopaminergic drug used) may not be linked to a biological function.

1527 EFFECT OF SYSTEMIC MORPHINE ON AMYGDALOID SEROTONIN CONTENT AND SERUM LEVELS OF LUTEINIZING HORMONE. Joan M. Lakoski and Gerald F. Gebhart, Dept. of Pharmacol., Univ. of Iowa, Iowa City, IA 52242.

Ascending serotonergic projections to the amygdaloid nuclei and hypothalamus have been implicated in the regulation of the secretion of luteinizing hormone (LH). Recent evidence defines a role for these serotonergic pathways in the depression by opioids of LH release in the rat (Maughan et al., Brain Res. 155:413, 1978). To evaluate the role the amygdaloid nuclei might play in the depression of serum levels of LH, the effect of acute parenteral administration of morphine (MOR) on levels of serotonin (5-HT) in 8 amygdaloid and 1 hypothalamic area was examined and correlated with MOR's effect on serum LH.

Male Sprague-Dawley derived rats maintained on a 12:12 light-dark cycle (lights on 0700 hr) were non-stressfully injected with MOR (20, 7.5, or 1 mg/kg), naloxone (NAL; 1 mg/kg), MOR plus NAL (7.5 + 1 mg/kg), or saline I.P. at 0800-0900 hrs. Animals were decapitated 60 minutes after injection and the brain tissue was rapidly removed, frozen and stored at -80°C. Blood from the decapitated trunk was collected, the serum separated, and stored at -80°C. Samples were punched from the following 8 amygdaloid nuclei (584 μ dia) and 1 hypothalamic area (508 μ dia) from frozen coronal brain sections and assayed for 5-HT employing an enzymatic-isotopic method (Saavedra et al., JPET 186:505, 1973): medial, cortical, basolateral, lateral posterior, central and the cortical, basolateral and lateral posterior caudal amygdaloic nuclei and the hypothalamic ventromedial nucleus (VMN). Serum LH was determined using a double antibody radioimmunoassay method (NIAMDD). Control serum LH levels (28.7 ± 2.4 ng/ml, n=15) were depressed by MOR in a dose-related manner. Serotonin content in the amygdaloid nuclei and VMN after MOR administration did not significantly differ from control levels of serotonin at any of the MOR doses employed. These data suggest the amygdaloid nuclei may not be the primary site of opioid regulation of LH secretion by ascending serotonin pathways. Supported by NIH grant NS 12114.

1528 DISCRETE LESIONS OF THE DORSAL ROSTRAL PONS PREVENT ACTH INCREASES AFTER HEMORRHAGE. Alan M. Lefcourt*, David G. Ward, Helen Santana*, Virginia S. Rudrow* and Donald S. Gann. The Johns Hopkins Univ. School of Medicine, Baltimore, MD 21205.

The region of the locus coeruleus has been implicated in the ACTH response to hemorrhage, but alternative pathways have not been excluded. Electrical stimulation of this area leads to changes in release of ACTH (Endocrinol. 99:1220, 1976; Fed. Proc. 38:1200, 1979) and neurons in the same area respond to decreases in venous return (Fed. Proc. 37:743, 1978). However, stimulation of more lateral and medial regions also lead to changes in ACTH (Fed. Proc. 38:1200, 1979), and the lateral pons receives projections from the solitary nuclei (J. Comp. Neurol. 181:421, 1978). To determine whether the region of the locus coeruleus is essential for the ACTH response to hemorrhage, bilateral electrolytic lesions (11 cats) or sham lesions (3 cats) were placed chronically in the pons of 14 anesthetized cats. Four days later the animals were anesthetized with chloralose/urethane and four hours later were hemorrhaged 15 ml/Kg/3 min. Arterial blood samples were collected over 3 min periods for 30 min beginning 6 min before hemorrhage and were assayed for ACTH by RIA. Responses were analyzed by analysis of variance. Data were grouped both according to the presence or absence of responses of ACTH and according to whether the lesions included the locus coeruleus bilaterally. Both methods for categorizing the data led to identical groups as shown:

ACTH pg/ml

	before Hem		after Hem				
	-6 min	-3 min	3 min	6 min	9 min	15 min	21 min
sham (n=7)	59±16	69±18	126±28	125±22	125±11	124±20	114±20
lesion(n=7)	73±11	66±9	61±10	43±4	61±11	75±11	90±22

The sham group includes sham lesions or lesions of areas surrounding the locus coeruleus and shows a significant response of ACTH to hemorrhage ($P<0.05$). In contrast, the lesion group includes bilateral lesions of the region of the locus coeruleus and shows no response of ACTH ($P>0.5$). The common overlap of the lesions in the lesion group is a region of less than 1 mm^3 that is within an area from which stimulation leads to changes in ACTH. The region includes the area of the ventral locus coeruleus and of the locus subcoeruleus that contains neurons responding to decreases in venous return, and an area immediately dorsal. The present data indicate that, under the conditions of the experiments, a discrete region within the locus coeruleus and locus subcoeruleus is essential for the response of ACTH to a 15 ml/Kg hemorrhage. (Supported in part by NIH grants AM14952 and GM7031).

1529 TESTOSTERONE EFFECTS ON ENZYMES IN CENTRAL AND PERIPHERAL TARGET SITES IN Tfm MUTANT MICE. V.N. Luine, N.J. MacLusky* and B.S. McEwen. Rockefeller University, New York NY 10021.

The testicular feminization mutation (Tfm) in mice is a mutation on the X chromosome conferring an insensitivity to testosterone (T) action in target organs. Affected mice show deficiencies in cytosol androgen receptors (Attardi et al., Endo 98: 864, 1976; Gehring et al., Nature New Biol. 232:106, 1971) and in the response of a number of enzymes to T in peripheral target sites (Ohno et al., Clin. Gen. 1:35, 1970).

We have examined the response of enzymes in central and hypophyseal sites to androgen as well as confirmed previous work in peripheral targets. Normal Balb/C and Tfm males were castrated and received s.c. implants of Silastic capsules containing cholesterol or T. Five days later they were sacrificed and cerebral cortex (Ctx), whole hypothalamus, preoptic area and amygdala (HPA), pituitary and kidney were removed and homogenized. The following enzymes were measured: monoamine oxidase (MAO) in Ctx and HPA; glucose-6-phosphate dehydrogenase (G6PDH) and lactate dehydrogenase (LDH) in pituitary; and G6PDH and alcohol dehydrogenase (ADH) in kidney.

In the brain, MAO activity in the HPA from normal males was increased approximately 20% by T. T was without effect on MAO in the HPA from Tfm males. In the Ctx, a brain region which contains far fewer T receptors than the HPA, T administration did not alter MAO activity in either normal or Tfm males. Kidney ADH activity was dramatically increased, approximately 6-fold, in normal males, but activity in T treated Tfm's was unchanged. G6PDH activity in the kidney also increased after T in normal but not Tfm mice. The change in normal males, approximately 30%, was modest compared to the ADH change. T effects on pituitary enzymes were not noted in either normal or Tfm mice.

The ability of T to increase activities of ADH and G6PDH in kidney and MAO in the HPA of normal but not Tfm mice led us to measure these enzymes in intact normal and intact Tfm mice. Kidney ADH levels were higher in intact mice, but G6PDH and MAO were identical in the two groups.

While further experiments are needed to clarify the hormonal regulation of G6PDH and MAO, it is clear that the response to T is abnormal in Tfms. Results with MAO are important because this is the first central enzyme shown to be androgen resistant in the Tfm, and it may provide a marker for exploring how alterations in levels of central androgen receptors may affect androgen sensitive neurochemical events in neuroendocrine regulation.

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1530 MELATONIN SPECIFICALLY INHIBITS PITUITARY RESPONSIVENESS TO LH-RELEASING HORMONE. Jeanne E. Martin and Carol Sattler.* Department of Pharmacology, Washington University Medical School, St. Louis, Missouri 63110.

Previous studies from this laboratory have shown that the pineal indole melatonin (MEL) can suppress the neonatal rat pituitary LH and FSH responses to LH-releasing hormone (LHRH). The present study has examined the specificity of MEL inhibition for the gonadotropins by measuring the effects of MEL on thyrotropin-releasing hormone (TRH) induced release of TSH and prolactin (PRL) and on basal release of TSH, PRL, and GH by pituitary cells in culture. Neonatal rat anterior pituitary cells were dissociated with collagenase and hyaluronidase and cultured for 22h. At the time of use, the dispersed cells were washed with fresh medium and incubated for 3h in control medium or medium containing TRH (0.1-1000 nM) or LHRH (1-1000 nM) either alone or in the presence of MEL (10 or 1000 nM). Medium concentrations of TSH, PRL, GH, and LH were determined by double antibody radioimmunoassay with the use of materials supplied by the NIAMDD. As previously, MEL (10 nM) significantly ($p<0.01$) suppressed LH release by all concentrations of LHRH with inhibition of the response ranging from 17-56%. This concentration of the indole consistently produces maximal suppression of both LH and FSH responses to LHRH. By contrast, MEL at a 100-fold greater concentration (1000 nM) had no effect on either TSH or PRL release induced by any dose of TRH. In the absence of releasing hormones, medium LH increased only about 50% over zero-time values during the 3h incubation whereas the levels of TSH, PRL and GH increased 200-300%. While this apparent spontaneous secretion of TSH and GH was not affected by MEL (1000 nM), the indole has consistently induced a slight (16-18%), but significant ($p<0.05$), suppression of PRL release. These findings reveal that MEL inhibition of releasing hormone-induced pituitary secretion apparently is specific for LHRH. The data suggest, however, that MEL may also exert a regulatory effect on PRL secretion by the pituitary gland. (Supported by The Population Council).

1531 CNS ACTIVITY DURING THE ESTROUS CYCLE OF THE RAT. J. F. Masken and R. J. Morgan. Dept. Physiol. and Biophys., Colo. State Univ., Fort Collins, Colo. 80523.

Sprague-Dawley rats were implanted with chronic electrodes in the amygdala, pre-optic area, and arcuate nucleus-median eminence region in order to learn about electrical communication in the limbic system during the estrous cycle. Electrical signals were recorded simultaneously for ten minutes of each half-hour between 10:00 h and 15:30 h on each day of the estrous cycle. These signals were analyzed for amount and direction of signal traffic between pairs of electrodes and for transmission times between pairs of sites. Signal traffic from the amygdala to the preoptic area lasts longer and takes longer en route during the "critical period" on the day of proestrus than at other times of the cycle. This is statistically significant at $p < 0.0002$ and implies a change in the signal pathway during this time. These data also imply that the arcuate-nucleus median eminence is probably not directly involved in initiating the release of gonadotropin-releasing hormone as a key event in the estrous cycle in the rat.

1532 EFFECTS OF GAMMA-AMINOBUTYRIC ACID (GABA) ON LORDOSIS BEHAVIOR AND DOPAMINERGIC ACTIVITY IN ESTROGEN PRIMED SPAYED FEMALE RATS. Marilyn Y. McGinnis*, John H. Gordon and Roger A. Gorski (Spon: M. Krieger). Dept. Anat. Sch. Med. UCLA, Los Angeles CA 90024.

To examine alterations in lordosis behavior (measured by the lordosis quotient: LQ) and dopaminergic function as they relate to changes in GABA activity, two drugs which alter GABA function were studied: picrotoxin, a GABA receptor blocker, and hydrazinopropionic acid (HPA) which elevates endogenous GABA levels. Because spayed female rats show low LQ's when primed with estrogen only, a suppression of lordosis would not be detected. Therefore, septal lesioned (SL) rats were used for detecting decreases in lordosis behavior as they show high LQ's after treatment with estrogen only. Also the estrogen-primed SL rat is a useful model in that it is known to have lower dopamine (DA) turnover and higher GAD activity than sham-operated (Sham) controls. To test whether alterations in GABA were correlated directly with changes in LQ, spayed SL and Sham rats were primed with estradiol benzoate (EB: 2µg/day for 3 days) and tested for behavior on the fourth day before and after bilateral infusion of drugs directly into the substantia nigra. SL rats received picrotoxin or saline; Sham rats received HPA or saline. In SL rats, the LQ was suppressed 30 min after picrotoxin but was again elevated by 120 min post infusion. Conversely, HPA resulted in a moderate increase in the LQ at 30 min and reached high levels by 120 min. The LQ was again low by 360 min post infusion demonstrating that the effect was reversible. Saline had no effect on behavior. In experiment II, SL and Sham rats were primed with EB and infused with the drugs as in Experiment I, but were sacrificed at the time the maximal behavioral effect had been observed in order to determine the effects of these same drugs on DA activity. Tyrosine hydroxylase (TH) activity and DA and homovanillic acid (HVA) levels were measured. No effect on TH activity was found, but Sham rats receiving HPA infusions had lower DA and HVA levels compared to those receiving saline, and SL rats receiving picrotoxin infusions had higher DA and HVA levels than those of saline controls. SL saline infused rats also showed decreased DA and HVA levels relative to Sham saline infused animals. Thus, these three variables tend to be related in a particular way: when DA is low and GABA is high, the LQ is high, and conversely, when DA is high and GABA is low, the LQ is also low. These results support the concept of a GABA inhibitory neuronal feedback system which modulates DA turnover and perhaps plays a critical role in the neural control of lordosis behavior.

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1533 PINEAL, TESTES, ADRENAL AND THYROID DATA IN INSULIN DEFICIENT RATS AFTER INJECTIONS WITH ANTERIOR HYPOPHYSEAL EXTRACT. M. Evelyn McNeill, Carl R. Morgan* and Everett C. Simpson*. Depts. of Anat. (Sch. Med.) and Biology, East Carolina University, Greenville, N. C. 27834.

In conjunction with an investigation of the pancreotropic effect of anterior hypophyseal extract (beef), we have initiated a study of the effects of this extract on other endocrine tissue with particular interest focused on the pineal - testes axis. Male Sprague-Dawley rats (18), made insulin deficient with i.v. injection of 2% alloxan, 40 mg/kg body weight, were paired with 18 normal rats of comparable weight. Half of the alloxan-diabetic rats and half of the normal rats were injected (2 ml, s.c.) daily x 14 with a crude alkaline extract of beef anterior hypophysis. On day 14 the animals were weighed, bled, anesthetized with Diabutal (60 mg/kg) and perfused with 10% formalin for light microscopic observations or with 0.25 Karnovsky's fixative for EM observations. The pineal, testes, adrenals and thyroid were harvested from alloxan-diabetic (AD), alloxan-diabetic plus extract (AD+E), normal (N) and normal plus extract (N+E) groups. The initial body weight, final body weight, wet weight of the pineal, testes, adrenals and thyroid were recorded in gms. Statistical analysis of the numerical data was carried out with an electron desk computer (Sharp Compet 364 P-II) using magnetic cards containing programs for multiple analysis of variance.

Group & Number	$\bar{x}$ Initial Body Wt. Gms.	$\bar{x}$ Final Body Wt. Gms.	$\bar{x}$ Pineal Wt. Gm.	$\bar{x}$ Testes Wt. Gms.	$\bar{x}$ Adrenal Wt. Gm.	$\bar{x}$ Thyroid Wt. Gm.
AD(n=8)	229	234	.00128	3.17	.06789	.01808
AD+E(n=9)	220	239	.00117	3.18	.14049	.01813
N(n=9)	219	257	.00189	3.51	.05691	.01990
N+E(n=9)	219	284	.00203	3.14	.12304	.02838
	$p>.05$	$p<.01$	$p<.01$	$p>.05$	$p<.01$	$p<.01$

Comparison of the mean testes weights for the 4 groups revealed no significant differences. Pineal weights in groups AD and AD+E were both significantly different from groups N and N+E ($p<.05$). In addition adrenal weights in groups N and AD were significant at the $p<.05$ level from groups N+E and AD+E. The only significant difference ($p<.05$) found in the thyroid weights was confined to the N+E group. Group comparisons were determined by the Neuman-Keul analysis.

1534 INHIBITION OF MELANOCYTE-STIMULATING HORMONE SECRETION BY NALOXONE IN AMPHIBIANS. S. P. Mennin* and L. C. Saland. Dept. Anat., Sch. Med., Univ. of New Mexico, Albuquerque NM 87131

It has recently become apparent that opiate peptides interact with several neuroendocrine systems. In view of the similarities between the proposed control mechanisms of prolactin in mammals and melanocyte-stimulating hormone (MSH) in amphibians we undertook a series of experiments to determine if opiate peptides were involved in the control of MSH secretion. Pars intermedia secretory cells contain MSH, beta-lipotropin, endorphins, and adrenocorticotrophic hormone, which may be present in the same secretory granule and released simultaneously. Melanophore changes may therefore be a valid biologic assay in amphibians for release of opiate peptides. Naloxone hydrochloride (20mg/kg, Endo Laboratories) was injected into the dorsal lymph sac of light adapted frogs at hourly intervals for three hours. Animals were placed on a dark background after the first injection, and the melanophore index (MI) was determined (Hogben and Slome, 1931, Proc. Roy. Soc. B, 108:10). The mean MI of naloxone injected frogs (2.1 ± 0.2) (mean $\pm$ S.E.M.) was significantly lower than control MI's (3.1 ± 0.1) after one hour and remained significantly less for three hours (naloxone: 2.1 ± 0.1, control: 4.1 ± 0.1). Naloxone also prevented the transient lightening observed routinely after handling. In contrast, the MI of animals already dark adapted and treated with naloxone was not significantly different from that of control dark adapted frogs at any time up to four hours. Ultrastructural examination of intermediate lobes from frogs moved from light to dark backgrounds and treated with naloxone revealed cellular characteristics of light adaptation. Controls acquired the endoplasmic reticulum and Golgi zone expansions typical of dark adaptation. These data support a role for endogenous opiates in amphibians. The data further indicate that naloxone may enhance the neural inhibition of MSH release. We suggest that an opiate-receptor dependent mechanism may modulate hypothalamic control of pars intermedia peptides. (Supported by a grant from NSF PCM 78-16224 and R-3002 from University of New Mexico.)

1535 RELEASE OF α-MSH-ACTIVITY FROM ISOLATED RAT NERVOSA-INTERMEDIA (NI) FOLLOWING ELECTRICAL STIMULATION AND IONIC CHANGES. H. J. Mitchell* and J. V. Milligan. Dept. of Physiology, Queen's University, Kingston, Ontario K7L 3N6.

Release of α-MSH from rat pituitary is thought to be under inhibitory control by the hypothalamus. This hypothesis is based on indirect evidence. We tested the theory directly using a modification of the in vitro procedure first described by Mikiten (1967). The NI were separated from the pars distalis with a bent needle using 10X magnification. The total time from decapitation of the rat to the end of tissue isolation did not exceed 40 sec. One or more isolated rat NI were skewered on a #22 stainless steel hypodermic needle and set up so that field stimulation (40 Hz, 80 V, 1.75 msec, bipolar cathodal pulses) could be applied every other minute. Incubation media was Krebs Ringer bicarbonate supplemented with 1% BSA and 2% glucose. A pH of 7.4 was maintained by gassing with a humidified gas mixture of 95% O_2-5% CO_2. The tissue and media content of α-MSH were determined by a sensitive bioassay (100 ± 30 pg in 50 µl) modified from Tilders et al. (1975). Total α-MSH ranged from 2.0 to 5.3 µg/NI. Spontaneous release was followed for periods of 120 minutes with media changes every 10 min. After a maximum of 30 minutes a steady release ranging between 0.1% and 1.0% of the total α-MSH (0.5% average) was observed. This is much smaller than values reported by others. Electrical stimulation during a 10 minute period promptly produced either a small but significant increase or a small decrease in release (0.7-2.0 fold). Incubation in low Na^+ (25 mM), produced by equiosmolar substitution with sucrose, caused a prompt decrease (.27-.13 of control values) in α-MSH release. Electrical stimulation produced a doubling, on the average, of this release. When Ca^{++} was reduced to 15% of normal, by omitting the appropriate amount from the medium, the release decreased to about 0.4 of the control value and electrical stimulation caused a further drop to about 0.2 of the control value. We cannot exclude the possibility of a direct effect of the ionic changes upon the pars intermedia cells. However, we suggest that our isolated in vitro preparation of rat NI is being tonically stimulated by both spontaneous inhibitory and excitatory nervous activity. In control circumstances the inhibitory activity predominates. Modification of the ionic environment would appear to preferentially affect the activity of either excitatory or inhibitory nerves allowing the respective single effect to be seen more clearly.

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1536 EFFECTS OF PERINATAL ACTH-NALOXONE TREATMENT ON LH LEVELS, AND PHYSICAL AND BEHAVIORAL DEVELOPMENT. H. Monder, N. Yasukawa*, S. D. Michael* and J. J. Christian*. Dept. Biol. Sci., S.U.N.Y. Binghamton, Binghamton, NY 13901.

A previous study from this laboratory (Life Sci. 22: 1381, 1978) implicated opioid receptors in the role of ACTH in reproductive maturation. Rate of maturation is an important factor in the control of rodent populations. The detrimental effects of high population densities are transmitted to the young even if the parents are removed from the population. In order to determine if a naloxone sensitive ACTH system mediates these effects, pregnant mice were treated with ACTH extract and Naloxone from about 5 days prior to parturition. ACTH injections were continued in the mothers to 5 days postpartum.

Observations of physical development, such as body weight, age of eye opening and vaginal opening were made. LH levels in trunk blood at 60 days of age were measured. Behavioral observations included ability and time for turning over for pups after being placed on back at 3 days of age; squares crossed in an open field task at 32 days of age; and latency to react to heat on a hot plate at 50 days of age.

ACTH treated animals showed a lower body weight from parturition to sacrifice at 60 days of age. Eye opening and vaginal opening were delayed in these animals and LH levels were depressed in the blood samples taken when the animals were sacrificed. These effects were not observed in the offspring of animals treated with both ACTH and naloxone. Open field activity was depressed in the naloxone treated group, but not in the offspring of mothers treated with both ACTH and naloxone. Latency on a hot plate at 50 days of age was significantly reduced by the naloxone treatment, while ACTH had no effect.

ACTH has not been shown to pass the placental barrier. Therefore, a separate class of ACTH mediated receptors, probably in the mother, may affect reproductive development of the fetus. These receptors may be preferentially blocked by naloxone, preventing the ACTH-induced delay of maturation. Naloxone also affects locomotor as well as nociceptive systems, while ACTH does not affect the development of these systems. The interaction of ACTH and naloxone on square crossing activity in the open field may be due to changes in the emotional reactivity of the animals. This research was supported by NICHHD 10515.

1537 COINCIDENCE MODELS AND HAMSTER PHOTOPERIODISM. L. P. Morin. Dept. Psychol., Dartmouth College, Hanover, N.H. 03755.

The external coincidence model of photoperiodic time measurement predicts that the testes of male hamsters which have experienced prolonged exposure to long days will always atrophy in the absence of photic stimulation. Three experiments were designed to test this prediction. Groups of reproductively mature male hamsters entrained to a long photoperiod were subjected to one of a variety of light cycle phase shifts followed several days later by blinding. Across all experiments, full gonadal atrophy occurred in 100% of the animals (N=58) not experiencing a shift or experiencing a phase advance, but failed to occur in 44% of animals (N=27) experiencing a 4 hr phase delay maintained for 10 days prior to blinding. A decrease or increase in the period of the circadian locomotor activity rhythm was an after-effect of a phase advance or delay, respectively. Animals with atrophied testes showed relative compression of the nocturnal locomotor activity phase regardless of the type of phase change. The fact that blinding does not necessarily lead to gonadal regression cannot be easily explained by the external coincidence model. Both gonadal size and wheelrunning patterns in blind hamsters are lighting history dependent. The data are consistent with an internal coincidence explanation of hamster photoperiodic time measurement.

1538 RELEASE OF ACTH AND β-LPH BY HUMAN ANTERIOR PITUITARY CELLS IN VITRO. G.H.Mulder* and D.T. Krieger, Mount Sinai School of Medicine, Division of Endocrinology, New York, N.Y., 10029

Collagenase dissociation of postmortem (6-36 hrs) normal human anterior pituitary tissue yields viable (65-95% Trypan Blue exclusion) cells that were used in the study of the regulation of ACTH, β-EP and β-LPH release. Two or more cell columns from one batch of pituitary cells were superfused simultaneously according to our previously published method (Endocrinology 100, 1143, 1977). Effluent was collected at 2 min intervals and assayed by RIA for ACTH, β-EP and β-LPH, using antibodies specific for ACTH, for β-EP+β-LPH and for β-LPH. We have previously shown that such human cells release ACTH in a log.dose-response fashion after stimulation with a rat hypothalamus extract (NIH-HME), that ACTH release can be inhibited by glucocorticoids and that such ACTH release represents active secretion (8th Neuroscience Meeting 1978). Serotonin at 5 x 10^{-6}M increased ACTH release to 3-4 times basal levels (n=5). Norepinephrine at 5 x 10^{-6}M and Dopamine at 10^{-6}M had no effect (n=5 and n=3). AVP at 20-40 mU/ml doubled to tripled ACTH release (n=4). In all cases cited, release of β-EP+β-LPH was not affected by the substances that increased the release of ACTH. Recovery of ACTH, β-EP or β-LPH, all added at 2 x 10^{-10}M, was 70-80, 70-85 and 60-70% respectively. In experiments with rat pituitary cells, both rat NIH-HME and AVP stimulated the release of both ACTH and β-EP+β-LPH, indicating that the methodology of the superfusion system was not the cause of our inability to detect concomitant release of these peptides by human cells in vitro. In contrast, a partially purified human hypothalamus extract (5M acetic acid extraction followed by Sephadex G-50 chromatography) is the only substance thus far found to stimulate the release of human β-EP+β-LPH, though dose-response curves have not yet been constructed. The main component (+ 90%) of the released β-EP+β-LPH was β-LPH; little or no stimulation of β-EP release was observed, indicating that there was little or no conversion of β-LPH to β-EP. These results indicate that, contrary to our earlier findings, it is possible, using a human hypothalamus extract, to induce concomitant secretion of ACTH and β-LPH by human anterior pituitary cells in vitro.

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1539 THE EFFECT OF HIPPOCAMPAL LESIONS ON THE HYPOTHALAMIC-PITUITARY-THYROID-TARGET ORGAN AXIS. Helen M. Murphy and Cyrilla H. Wideman, John Carroll Univ., Cleveland, OH 44118, Donald W. Long*, Consolidated Biomedical Laboratories, Columbus, OH 43216, and Thomas S. Brown*, DePaul University, Chicago, ILL 60614.

Two experiments were conducted in an attempt to ascertain the effect of hippocampal lesions on the hypothalamic-pituitary-thyroid-target organ axis. In the first experiment total T_4 (RIA), total T_3 (RIA), cholesterol, triglycerides and albumin were measured in animals with hippocampal lesions (H) and in cortical control (C) and normal animals (N). The H animals had significantly less total T_4, significantly lower serum cholesterol levels and significantly lower triglyceride levels than control animals. There were no significant differences in total T_3 or serum albumin levels. In the second experiment total T_4, total T_3, free T_4 (RIA) and TSH (RIA) were measured in H, C and N animals as well as in thyroidectomized animals (T) and animals made hyperthyroid (I) by means of thyroxine injections. The H animals had significantly less total T_4 than C, N and I animals. The T group was significantly lower than all of the other groups. The H animals were not significantly different from C and N animals in total T_3, but were significantly lower than I animals and significantly higher than T animals. The H group had significantly less free T_4 than the C, N and I groups. The T animals were significantly lower than the other groups. Animals with hippocampal lesions were not significantly different from C and N animals in plasma TSH concentrations. The H animals had significantly more TSH than the I group and significantly less TSH than the T group. T_4 (RIA) to T_3 (RIA) ratios were calculated for all five groups. The H group was significantly lower than the C, N and I groups and significantly higher than the T group. The results of the two experiments present a paradox. From the total T_4 and free T_4 tests it would appear that H animals may be tending toward hypothyroidism. From the cholesterol and triglyceride tests it would appear that these animals may be tending toward hyperthyroidism. It is hypothesized (particularly from the TSH results and the calculation of the T_4:T_3 ratios) that animals with hippocampal lesions have an increased utilization of thyroid hormones. This hypothesis could explain many of the behavioral studies in which animals with hippocampal lesions resemble hyperthyroid animals.

(Rat TSH standard was provided by NIAMD.)

1540 DIFFERENTIAL CHANGES IN VASOPRESSIN IN SPECIFIC HYPOTHALAMIC NUCLEI AND CIRCUMVENTRICULAR ORGANS AFTER PROGRESSIVE DEHYDRATION. A. Negro-Vilar* and W. K. Samson* (SPON: J. M. Lipton). Dept. Physiol. Univ. of Tx. Hlth. Sci. Ctr. at Dallas, TX 75235.

Increased extracellular osmolarity after water deprivation (WD) is a well known stimulus for vasopressin (AVP) release. Taking advantage of a highly sensitive radioimmunoassay for AVP combined with microdissection techniques, we have analyzed the sequential changes in AVP in specific brain areas, in the posterior pituitary (PP) and in the serum of rats after 1-7 days of WD. Serum AVP increased progressively up to 3 days after WD, was still high by 5 days and declined to low levels by day 7 of WD. AVP in the PP declined steadily to values below 10% of controls by day 7 of WD. AVP levels in paraventricular (PVN), suprachiasmatic (SCN) and arcuate (ARC) nuclei, declined slightly by 3 days and clearly fell below control levels by day 7. ME levels of AVP were also diminished after 7 days of WD. Levels of AVP in the SON remained unaltered throughout the 7 days of WD. AVP was also measured in the organum vasculosum lamina terminalis (OVLT), subfornical organ (SFO) and in the pineal gland. Changes in these circumventricular structures mirrored the changes in serum AVP, i.e. high values were seen at 3-5 days of WD and by 7 days levels had returned to low control values. To determine if the increased AVP content in the OVLT after 3 days of water deprivation was due to an increased delivery of AVP from the SCN, bilateral lesions were generated in this nucleus. Preliminary results suggest that SCN lesions reduced AVP levels in the OVLT in normally hydrated rats. It was also found that AVP levels in the OVLT did not increase 3 days after WD. If confirmed, these results would suggest that the AVP in terminals of the OVLT may originate in SCN cell bodies. The decline in serum AVP levels by day 7 of WD may reflect an exhaustion of AVP stores as indicated by the drop in AVP in the PP, PVN, ARC, ME and SCN. The lack of change in AVP levels in the SON even after 7 days of WD is puzzling, and suggests that these nuclei may play a role different from that of the PVN in the release of AVP. The increase in AVP levels seen in the circumventricular organs may be a passive reflection of peripheral AVP levels or may represent an epiphenomenon of some central action of the hormone. (Supported by NIH AM10073 and HD09988 and the Ford Foundation).

1541 QUANTIFYING SEXUAL BEHAVIOR IN FEMALE HAMSTERS. Nancy L. Ostrowski and Ralph G. Noble*. Dept. Psych. Rensselaer Polytechnic Institute, Troy, N.Y. 12181.

Females of many mammalian species display a vaginal orienting response to tactile stimulation of the perineum during coitus. This response aids the male in achieving intromission and successfully impregnating the female. A system for measuring this response in the female hamster has been developed.

The measurement system: The lateral component of this response can be measured using a technique which simulates tactile stimulation during coitus. Application of repetitive stimulation to an anatomically defined region results in a lateral displacement toward the point of contact. The magnitude of the reflex is a linear function of the log of the amount of force applied (ranging from .009 - 66.8 g of force), with the mean interobserver reliability (R) typically greater than .90.

The response: This lateral displacement response can only be obtained from females in the lordosis posture. This measure will discriminate among "receptive" females. Those which are active sexual participants display a greater amount of displacement than either (a) females mated to 30 or more intromissions and ejaculations, or (b) pregnant females which show lordosis. The magnitude of the response increases rapidly during the early stages of the estrus period; it decreases gradually toward the end of the estrus period and can be used to predict the termination of estrus.

Other manipulations which are known to alter the neuroendocrine state of an animal also produce changes in magnitude of this response including inescapable foot shock, morphine, ethanol, naloxone and voluntary exercise.

It is suggested that this measure may reflect subtle changes in the neuroendocrine status of the "receptive" female, changes which are not usually detected by other currently used measures.

1542 THE ONTOGENY OF THE LHRH NEUROSECRETORY SYSTEM IN THE ALBINO RAT: AN IMMUNOCYTOCHEMICAL STUDY. C.M. Paden* and A.J. Silverman (SPON. D. Micco). Rockefeller University and Dept. Anat., P&S, Columbia University, New York, NY 10032.

A study on the ontogeny of the LHRH neuroendocrine system was carried out on fetal and neonatal rats (Charles River, CD). Both male and female brains from 18 day fetuses to 20 day neonates were studied. Fetal brains were fixed by immersion in Bouin's solution; neonates were perfused with Bouin's solution and fixation continued by immersion for 48 hr. Brains were cut at 50um on a Vibratome, sections washed in PBS and incubated sequentially with: (1) rabbit antiserum to LHRH (48hr); (2) sheep anti-rabbit globulin serum (60min); (3) the PAP complex (90min) and (4) DAB-H_2O_2 (15min). All sera contained 0.4% Triton X 100. After immunostaining sections were mounted on glass slides, counterstained with cresyl violet and coverslipped. Specificity controls were performed on paraffin sections.

On day 21 of fetal life (day 1=day of sperm plug, and birth occurs on day 23) there is a dense fiber plexus around the organum vasculosum lamina terminalis (OVLT); only a few isolated fibers were present in the median eminence (ME) at this time. The number of LHRH positive axons in both structures increased throughout the first postnatal week, though the increase was more dramatic in the ME.

In the neonatal rat, LHRH cell bodies have been observed in the medial preoptic area at the level of the OVLT and in the n. of the tract of the diagonal band. Perikarya measured 10-12um in diameter and the majority had a single dendritic process extending for 60 to 250um. Some of these dendrites branched approximately 100um from the cell body. Although no LHRH neurons were observed in the arcuate nucleus of the neonatal rat, our antiserum does stain LHRH neurons in this region in the neonatal monkey in similarly prepared tissue sections.

By using Vibratome sections we have been able to enhance the immunostaining of LHRH fibers and perikarya in the perinatal rat. Furthermore the use of thick sections aids in observing the dendritic arborization of these neurosecretory neurons.

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1543 FURTHER CHARACTERIZATION OF PINEAL MONOLAYER CULTURES. J. Parr* V.D. Rowe Veterans Administration Medical Center, Kansas City, Missouri 64128, and Univ. of Kansas Medical Center, K.C., KS.

Monolayer cultures of cells derived from neonatal rat pineal glands have been shown to share certain characteristics with the pineal gland in vivo and in organ culture. In particular, serotonin N-acetyltransferase (NAT) activity is markedly stimulated by norepinephrine (NE) and by dibutyryl cyclic AMP (dBcAMP). These cultures, however, have been found to differ from organ explant cultures in at least 3 important respects: (1) Despite the many-fold increase in NAT activity with NE and dBcAMP stimulation, no formation of N-acetylserotonin or melatonin from either tryptophan or serotonin could be demonstrated. (2) Stimulation of NAT activity was potentiated by serum in a dose-dependent fashion. (3) L-propranolol was found to cause a small but significant stimulation of NAT activity, and this stimulation could be blocked by cycloheximide and actinomycin D. These differences may reflect the effect of the absence of neuronal influence on the developing pineal.

1544 THE TEMPORAL RELATIONSHIP BETWEEN ESTROGEN-INDUCIBLE PROGESTIN RECEPTORS IN THE BRAIN AND THE TIME COURSE OF ESTROGEN ACTIVATION OF MATING BEHAVIOR. B. Parsons*, N.J. MacLusky*, L. Krey, D.W. Pfaff and B.S. McEwen. Rockefeller Univ., New York NY 10021

Estradiol (E_2) has been shown to induce progestin (P) receptor synthesis in those areas of the brain which mediate sexual behavior, the mediobasal hypothalamus (MBH) and the preoptic area (POA). In this study, we explored the temporal relationship between inducible P receptors in the MBH-POA and pituitary (PIT), and the time course of estrogen activation of mating behavior.

Ovariectomized (OVX) female rats received Silastic capsules of E_2. Animals either were given mating tests after progesterone (P), or were sacrificed. Radioimmunoassay (RIA) indicated that plasma E_2 levels were 40.0 ± 4.6 pg/ml at 12h, and remained constant. Initial receptivity was seen after 18h (LQ=22 + 10). LQ's increased between 18 and 48h, approaching maximal levels (LQ=78 ± 13). In both MBH-POA and PIT, an increase in ^{3}H-R5020 cytosol binding was seen at 12h. When receptivity is first observed (18), inducible P receptors are 26% maximal. Increases in ^{3}H-R5020 binding were seen at 24, 36 and 48h, reaching maximal levels at 48h.

OVX females which had been implanted with E_2 capsules for 7 days had their capsules removed. All animals either were tested with E_2 and E_2 + P, or were sacrificed. At time 0, animals tested with E_2 and E_2 + P gave LQ scores of 98 and 100, respectively. Thus E_2 alone may induce full receptivity as measured by the LQ score. Behavior without P showed a more rapid decline than behavior with P. LQ's decreased progressively at 12, 24, and 36h, reaching 10% (+P) and 1% (-P). Receptivity was not seen at 48, 72 or 120h. In the MBH-POA and PIT, a decrease in ^{3}H-R5020 binding was observed at 12h. We analyzed mathematically the time course of decay of E_2 inducible P receptors. The half life of the inducible P receptor, a first order process, is 24.7h in MBH-POA, and 26.6h in PIT. When receptivity is last observed (36h), ^{3}H-R5020 binding was 34% maximal. RIA indicated that plasma E_2 had declined to ADX-OVX levels (6.8 ± 1.0 pg/ml) within 12h.

Three observations suggest a complex, non-linear relationship between inducible P receptors and sexual receptivity. 1) Continuous estrogenic stimulation for 7 days results in LQ's of 100 in the absence of P. 2) While the decay of inducible P receptors follows first order kinetics, the complex decay of mating behavior clearly does not. 3) Receptivity is first observed when inducible P receptors in brain are 26% maximal; sexual receptivity is last observed when inducible P receptors are 34% maximal. A threshold level (25-35% maximal) of P receptors in the MBH-POA of the rat brain thus appears to be a good index of the competency to express feminine sexual behavior.

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1545 EFFECTS OF PREOPTIC AND HYPOTHALAMIC STIMULATION ON SERUM LEVELS OF TESTOSTERONE AND CORTISOL IN INTACT AND GONADECTOMIZED MALE RHESUS MONKEYS. A. A. Perachio, J. G. Herndon*, M. Alexander and D. C. Collins*. Yerkes Regional Primate Research Center, Emory University, Atlanta, Georgia 30322.

Recent evidence has suggested that, in the rhesus monkey, social aggressive behavior may be correlated with fluctuations in blood concentrations of gonadal and adrenal steroids. Electrical stimulation of specific regions of the hypothalamus and preoptic area can be effective for eliciting both aggression and the release of prolactin and gonadotrophin (luteinizing) hormone. The purpose of the present experiments was to investigate the effects of electrical stimulation of the preoptic area and medial basal hypothalamus of unanesthetized male rhesus monkeys on testosterone and cortisol as measured in RIA of serum samples obtained through chronically implanted venous catheters. A single set of stimulus parameters (0.4 mA, 1.0 msec, 50 Hz, 10 sec/min trains) was used for all subjects and all stimulus sites. These parameters had been determined in preliminary experiments to be suprathreshold for producing manifest behavioral responses. Stimulation was delivered unilaterally through a monopolar electrode arrangement for 60 min. Blood samples were taken 30 min prior to and just before the onset of stimulation. Samples were obtained at 15, 30 and 60 min following stimulation onset and at 30 min intervals for two hours post-stimulation. Positive hormonal responses were observed as 30-500% increases in serum concentration of cortisol or testosterone during stimulation over the mean of the two pre-stimulation values. Stimulation of the medial basal hypothalamus in both castrated and intact males produced increments as great as 2 to 5 fold in levels of both testosterone and cortisol. In castrated males the time course of change of both hormones was parallel. Increases in steroid levels in some instances remained elevated for more than 2 hours post-stimulation. Of the 40 tested sites that were histologically verified to be located in the preoptic area or hypothalamus, most effective sites were found in the POA (15%) and VMH (12.5%), with additional active sites located in the SCH, ARC, LH and AHA. Negative sites, from which no response was obtained, were located in the MB, DMH, SEPT and OC. Behavioral observations were conducted during stimulation of some of the same sites while the animal was able to move freely in a social situation. Some, but not all, sites from which testosterone and cortisol release could be elicited also supported stimulation-bound aggressive behaviors. The magnitude of the hormonal response to hypothalamic and/or preoptic stimulation was equivalent to or greater than the elevation in testosterone that occurred after episodes of social aggression. (Supported by NIH Grants NS 09688 and RR 00165.)

1546 THE AFFERENT CONNECTIONS OF THE SUPRACHIASMATIC NUCLEUS: A HORSERADISH PEROXIDASE STUDY. Gary E. Pickard. Dept. Anat., College of Physicians & Surgeons,Columbia Univ., N.Y.,N.Y. 10032

The region of the hypothalamus that includes the suprachiasmatic nuclei (SCN) is known to be responsible for the entrainment of endogenous circadian rhythms to the external light-dark cycle. It has been shown that the SCN receives an input from the retina, the ventral lateral geniculate nucleus, and the midbrain raphe, however the precise anatomical location and morphlogical characteristics of these neurons are unknown.

The afferent connections of the SCN of the female hamster were examined using the method of retrograde transport of horseradish peroxidase (HRP). A 30% solution of HRP in 0.05 M Tris (pH 7.6) was iontophoretically applied to the SCN using 20-30 um (OD) micropipettes by a positive current of 2 uamps for 2-5 min. After a survival period of 24-72 hr, animals were perfused with 0.9% saline followed by 2.0% glutaraldehyde in 0.1 M phosphate buffer (ph 7.4). During the saline perfusion, both eyes were removed and the retinae were dissected from the choroid and pigment epithelium. The retinae were briefly fixed in 1.5% glutaraldehyde and rinsed in phosphate buffer. HRP in retinal whole mounts and 60 um thick frozen sections of the brains was demonstrated histochemically using tetramethyl benzidine.

HRP injected into the SCN was taken up by retinal ganglion cell terminals and transported to the retina. Labeled ganglion cells were large, relatively few in number and displayed no outstanding regional localization. After correcting for shrinkage resulting from fixation and histochemical procedures, the ganglion cell somata were estimated to average about 17 um in diameter. 3-4 proximal processes could often be followed a short distance from the labeled cells and entered the inner plexiform layer at a wide angle. Control injections into the optic chiasm resulted in the labeling of all sizes of retinal ganglion cells.

The ventral lateral geniculate nucleus (vLGN) was also labeled after HRP injections into the SCN. Labeled neurons were more often found in the lateral magnocellular subdivision, although the parcellation into magno- and parvocellular regions is not distinct in the hamster. There were 2-3 times more labeled cells ipsilateral vs. contralateral to the injection. The dorsal-caudal aspect of the vLGN contained the majority of labelled cells. Many large neurons were often clustered near the boundary between the dorsal and ventral LGN which is demarcated by traversing vessels.

HRP was also transported to neurons in the midbrain. Labeled cells were distributed bilaterally in the periaqueductal gray and in the dorsal and medial raphe nuclei.

Evidence exists that suggests all these afferent systems to the SCN modulate endogenous circadian rhythms. (Supported by a Fellowship from the Pharmaceutical Manufacturers Association).

1547 RELATIONSHIP OF DOPAMINE TURNOVER IN THE MEDIAN EMINENCE TO DOPAMINE CONCENTRATION IN HYPOPHYSIAL PORTAL PLASMA AND PROLACTIN RELEASE. Nancy S. Pilotte, Gary A. Gudelsky, and John C. Porter, Depts. Ob/Gyn. & Physiol., Green Ctr. for Reprod. Biol. Sci., Univ. of Texas Southwestern Med. Sch., Dallas, TX, 75235.

The release of prolactin from the pituitary gland is influenced by several substances, e.g., anesthetic agents and dopaminergic compounds. In the present study, we compared serum prolactin concentrations in urethane- or pentobarbital-anesthetized male rats with turnover of dopamine in the median eminence and concentration of dopamine in hypophysial portal plasma. The serum prolactin concentration in rats anesthetized with pentobarbital was 47 ± 7 ng/ml (mean and SE), whereas that in rats anesthetized with urethane was 16 ± 3 ng/ml. Inasmuch as the secretion of prolactin from the anterior pituitary gland is under tonic inhibitory control of dopamine released from tuberoinfundibular neurons, we examined the question whether the difference in serum prolactin concentrations in rats anesthetized with pentobarbital or urethane reflected differences in the release of dopamine from hypothalamic neurons. The effects of these anesthetic agents on the release of dopamine from tuberoinfundibular neurons were assessed by measuring the dopamine concentration in hypophysial portal plasma and dopamine turnover in the median eminence. Dopamine was determined in acidified extracts of plasma or median eminence fragments using a radioenzymatic procedure. Pituitary stalk blood was collected for 1 hr from rats anesthetized with urethane (1.5 g/kg, ip) or pentobarbital (40 mg/kg, ip). The dopamine concentration in hypophysial portal plasma from urethane-anesthetized rats was .49 ± .11 ng/ml, and was significantly greater than that in rats anesthetized with pentobarbital (.11 ± .03 ng/ml). The α-methyltyrosine (αMT)-induced decline of dopamine in the median eminence was used in the evaluation of dopamine turnover, using urethane- or pentobarbital-anesthetized male rats. The animals were injected with αMT (250 mg/kg, ip) 1 hr or 2 hr prior to removal of the median eminence. Anesthetized rats which were injected with 0.15M NaCl served as controls. The dopamine concentrations in the median eminence of saline-treated rats anesthetized with urethane or pentobarbital were the same. In addition, at no time after inhibition of dopamine synthesis with αMT did concentrations of dopamine in the median eminence of urethane- or pentobarbital-anesthetized rats differ significantly, indicating that the turnover of dopamine in the median eminence of these two groups of animals was similar. These results are suggestive that the release of dopamine from tuberoinfundibular neurons into pituitary stalk blood is greater in rats anesthetized with urethane than in rats anesthetized with pentobarbital, and are indicative of an inverse relationship between the dopamine concentration in hypophysial portal blood and the release of prolactin from the pituitary gland. Furthermore, it would appear that dopamine turnover in the median eminence is not necessarily an accurate index of the release of dopamine from those tuberoinfundibular neurons which secrete dopamine into hypophysial portal blood.

1548 THE LH-RELEASING ACTION OF N-METHYL ASPARTATE IS REVERSIBLE AND IS DEPENDENT ON ARCUATE HYPOTHALAMIC NEURONS. M.T. Price, J.W. Olney, M. Anglim*, V. Mitchell* and S. Buchsbaum*. Washington University School of Medicine, St. Louis, MO 63110.

Recently we reported that subcutaneous (sc) administration of a subtoxic dose (25 mg/kg) of N-methyl aspartate (NMA), a potent excitotoxic analog of glutamate and aspartate, to 25 day old male rats, induces a rapid elevation of serum luteinizing hormone (LH). The locus of NMA action is suprasellar, since NMA does not release LH from pituitary *in vitro* (Schainker and Cicero, Neurosci. Abstr. 4, 355, 1978). In toxic sc doses, NMA selectively destroys arcuate hypothalamic neurons (AH) which are thought to play a role in LH regulation. We have proposed that both the AH neurotoxic and LH releasing actions of NMA stem from specific interaction of NMA at excitatory receptors on the dendrosomal surfaces of AH neurons, the assumption being that irreversible depolarization underlies one phenomenon and reversible depolarization the other. Findings reported here further characterize the LH releasing action of NMA as rapid in onset, brief in duration, reversible and AH-dependent.

LH levels reach peak values 7½ min following NMA administration sc and return to base line within 30 min. Reversibility of the LH releasing action was explored by sacrificing groups of rats for LH determination 7½ min following either a 1st, 2nd, 3rd or 4th sc injection of NMA, the first 3 injections being at consecutive hourly intervals and the 4th being 24 hrs after the 3rd. The LH response to the 1st NMA injection was typical (12.7 times control values) but to the 2nd and 3rd was moderately and markedly attenuated respectively. The LH response to the 4th injection was the same as to the 1st. Thus, although repetitive stimulation of the pathway at hourly intervals reduces its response capacity, it is restored to normal after a 24 hr interval. We suspect this pattern reflects a transient depletion of LHRH stores with replenishment after a 24 hr rest period.

To further clarify the role of AH neurons in NMA-induced LH release, we destroyed approximately 80% of the neurons in AH by administering glutamate to neonatal rats, then challenged them with 25 mg/kg NMA at 25 days of age. LH levels in these animals 7½ min after NMA challenge did not differ from controls. It appears therefore, that AH neurons are a crucial part of this LH release pathway. This and other evidence, including the finding that alpha-aminoadipate, a specific antagonist of NMA excitation,blocks both the AH neurotoxic and LH releasing actions of NMA (Olney et al., Neurosci. Abstr. 1979), argues compellingly that the LH releasing action of NMA is not only AH dependent but stems from specific interaction of NMA with excitatory receptors (aspartergic?) on the dendrosomal surfaces of AH neurons. Supported by NIH grants DA-00259, MH-14677, NS-09156, a Huntington's Chorea Fdn. grant and RSD Award MH-38894 (JWO).

1549 BIOCHEMICAL AND PHYSIOLOGICAL EFFECTS OF UNPREDICTABLE AND PREDICTABLE STRESSFUL STIMULATION IN RATS. Carolos M. Quirce* and Mauricio Odio* (SPON: Roger P. Maickel). School of Pharmacy, University of Costa Rica, San Jose, COSTA RICA.

The biochemical and physiological effects of both unpredictable and predictable schedules of physical immobilization were determined in adult, male Sprague-Dawley rats. Both schedules were tested in isolated and group-reared animals; the total number of test sessions was also varied (15, 25, 36). Plasma levels of glucose and corticosterone were found to be significantly elevated for up to 48 hours after the last immobilization session in animals on the unpredictable schedules, but were essentially normal in those on the predictable schedules. In contrast, plasma levels of free fatty acids were significantly elevated in all animals on unpredictable schedules, but were significantly lowered in animals on the predictable stress schedules. Adrenal gland hypertrophy and relative decreases in total body weight gain were both greater in animals subjected to unpredictable stress schedules, as compared to those on predictable schedules. These findings suggest that elevations in biochemical and physiological stress markers can be achieved preferentially through the use of unpredictable schedules of stimulation. In contrast, predictable schedules appear to evoke a type of habituation or adaptation of stress marker levels.

1550 SYNTHESIS OF ACTH BY RAT NEURONAL CELL CULTURES. Richard J. Robbins*, Leonard P. Kapcala*, Richard H. Goodman*, and Seymour Reichlin. Dept. Med., Endocrine Div., New England Medical Center Hospital, Tufts University School of Medicine, Boston, MA 02111.

ACTH has been demonstrated by radioimmunoassay and immunohistochemistry in many areas of the brain including the amygdala, hypothalamus, hippocampus,midbrain and brain stem. Studies of brain ACTH after hypophysectomy have given contradictory results leaving unresolved the question as to whether ACTH can be synthesized by neurons themselves. In this experiment we determined serially the ACTH content of monolayer cell cultures derived from amygdala cells of day 1 neonatal rats. The amygdala was chosen for study because it is one of the limbic structures containing and regulating ACTH, our laboratory has shown somatostatin synthesis in these cell cultures and Pacold et al. had shown IR-GH production in amygdaloid cell cultures. Cells were dissociated by trituration and Papain-DNA-ase treatment, and the cultures maintained in a modified Eagles media with 10% heat inactivated horse serum at 35.5°C in 5% CO_2 in high humidity. The media were changed three times a week and the cells harvested at various times in distilled deionized water (DD) and sonicated. The initial cell suspension contained 80 pg of IR-ACTH/dish by radioimmunoassay and there was a progressive increase in cellular content up to day 23 when content was 257 pg/dish. This IR-ACTH exhibited parallelism to the standard curve. At no time was IR-ACTH release into the media shown. To demonstrate incorporation of precursor amino acid into ACTH ^{35}S-Met was incubated with day 14 cultures in Met-free media for 4 hrs. followed by 14 hrs. in normal media, or for 18 hrs. in normal media. The cells were harvested in DD water, sonicated, the extracts immunoprecipitated with anti-ACTH anti-serum and chromatographed on SDS gels which were analyzed by autoradiography. Each of the incubations gave rise to several bands, one of which was similar in mobility to $ACTH_{1-39}$. These findings are interpreted to indicate that amygdala neurons have the capacity to synthesize ACTH independent of the pituitary.

51 TWO EXCITABLE NEURONAL ELEMENTS IN THE PINEAL GLAND OF THE RAT: FURTHER EVIDENCE FOR A HABENULO-PINEAL PATHWAY. Oline K. Rønnekleiv*, Martin J. Kelly*, Wolfgang Wuttke* (SPON: D.L. Tomko) Western Psychiatric Institute & Clinic (OKR), Dept.of Physiology (MJK), Univ.of Pittsburgh Sch. of Med., Pittsburgh, PA. 15261, and Max-Planck Inst. for Biophysical Chemistry (WW), D-3400 Goettingen, W. Germany.

Recently we observed, using electron microscopy, degeneration of nerve terminals in the pineal following habenula lesions (Rønnekleiv & Møller, Exp.Brain Res.,in press). It was earlier believed that these central fibers running from the habenula and posterior commissures do not make synaptic contact in the pineal. We decided to further elucidate the nature of the central neural connection and the excitability of the pineal cells using conventional single unit recording techniques. Stimulating electrodes of Wood's metal (40-60µm tip) were placed in the habenula area of female rats. Extracellular AC recordings were made through glass-insulated tungsten electrodes (1-5µm, 8-14MΩ) placed in the pineal by penetrating the sagittal sinus from above. Throughout the recording period, single anodal pulses of 50-200 µsec duration and of 0.001-0.1mA strength were delivered to the habenula complex. Two distinct populations of pineal cells were found, 23 silent cells which were driven by habenula stimulation and 114 spontaneously active cells. In the former case 17 of the silent cells (median latency of 1.2msec) showed a positive-negative potential in response to habenula stimulation, and 6 of the 23 silent cells (∿1msec latency) showed only a positive potential of 1-2msec duration. The remaining cells (114) could not be driven by habenula stimulation but exhibited spontaneous activity with firing frequencies varying from less than 1Hz to greater than 100Hz with a median firing frequency of 10Hz. These experiments clearly demonstrate a direct habenulo-pineal fiber pathway and furthermore show that there are neuronal elements in the pineal which are only activated by habenula stimulation (∿17%). In view of the recent findings showing a direct peptidergic pathway from the suprachiasmatic nucleus (SCN) to the habenula complex (Sofroniew & Weindl, Am. J. Anatomy 153:391, 1978), our findings would indicate that a more direct innervation of the pineal by the SCN is possible than what had been proposed earlier. We do not know if the spontaneous activity is of pinealocyte origin since neurons have not been identified in the pineal, but intracellular recordings will reveal the nature of this activity and whether it is coupled to the secretory function of the pinealocyte.

OKR was an Alexander Von Humboldt Fellow and MJK an NIH Fellow at the Max-Planck Institute.

1552 PROJECTIONS TO THE LATERAL MEDULLA FROM THE MIDBRAIN AND PONS IN THE CAT. James D. Rose. Dept. Psychol., Univ. Wyoming, Laramie, WY 82071.

Evidence from single unit recording and lesion-behavioral experiments has implicated the lateral medullary tegmentum in the mediation of estrous vocalization and afterreaction responses to genital stimulation in the female cat. The principal densities of estrogen-concentrating neurons in the cat brain, however, are in hypothalamic, preoptic, and limbic regions. The present study was aimed at identifying descending pathways through which the activity of estrogen target neurons could be transmitted to lateral medullary cells. Cats received pressure-injections of 0.1-0.2µl of horseradish peroxidase (HRP) in a 50% saline solution, in the lateral medulla near nucleus ambiguus. After a 1-3 day survival, the brains were processed to demonstrate retrogradely transported HRP by the method of Mesulam (J. Histochem. Cytochem., 1978). At supramedullary levels, labeled neurons were concentrated mainly in the pons and midbrain. In the pons, labeled cells were most abundant in the ipsilateral medial and lateral parabrachial nuclei, the Kolliker-Fuse nucleus, and lateral tegmental field. Some nucleus raphe magnus neurons were also labeled. In the midbrain, the periaqueductal gray contained labeled neurons distributed widely, but unevenly along its rostro-caudal extent. The relative abundance of labeled central gray neurons varied substantially between animals. Labeled midbrain neurons were also found, in lesser numbers, just lateral to the central gray and in the oculomotor, trochlear and Edinger-Westphal nuclei. In non-midline midbrain regions most labeled neurons were ipsilateral to the injection site. Only a small number of labeled neurons, these in the lateral and periventricular hypothalamus, were found in the diencephalon. In view of variability of retrograde labeling of central gray neurons, electrophysiological verification of a medullary projection from this region was also undertaken. Central gray cells were antidromically-invaded in sufficient numbers by stimulation of the lateral or medial medulla to indicate the presence of a quantitatively significant number of neurons with descending axons. The existence of the central gray projection to the lateral medulla provides a potential route for activity of estrogen-concentrating neurons to be transmitted to neurons involved in vocal and other estrous reflexes, since some central gray neurons in the cat are estrogen-concentrating cells, and since the central gray receives a strong projection from the ventromedial hypothalamic nucleus, which contains numerous estrogen-concentrating cells.

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1553 DIURNAL VARIATION IN PITUITARY SENSITIVITY TO LHRH. K.B. Ruf, and J. McElhone*, Dept. Ob/Gyn., McGill Univ., Montreal H3A 1A1 Canada.

Gonadal steroids and LHRH itself have been recognized as the main modulators of the sensitivity of pituitary gonadotrophs. Recently, Caffrey et al (PSEBM 159,444,1978) reported that the neuropeptides oxytocin and vasopressin synergize with LHRH at the level of the anterior pituitary gland. Since it is possible that gonadotropin release is affected by additional brain peptides (some of which may be released in episodic manner), diurnal variations in pituitary sensitivity were investigated. In order to standardize the steroid milieu, groups of 5 adult female rats, ovariectomized for one month, were given estradiol benzoate (20 µg) at either 0700 h (AM), 1200 h (PM I) or 1700 h (PM II) colony time. Exactly 72 h later, 200 ng (D-Ala^6-des-Gly-NH$_2$^{10})-LHRH-ethylamide was injected i.v. and the LH and FSH response monitored over 4 h. Additional groups of 5 rats were pretreated with Dexamethasone phosphate (Dex. 100 µg/100 g b.w. i.p.) at -22h and -4 h. Results, expressed as areas under the respective curves and subjected to 2-way ANOVA are tabulated below:

Hormone measured	Treatment applied	AM	PM I	PM II
LH	LHRH	74*	100	80*
	Dex/LHRH	53*	72*	80*
FSH	LHRH	61*	100	71*
	Dex/LHRH	63*	78	74*

(Figures refer to areas under curves as percentage of PM I-response to LHRH; * denotes $p < 0.05$ compared to 100% response).

The results indicate that the sensitivity of the gonadotrophs is not governed solely by gonadal steroids and the administered LHRH, but shows marked diurnal variations which can be abolished by pretreatment with Dex. The nature of the factor(s) influenced by the glucocorticoid (ACTH, endorphins, half-life of LHRH, adrenal progesterone/DOC?) is under investigation. (Supported by grant MA-6235 from the Canadian Medical Research Council).

1554 CHROMATOGRAPHIC AND BIOLOGIC COMPARISONS OF THE LUTEINIZING HORMONE RELEASING HORMONE (LHRH) FOUND IN THE ORGANUM VASCULOSUM LAMINA TERMINALIS (OVLT) AND MEDIAN EMINENCE (ME). W. K. Samson*, and S. M. McCann. Dept. Physiol., Univ. of Tx. Hlth. Sci. Ctr. at Dallas, TX 75235.

LHRH is present in the OVLT and ME of the male (7.42 ± 3.30 pg/µg extracted protein, n = 10;25.06 ± 2.53, n = 9, respectively), ovariectomized female (3.01 ± 0.43, n = 11; 18.73 ± 3.27, n = 12), and random cycle female (3.04 ± 0.20, n = 40; 25.43 ± 1.04, n = 40) rats. In order to compare the physical properties of the LHRH in both structures, acid extracts of pooled tissue punches from the OVLT, ME and a punch of tissue surrounding the OVLT, as well as synthetic LHRH, were chromatographed on G-25 Sephadex (1.2 x 96.0 cm) using 0.2N acetic acid. Column fractions were measured for LHRH content by RIA. In two separate experiments the elution profiles of LHRH were seen to be similar for OVLT and ME extracts and the decapeptide. In the second experiment extracts of tissue surrounding the OVLT, including stria terminalis and preoptic area, revealed a similar elution pattern. Bioassay of LHRH activity in extracts of ME and OVLT tissue was conducted in cultures of both dispersed male rat anterior pituitary cells and an enriched gonadotropin fraction from 18 day old female rat pituitaries. In both systems extracts of rat cerebral cortex failed to stimulate LH or FSH release, while extracts of OVLT and ME tissues were capable of stimulating gonadotropin release with a similar dose response profile as that of the synthetic decapeptide.

We then examined the temporal fluctuations in LHRH content in these areas at 1000 hr and 1600 hr on all four days of the estrous cycle. Both ME and OVLT displayed marked temporal fluctuations in LHRH content throughout the estrous cycle with highest levels found in the ME at the 1000 hr sampling, and a precipitous decline between 1000 hr and 1600 hr on proestrous, however, the OVLT contained highest LHRH levels in the afternoons, particularly on proestrus and estrus and precipitous declines occurred between 1600 hr of estrus and 1000 hr of metestrus, and between 1600 hr of proestrus and 1000 hr of estrus.

In summary we have demonstrated the physical and bioassayable similarities of the LHRH residing within the OVLT and the ME and have seen a fluctuation in tissue LHRH content which is disparate between the two areas. These observations suggest either a phase shift in the delivery or release of LHRH by these structures or the possible existence of an entirely separate release mechanism within the two structures. (Supported by AM 10073 and HD09988 and HD07062).

1555 CHRONIC GLUCOCORTICOID ADMINISTRATION ALTERS AXON SPROUTING IN THE RAT HIPPOCAMPAL FORMATION. Stephen W. Scheff, Donald R. Thorne‡ Georgia Sasvary‡ Larry S. Benardo* and Carl W. Cotman. Dept. Psychobiology, Univ. California, Irvine, CA 92717.

The hippocampus has been widely used to study plasticity in the CNS. Previously we demonstrated that the sprouting reaction in the dentate gyrus of the hippocampal formation could be altered with pharmacological doses of a glucocorticoid, hydrocortisone. In the present study we tested whether or not chronic changes in the levels of glucocorticoids which more closely approximates physiological conditions might also alter this sprouting response. Thus, we examined the response of various hippocampal afferents following a unilateral lesion of the entorhinal cortex in control and hormone treated animals.

Young adult animals were adrenalectomized six to ten days prior to a subcutaneous implantation of a pellet containing a specified concentration of corticosterone utilized for chronic glucocorticoid replacement. Adrenalectomized animals were maintained on levels of corticosterone which corresponded to those in naive young rats. In addition animals were maintained on levels which were approximately twice those present in control animals. Five days following implantation the animals were subjected to unilateral removal of the entorhinal cortex. The brains were examined for changes in septal input by means of AChE staining and for changes in the commissural-associational fiber plexus by means of the Holmes fiber stain. Control animals showed a sprouting response in agreement with previous results. It was found that animals maintained at the high levels of corticosterone showed significantly less sprouting than controls and animals maintained on low levels of hormone. Astrocytes appeared markedly hypertrophied in the high level group.

The present findings support our previous results with the administration of hydrocortisone. These findings suggest that steroid hormones may regulate synaptogenesis in the mature brain. Moreover, these results may provide a clue for understanding the mechanism underlying changes in synaptogenesis in aged animals. Previously we found that axon sprouting is reduced in aged rats and that glucocorticoids are elevated in these animals. The elevated glucocorticoids may be responsible for reduced sprouting in the aged animals. (Supported by research grant AG 00538)

1556 DECREASED DOPAMINE TURNOVER IN THE MEDIAN EMINENCE IN RESPONSE TO SUCKLING IN THE LACTATING RAT. Michael K. Selmanoff and Phyllis M. Wise*. Department of Physiology, University of Maryland, School of Medicine, Baltimore, Maryland 21201.

The effects of suckling on the turnover of dopamine (DA) and norepinephrine (NE) were studied in terminal projection fields of the tuberoinfundibular (median eminence, ME) and nigrostriatal (caudate nucleus, CN) dopaminergic neurons. Lactating rats with their litters were received between 1 and 5 days postpartum from the supplier. On day 9 postpartum the litters were culled to 8 pups and the mothers fitted with indwelling right atrial cannulae under ether anesthesia. On day 10 postpartum pups were removed from their mothers for 4 hours and then returned. The onset of suckling was operationally defined as the time when 6 or more of the 8 pups started suckling. This occurred with a latency of 1-5 minutes from the time of pup return. After 30 minutes of suckling fresh litters were substituted to ensure continuation of the stimulus from 30 to 60 minutes.

Catecholamine turnover was assessed by using the synthesis inhibitor α-methyl-para-tyrosine (αMPT). The drug was administered in acidic diluent (pH 5.2-6.5, 0.5-1.2ml volume) via the indwelling catheter (300mg/kg body weight) and the animals killed at 0, 30 and 60 minutes thereafter. The ME and CN were microdissected and DA and NE concentrations determined by radioenzymatic and protein assays. Trunk blood was collected and serum prolactin levels determined by double-antibody radioimmunoassay.

Two protocals were employed. In one, αMPT was administered 30 minutes after the onset of suckling, and in the other, at the same time the pups were returned. In both situations the results were similar. Comparison of the rates of DA depletion after αMPT in suckled and non-suckled mothers revealed a decrease in ME DA turnover in the suckled mothers. In contrast, dopamine turnover in the CN was not affected by suckling. Similarly, NE turnover in suckled mothers did not differ from non-suckled mothers in the ME. These results are consistent with the hypothesis that dopamine is a physiological prolactin inhibitory factor mediating suckling-induced prolactin release. (Supported in part by NIH grants NS-14611, HD-02138 and HD-00435).

1557 ESTROGEN NULLIFIES LORDOTIC REFRACTORINESS TO PROGESTERONE IN SPAYED RATS. B.D. Shivers, R.E. Harlan* and R.L. Moss. Dept. Physiol., Univ. Texas Health Sci. Ctr., Dallas, TX 75235

Experiments were conducted to investigate the interaction between estrogen and progesterone in the display of feminine sexual behavior. Spayed rats received 5mm Silastic capsules containing 17β-estradiol (E_2) and sc injections of progesterone (P;2.5mg) or oil (O) at 1200h, and were tested at 18-2000h for lordotic responsiveness (LR) as measured by the lordosis quotient (LQ) in 20-mount tests (lights off: 13-2300h). Results are reported as mean LQ±1 SEM (N). A preliminary study revealed that in rats exposed to E_2 for 27 h, P given 24 h after capsule removal (1200h) could not facilitate LR (at 18-2000h) [P:32±10 (11); O:21±6 (10)]. In Experiment 1, E_2 capsules were removed 48 h after implantation at 0900h; P or O was injected at 1200h on this and the following day; and the rats were tested at 18-2000h on both days. Results indicate that while the first P injection facilitates LR, the rats are refractory to a second P injection given 24 h after the first [O,O:36±9; 21±9 (11); P,P:88±6; 6±3 (11); P,O:97±3; 2±2 (9); O,P: 43±11; 54±12 (10)]. In Experiment 2, E_2 capsules were left in vivo throughout the experiment. P or O was injected at 1200h on the third and fourth days after implantation and the rats were tested at 18-2000h on both days. Results show that a second P injection 24 h after the first can facilitate LR if E_2 capsules are present [O,O:47±14; 64±12 (10); P,P:98±1; 98±1 (10); P,O: 96±2; 66±12 (10); O,P:59±12; 100±0 (9)]. In Experiment 3, E_2 capsules were either left in vivo or removed 3 h before or 1, 11 or 20 h after the first P injection (given at 1200h). Results suggest that for the second P injection (given 24 h later) to facilitate LR, E_2 capsules must be in vivo either for longer than 1 h, with 11 h sufficing, following the first P injection or for longer than 52 h, with 62 h sufficing [second test: E_2 removed 3 h before P:37±14 (6); 1 h after P:18±7 (10); 11 h after P:90±3 (10); 20 h after P:99±1 (9); E_2 not removed:99±1 (6)]. Experiment 4 was conducted to distinguish between these two possibilities. E_2 capsules were left in vivo 62 h with the E_2 present for either 1 or 11 h after the first P injection. Results show that the presence of E_2 for 11 h, but not 1 h, following the first P injection permits the second P injection to facilitate LR [second test: E_2 removed 1 h after P:49±16 (8); 11 h after P:97±2 (7)]. In summary, the results of the present experiments (a) reveal that 5mm E_2 capsules must be in vivo for longer than 27 h for P injected 24 h later to facilitate LR; (b) demonstrate the lordotic refractoriness developed in response to sequential P injections; and (c) establish that E_2 must be present longer than 1 h, with 11 h sufficing, following the first P injection for a second P injection given 24 h later to facilitate LR. (Supported by HD 11814, HD 05585 and HD 05737).

1558 THE ONTOGENY OF THE MAGNOCELLULAR NEUROSECRETORY SYSTEM OF THE MOUSE. A.J. Silverman and R. Goldstein*. Dept. Anat., P&S, Columbia Univ., New York, NY 10032.

Four aspects of the development of the supraoptic (SO) and paraventricular (PV) nuclei of the mouse were studied: (1) appearance of neurophysin (NP) immunoreactivity; (2) axonal outgrowth to neural lobe (NL) and median eminence (ME); (3) synaptogenesis in SO and (4) dendritic growth of NP positive neurons. Light microscopic immunocytochemistry on 6 um paraffin and 50um vibratome sections and electron microscopy were used. Adult females were placed with males at 1700 hr and checked for sperm plugs at 0800 hr the following day; this equals day 1/2 of gestation. NP positive perikarya were first seen on fetal day 13 1/2 either in the presumptive SO or in migration to the SO from the ependymal wall. No positive axons were observed in either the brain or NL. A substantial increase in the no. of positive cells in the pricipal SO was seen on day 14 1/2; this is also the first day on which NP axons were seen in the NL. These fibers were only present in the lateral aspect of the gland. Days 14 1/2 and 15 1/2 were similar except for the appearance of NP in cells of the dorsal and retrochiasmatic SO. Day 16 1/2 marks the appearance of NP cells in the PV, mainly in the dorso-medial aspect. By day 17 1/2 NP axons are evenly distributed in the NL and are present in the zona externa of the ME. By this time all cells in the SO are NP positive. This is not true of the PV; appearance of NP positive cells occur throughout the remainder of gestation and the first week of neonatal life.

Synapses were almost completely absent in SO just prior and after birth (18 1/2 day = 0.07/250um^2; day 1 neonate = 0.26 ± 0.19/250um^2) compared to the adult (13.03 ± 1.0/250um^2). These first synapses were on the dendrites. There is a significant increase in the no. of synapses by day 5 (1.77 ± 0.45/250 um^2) which continues on day 15 (6.8 ± 0.62/250um^2). Synaptogenesis is complete on day 24 (10.04 ± 0.8/250um^2). On day 5 axo-somatic as well as axo-dendritic synapses are present. By day 15 axo-axonic synapses appear. On the day of birth, very little dendritic arborization can be seen in thick, immunostained sections. There is a progressive increase in dendritic complexity from birth to day 24 post-natal.

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1559 DRINKING, VASOPRESSIN SECRETION, AND ACTH SECRETION INDUCED BY INTRACRANIAL ANGIOTENSIN. J.B. Simpson, M. Reed, L.C. Keil, T.N. Thrasher, and D.J. Ramsay. Dept. of Physiology, University of California School of Medicine, San Francisco, CA 94143

178 male Long-Evans rats were stereotaxically implanted each with one 26-33 ga cannula system. On the fifth and sixth days following surgery, each rat received an intracranial injection of 5 ng Ile^5-angiotensin II (AII)/0.5 ul/10 sec. Water intakes for the following 30 min were measured. On the seventh day, following the same injection procedure, each rat was decapitated at 60 sec following AII administration. Trunk blood was collected for subsequent measurements of arginine vasopressin (AVP) and ACTH by radioimmunoassay. Nissl-stained sections were prepared showing the cannula track from each brain.

Rats were assigned to either of two principle groups: ventricular loci or tissue loci. Differentiation was made on whether the cannula ruptured lateral or third ventricular ependyma at its tip or along its trajectory. Ventricular loci were: lateral; dorsocaudal third; rostrodorsal third; mid (D/V) third; ventral third; and optic recess. Cannulae not perforating ependyma were classified according to the tissue surrounding the cannula tip.

Drinking (4 ml or more) and elevated plasma AVP (8 pg/ml or more vs. control of 2.4 pg/ml) followed anatomically different distributions. Drinking and increased AVP were observed at subfornical organ, lateral ventricle and rostrodorsal third ventricle. Drinking but not increased AVP was found in ventral third ventricle and optic recess. Increased AVP but not drinking was found in diagonal band and medial preoptic area. Neither drinking nor increased AVP was found in corpus callosum, fornical commissure, septum (medial, lateral, triangular and fimbrial nuclei), lateral preoptic area, anterior hypothalamus, supraoptic nuclei, and dorsocaudal third ventricle. Increased plasma ACTH was not found to occur reliably following AII at any tissue or ventricular locus tested.

These results indicate that AII may or may not provoke drinking independently of enhanced AVP secretion. Previous results (Simpson and Mangiapane, 1978) have shown likewise that drinking may or may not be provoked independently of a primary increase in arterial pressure. Application of AII unilaterally to the supraoptic nuclei did not provoke AVP secretion, suggesting that AII does not act there. It was also found that application of AII to those loci positive for drinking and/or AVP secretion did not provoke increased ACTH secretion. At the subfornical organ, the only tested site which is accessible to circulating AII, application of AII causes drinking, increased arterial pressure, and increased secretion of AVP. Such data, then, suggest different central sites of action of AII at which drinking and/or AVP secretion and/or increased arterial pressure may be provoked. Supported by NIH grant AM 06704.

1560 NORADRENERGIC CONTROL OF VASOPRESSIN RELEASE BY THE ORGAN CULTURED RAT HYPOTHALAMO-NEUROHYPOPHYSEAL SYSTEM. Celia D. Sladek and John R. Sladek, Jr. Depts. of Neurology and Anatomy, Univ. of Rochester Sch. of Medicine and Dentistry, Rochester, NY 14642.

It is well established that the supraoptic nucleus (SON) receives a rich noradrenergic innervation, but contradictory evidence exists as to the role of norepinephrine (NE) in the control of vasopressin (VP) release. The organ cultured rat hypothalamo-neurohypophyseal system (HNS) has been used as an *in vitro* system for investigating the effect of NE on VP release. Each of these organotypic explants includes the SON, median eminence, and neural lobe. It has been shown previously that the HNS releases VP at a constant rate *in vitro*, and releases VP in response to acetylcholine and other stimuli. In this study, NE inhibited VP release from 3-day cultured HNS explants. Addition of 10^{-5}M NE to the culture medium reduced VP release to 56±7% of control VP release. Addition of NE (10^{-5}M) concurrently with acetylcholine (10^{-5}M) inhibited cholinergic stimulation of VP release. These data are consistent with previous observations of inhibition of electrical activity of supraoptic neurons by NE (Barker et al., Science 171:208, 1971; Sakai et al., J.Pharm. Exp.Ther. 190:482, 1974).

HNS explants were examined for formaldehyde-induced catecholamine (CA) fluorescence before and after 1,3 and 5 days in culture. A rich pericellular pattern of CA varicosities were observed in the SON prior to culture. Pericellular fluorescent varicosities persisted in the SON in explants maintained 1 or 3 days in culture, but were not detected after 5 days. Microspectrofluorometric analysis of the fluorescence persisting in 3-day cultures confirmed the presence of CA. Thus, agents inhibitory to VP release are endogenous to the HNS explant at least through day 3 of culture.

These data indicate that the noradrenergic input to the SON is inhibitory to VP release. Noradrenergic afferents may transmit information from cardiovascular volume and baroreceptors. Additionally, the effect of NE on acetylcholine stimulation of VP release suggests that noradrenergic control mechanisms may modulate VP release in response to osmotic and other control mechanisms for VP release.
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1561 EVIDENCE FOR BRAIN HORMONE CONTROL OF LONG-DAY-INDUCED MATURATION IN A TERRESTRIAL MOLLUSC. Phillip G. Sokolove and Elinor J. McCrone*. Dept. Biol. Sci., UMBC, Catonsville, MD 21228.

Maturation of the reproductive tract of the terrestrial slug, *Limax maximus* is photoperiodically controlled: Long days (LD 16:8) promote growth of the hermaphrodite gonad and of the accessory sex organs such as the penis and albumen gland. Animals kept on short days (LD 8:16) fail to mature sexually regardless of age. (Sokolove and McCrone, J. Comp. Physiol. 123:317 1978). Results of implantation experiments suggest that reproductive tract development is controlled by a "maturation hormone" (or hormones) produced by the *Limax* CNS. Slug "brains" (circumesophageal ring of 9 fused ganglia) were removed from maturing animals that had been exposed to LD 16:8 lightcycles for 13-15 weeks. These were implanted in the haemocoels of immature recipient slugs that were hatched in the laboratory and had seen only LD 8:16. Host slugs were replaced on short days, and, when their reproductive tracts were examined 9-10 weeks later, all were found to be maturing. In contrast, only immature tracts were found in control slugs that received muscle implants or were sham operated. In another experiment, short-day recipients were implanted either with brains from long-day (maturing) donors, or with brains from short-day (immature) animals. Reproductive tract development was later found in hosts receiving long-day brains but not in those receiving short-day brains. The presumptive neuroendocrine factor thus appears to be synthesized and/or secreted only by the brains of animals exposed to long days.

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1562 EFFECT OF INTRAVENTRICULAR ANGIOTENSIN II AND BRADYKININ ON RELEASE OF ANTERIOR PITUITARY HORMONES IN THE RAT. M. K. Steele, A. Negro-Vilar* and S. M. McCann. Dept. Physiol., Univ. of Tx. Hlth. Sci. Ctr. at Dallas, TX 75235.

Angiotensin II (A II) and Bradykinin (BK) have been localized within the hypophysiotrophic area of the rat (Correa, et al. Neurosci. Abstracts 1280, 1978; Fuxe, et al. Neurosci. Letters 2: 229, 1976). Although the physiological significance of these peptides is as yet unclear, their predominence within the hypothalamus may suggest an endocrine function. We therefore proceeded to evaluate the possible role of A II and BK on pituitary hormone release in ovariectomized rats bearing chronically implanted third ventricular cannulae. On the day prior to testing, silastic cannulae were inserted into the jugular vein. On the test day, blood samples were taken from the unrestrained, conscious rats according to the following schedule: 2 pre-drug samples 30 min apart; post-drug samples at 5,15,30,60 and 120 min. Infusions of saralasin, an A II blocker, preceeded A II infusion by 30 min and an additional blood sample was taken just prior to A II administration. Control animals were infused with the saline diluent. In the A II experiments, water intake was monitored throughout the testing period.

Results: Infusion of 5 µg A II significantly reduced plasma LH at 15,30,60 and 120 min. Prolactin levels were also reduced at these time points as well as at the 5 min sample. Intravenous A II (5 µg) had no significant effect on LH. However, prolactin levels were increased at the 5 min sample, returning to baseline by 15 min. No consistent effects were observed on GH, TSH or FSH release. Infusions of saralasin (25 µg) attenuated the A II-induced LH suppression and totally blocked the decrease in prolactin levels.

BK infusion (5 or 25 µg) resulted in a dose-related increase in GH levels at 30 and 60 min. No effects on LH or prolactin concentrations were seen.

Current research is investigating whether A II and BK act directly upon the pituitary or via the hypothalamus to produce these endocrine effects. (Support by NIH AM10073, HD09988 and HD05492).

1563 DIVERSITY OF DISTRIBUTION OF CORTICOTROPIN AND β-LIPOTROPIN IN HYPOTHALAMUS AND ANTERIOR PITUITARY. Ludwig A. Sternberger*, Thurma V. McDaniel* and Shirley A. Joseph* (SPON: Leo G. Abood). Ctr. Brain Res. and Dept. Anatomy, Univ. Rochester Sch. Med., Rochester, NY 14642.

With the PAP method two antigens in the same paraffin or vibratome section can be stained in consecutive reaction sequences, using diaminobenzidine as electron donor for peroxidase in the first sequence to obtain a brown color, and chloronaphthol in the second sequence to obtain a blue color. No removal of the immunoreagents is necessary between the two sequences even though anti-immunoglobulin and PAP are used in each. Anti-ACTH 1-24 and 1-39 stained fibers in the dorsomedial nucleus and fibers and perikarya in the arcuate nucleus. Fibers were distinct from those containing somatostatin, vasopressin and luteoliberin. Anti-ACTH 17-39 failed to stain these areas even though all anti-ACTH sera used stained cells in the pars intermedia (PI) and pars distalis (PD). Antiovine β-lipotropin (LPH, courtesy of Dr. C. H. Li) stained in the PI the same cells as the various anti-ACTHs, yielding mixed color reactions. Anti-LPH and anti-ACTH 1-39 stained in the magnocellular system and the internal zone of the median eminence the same cells and fibers as vasopressin, again giving mixed color reactions. Pars nervosa was either unstained or weakly stained with anti-LPH or anti-ACTH 1-39. No hypothalamic LPH reactivity was seen outside the magnocellular system. Anti-ACTH 17-39 and 1-24 failed to stain the magnocellular system. In the PD separate cells were stained brown and blue when pairs of antisera to prolactin, growth hormone, luteinizing hormone and ACTH were applied, except for a few cells that stained both for growth hormone and luteinizing hormone. Mixed colors were obtained in the PI when pairs of anti-LPH and various anti-ACTHs were used in sequence. However when anti-ACTH 1-24 was followed by anti-LPH, separate cells stained brown and blue in the PD. On the basis of mixed color reactions the PD cell reactive with anti-LPH was identified as a glycoprotein-containing cell. The data suggest that ACTH-like peptides in various areas of brain may not always be identical to ACTH and that ACTH or β-LPH precursors are not necessarily coded by the same genes in different areas of brain and pituitary.

1564 TYROSINE INJECTION LOWERS SERUM PROLACTIN LEVELS IN CHRONICALLY RESERPINIZED RATS. Alan F. Sved*, John D. Fernstrom & Richard J. Wurtman MIT, Cambridge, MA 02139.

Work in our laboratory has indicated that catecholamine synthesis depends, in part, on the availability of the precursor amino acid, L-Tyrosine (TYR). Since dopamine neurons play a major role in the inhibitory control by the hypothalamus of pituitary prolactin (PRL) release, we tested the effects of TYR administration on serum PRL levels. However, previous work has suggested that nigro-striatal dopamine neurons release more dopamine in response to TYR injection only following treatments that increase dopamine turnover. Therefore, we also tested the effects of TYR in chronically reserpinized rats. The injection of TYR (200 mg/kg ip) to male Sprague-Dawley rats had no effect on serum PRL levels. In contrast, TYR (200 mg/kg ip) significantly reduced serum PRL levels in chronically reserpinized rats (2.5 mg/kg twice daily for 4.5 days) (vehicle 138±16 ng/ml; TYR 67±7 ng/ml; $p<.05$) 1 hour after its injection. That this effect is mediated via increased hypothalamic dopamine release is suggested by the concomitant increase in hypothalamic levels of the dopamine metabolites dihydroxyphenylacetic acid (DOPAC; +300%, $p<.05$) and homovannylic acid (HVA; +54%, $p<.05$) following TYR injection. TYR injection to non-reserpinized rats did not alter hypothalamic DOPAC or HVA levels. These studies demonstrate that a physiologic variable dependent upon catecholaminergic neurons can be affected by the availability of the catecholamine precursor, TYR. Furthermore, they suggest that TYR may be a useful treatment for lowering chronically elevated PRL levels found in hyperprolactinemia.

1565 **EFFECT OF MUSCIMOL ON PROLACTIN GROWTH HORMONE AND TSH SECRETION IN THE CONSCIOUS MALE RAT.** **C.A. Tamminga, M. Szabo & L.A. Frohman, NIH, NINCDS, Bethesda, MD 20205 and Michael Reese Hospital, University of Chicago, Chicago, Ill.**

The role of GABA in the regulation of anterior pituitary hormone secretion has been incompletely studied. Results of pharmacologic studies have varied depending on the GABA drug used, route of administration and type of animal preparation. In the present studies, estrogenized male rats with indwelling intraatrial cannulae were used. Catheters were implanted at least 3 days prior to testing and estradiol valerate (1.25 mg/kg) was administered 3-14 days before each experiment. On the day of the experiment, extension catheters were attached to the indwelling cannula, the animals placed in individual cages, and a 45 min period of accommodation allowed. Muscimol (1.5 mg/kg) was administered i.v. to the conscious rats while blood samples were drawn into heparinized syringes at specified intervals prior to and following the drug. The samples were centrifuged and the plasma frozen for subsequent radioimmunoassay. Prolactin (PRL), growth hormone (GH) and TSH were measured by double antibody radioimmunoassay. Plasma PRL response to muscimol described a biphasic curve. An initial elevation which peaked at 5-10 min was followed by a subsequent prolonged suppression at 30-60 min. The PRL elevation was blocked by both haloperidol and picrotoxin pretreatment, whereas the PRL suppression occurred even with haloperidol blockade. GH secretion was not altered in the animal preparation either in the conscious rat or in urethane anesthetized animals. TSH briskly rose following muscimol in the conscious rat, however, was blocked by urethane anesthesia. GABA could alter pituitary hormone secretion through a direct effect of GABAergic pathways on hypothalamic regulatory hormones or through an indirect effect on another neurotransmitter possibly dopamine which regulates pituitary function.

1566 IS ACETYLCHOLINE A RELEASING HORMONE: EVIDENCE FOR MUSCARINIC RECEPTORS IN ANTERIOR PITUITARY. Richard L. Taylor* and David R. Burt. Dept. Pharmacol. Exptl. Ther., U. Md. Sch. Med., Balto., MD 21201, U.S.A.

One of the clearest pieces of evidence that dopamine must have direct effects on the pituitary as a prolactin-inhibitory factor was the demonstration of dopamine receptors there by binding studies. Two recent reports of cholinergic effects on the anterior pituitary in vitro (Vale et al., 1976, In Hypothalamus and Endocrine Functions, Plenum, p. 397; Pawlikowski et al., 1978, Neuroendocrinol. 26:85) led us to look for muscarinic receptors in the pituitary by binding of [^{3}H]quinuclidinyl benzilate (QNB), a potent and specific muscarinic antagonist. We have detected [^{3}H]QNB binding sites in membranes of sheep anterior pituitary whose characteristics are closely similar to those reported for muscarinic receptors in other tissues. They have an equilibrium dissociation constant in the 20-40 pM range, a rate constant for association at 37°C of about 2×10^8 $M^{-1}min^{-1}$, a rate constant for dissociation at 37°C of about 4×10^{-3} min^{-1}, and the expected specificity for a variety of cholinergic and other drugs. Since there is no established cholinergic or other innervation of the anterior pituitary, the presence of typical muscarinic receptors there, assuming that they are functional, suggests that acetylcholine reaches the tissue through the hypophyseal portal circulation and that it may thus act as a releasing hormone or otherwise regulate pituitary function. An alternative possibility is that there is a minor cholinergic innervation of the vasculature, which contains all the receptors. Histological examination of receptor localization is needed for a definitive answer, but a vascular or innervation-related localization in the anterior pituitary is argued against by the fact that both the posterior pituitary (with a known innervation and heavy vascularization) and lower infundibular stem have less concentrations of [^{3}H]QNB binding sites than the anterior pituitary by at least a factor of two. The concentration of apparent muscarinic receptors in the sheep anterior pituitary (5-7 pmol/g wet weight) still is relatively low compared to receptors for thyrotropin releasing hormone (20 pmol/g wet weight) or dopamine (20-30 pmol/g wet weight) in the same membrane preparations or compared to muscarinic receptors in similarly prepared membranes from sheep whole hypothalamus (25-30 pmol/g wet weight) or median eminence (10-15 pmol/g wet weight). These regional differences may explain in part the failure of most investigators to detect the physiological correlates of our biochemical findings. Relatively prominent effects of muscarinic agents at the hypothalamic level in vivo could obscure any direct effects at the pituitary level. (Supported in part by U.S.P.H.S. grant MH-29671 to D.R.B.)

1567 FURTHER STUDIES OF LESIONS OF THE MEDIAL PREOPTIC NUCLEUS (MPN) ON THE CONTROL OF LUTEINIZING HORMONE (LH) RELEASE IN FEMALE RATS. E. Terasawa, S.J. Wiegand, L.V. Rubens* and W.E. Bridson*. Wis. Reg. Primate Res. Ctr., Univ. of Wis., Madison, WI 53706

Previously we have reported that the MPN, a small periventricular structure located immediately caudal to the organum vasculosum lamina terminalis is indispensable for the cyclic release of LH in the female rat. In the present study, effects of MPN lesions on I) the estrogen (EB)-induced daily surge of LH and on II) LHRH content in the medial basal hypothalamus (MBH) following injections of EB and P, were determined. Regular cyclic rats housed under standard lighting conditions were ovariectomized 4-6 wks after brain lesions, and 2 wks later were given ovarian hormones. Circulatory LH and hypothalamic LHRH were measured by RIA. (I) Small electrolytic lesions were placed in 4 loci of the suprachiasmatic area. Lesions of MPN and suprachiasmatic nucleus (SCN) induced persistent estrus. Lesions in the locus anterior to MPN (VPC) and lesions in the locus between MPN and SCN (ASR) as well as sham lesions did not abolish estrous cyclicity. However, after ovariectomy and 50 µg EB, the daily surge of LH evident at 1730 on days 1 and 2 in sham controls was blocked in all lesioned groups. After 1.5 mg P on day 3, a surge of LH comparable to that of sham controls was induced in VPC and ASR lesioned groups, and an attenuated surge was seen in SCN lesioned groups. MPN lesioned groups did not respond to P. (II) Lesions were made in the MPN and in VPC with partial damage to MPN. MPN lesions, but not VPC lesions, abolished estrous cyclicity. After ovariectomy, lesioned animals and controls were treated with 5 µg EB (day 0). On day 2 at 1000 half of the animals in each group were decapitated, tissue blocks from the MBH were removed for LHRH, and trunk blood was collected for serum LH. Remaining animals received P on day 2 and were sacrificed at 1730. MBH content of LHRH in controls decreased from 1000 to 1730 ($p<0.001$) with a concomitant increase of serum LH. However, LHRH contents in both MPN and VPC lesioned groups were well below those of controls at 1000 and 1730 ($p<0.001$), despite the facts that MPN lesions entirely abolished the P-induced LH surge while VPC lesions partly attenuated it. It is concluded that the P-induced LH surge can be blocked only by MPN lesions, while the EB-induced daily surge of LH can be eliminated by lesions anywhere in the suprachiasmatic region, suggesting that the neural mechanism mediating the facilitatory action of EB is diffusely organized. Reduction of LHRH content in the MBH is caused not only by MPN lesions, but also by VPC lesions, indicating that the LHRH neuronal system is also diffusely organized in the vicinity of the MPN. Thus, the neural component sensitive to P in the MPN may be the key factor for the cyclic release of LH. (NIH RR00167)

1568 MODULATION OF HIPPOCAMPAL EXCITABILITY IN ADULT CASTRATED AND OVARECTOMIZED RATS. Timothy J. Teyler, Michael Foy* and Richard M. Vardaris. Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272 and Kent State University, Kent, Ohio 44240.

We previously demonstrated the modulating effects of 100 pM concentrations of 17-beta-estradiol (E_2) and testosterone (T) on in vitro slices of normal adult rats (Vardaris and Teyler, Neurosci. Abs., 1978, 4, A1145). In normal males and proestrus females E_2 enhanced the amplitude of an extracellular population spike from the CA1 area of the hippocampal slice. In non-proestrus females T enhanced the amplitude of the same monosynaptic responses. This differential responsivity to physiologically appropriate levels of gonadal steroid may reflect sexual differentiation in the hippocampus as is suggested by the presence of androgen and estrogen receptors in the embryonic brain (Vito & Fox, Neurosci. Abs., 1977, 3, A359; Science, 1979, 204, 517) or it may result from the priming effect of plasma gonadal steroids.

To investigate this question adult rats were castrated or ovarectomized prior to testing with 100 pM E_2 and T. After allowing two days for plasma gonadal steroid levels to decline, hippocampal slices were prepared and were tested to both E_2 and T in an ABA design. Electrophysiological measures were taken at 10 and 20 minutes following addition of steroid to the bathing ringer solution. CA1 excitability to monosynaptic stimulation of stratum oriens displayed the same properties as seen in the gonadally intact animals. That is, E_2 enhanced the amplitude of the population spike in the castrated male. T enhanced the same response in the ovarectomized female. A tendancy was also noted for T to depress responsivity in castrated males and for E_2 to depress responsivity in ovarectomized females.

These data are consistent with the hypothesis that hippocampal modulation by gonadal steroid hormones reflects sexual differentiation of hippocampal tissue rather than a response to circulating levels of hormone. The rapid onset of the steroid effect, within 10 min, may suggest a membrane effect of the steroid and is similar to the effect of E_2 on hypothalamic cells (Dufy et al., Neuroendocrin., 1976, 22, 35). These results indicate that steroid fluxes modulate the excitability of the rodent hippocampus and may do so by acting upon sexually differentiated neurons. (NSF grants BNS-77-28497 and BNS-78-23947 (TJT)).

1569 EFFECTS OF ESTRADIOL ON CATECHOLAMINE SYNTHESIZING ENZYMES, LUTEINIZING HORMONE (LH) AND PROLACTIN IN THE OVARIECTOMIZED RAT. Harriet Tobias*, Laurence Carr and James Voogt*. Dept. Pharmacol., Univ. Louisville, Louisville, KY 40232 and Dept. Physiol., Univ. Kansas Medical Center, Kansas City, KS 66103.

Hypothalamic catecholamines have been postulated to play a role in the effects of estrogen on LH and prolactin release. In the present study the actions of estradiol benzoate (EB) on tyrosine hydroxylase (TH) and dopamine-B-hydroxylase (DBH) activity were studied to determine their relationship to the plasma levels of LH and prolactin. Female Sprague-Dawley rats ovariectomized 3-4 weeks previously were injected with 10 µg EB or oil and decapitated 1, 2, or 3 days later. The medial basal hypothalamus (approx. 30-90 µg protein) was dissected and assayed for TH and DBH activity. Trunk blood was assayed for LH and prolactin by radioimmunoassay. Injection of EB caused a significant decrease in LH levels 1 day later but LH returned to control (untreated) levels on the second day. LH levels decreased again on the third day. Plasma prolactin increased steadily over the first two days and remained elevated on the third day. TH activity was increased 1 day after injection of EB but returned to control level on the second day. The release of LH appeared to be inversely correlated with TH activity throughout the 3 day period whereas no consistent correlation with prolactin levels could be demonstrated over the same time period. DBH activity remained unchanged except for a significant decrease 2 days after administration of EB. The results suggest that the effects of EB on LH release may be mediated by selective alteration of TH activity in the hypothalamus.

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1570 INACTIVATION OF BRAIN CYTOSOL GLUCOCORTICOID RECEPTOR BY ATPase. Andrew C. Towle* and Paul Y. Sze (SPON: B. E. Ginsburg). Dept. of Biobehavioral Sciences, Univ. of Connecticut, Storrs, CT 06268.

Na^+-K^+-ATPase from porcine cerebral cortex inactivates the glucocorticoid binding capacity of 104,000 x g soluble preparations from rat hippocampus and liver. Pre-incubation of the hippocampus preparation at 30°C in the presence of ATPase (10 u/ml) results in a rapid loss of specific ^{3}H-triamcinolone binding, with a half-life of approximately 10 min. The ability of ATPase to inactivate the glucocorticoid receptor is dependent on the concentration of the enzyme, requires magnesium, and is inhibited by ouabain. The unbound receptor is sensitive to inactivation, whereas the steroid-bound receptor is unaffected. The inactivation results in a loss of the binding capacity rather than an alteration of the binding affinity for the steroid. Similar inactivation by ATPase can be obtained in liver cytosol preparations.

Alkaline phosphatase is known to inactivate the glucocorticoid binding capacity of rat liver cytosol preparations (Nielsen et al., PNAS, 74, 1398, 1977). This effect of alkaline phosphatase has also been demonstrated with hippocampal glucocorticoid receptor in our study. Similar to ATPase, alkaline phosphatase produces its effect only on unbound receptor, and the inactivation reflects the decrease of binding capacity rather than binding affinity, in both liver and brain preparations.

Since ATP is a substrate of alkaline phosphatase as well as ATPase, the inactivation of glucocorticoid receptors by the two enzymes may have a similar mechanism. Nielsen et al. proposed phosphorylation-dephosphorylation of receptor protein as a regulatory process on the basis of the phosphatase effect. Alternatively, as suggested by the ATPase effect, it may be ATP instead of protein-bound phosphate that is required for receptor binding activity.

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1571 THE EFFECT OF DARK PULSES ON THE CIRCADIAN RHYTHM OF LOCOMOTOR ACTIVITY IN HAMSTERS MAINTAINED IN CONSTANT LIGHT. Fred W. Turek and Gary B. Ellis*. Northwestern Univ., Evanston, IL 60201.

An important approach in the elucidation of formal properties of circadian systems has been the characterization of how a free-running rhythm is altered following a brief perturbation. In the most commonly used paradigm, organisms free-running in constant darkness are exposed to a single light pulse, and the resulting phase shift of the rhythm is measured. The interpretation of phase response curves generated from such studies is at present the theoretical basis for the entrainment of circadian systems by environmental signals. Despite the theoretical importance attached to phase response curves generated via light pulses on a background of constant darkness (DD), little is known about the effects of dark pulses on a background of constant light (LL).

To assess the response of the circadian rhythm of wheel-running activity to dark pulses, 22 male golden hamsters maintained in LL were exposed to a 3h dark pulse at various circadian times. The dark pulses were administered at 3 wk intervals through 150 days of LL. The effect of the dark pulse on the activity rhythm depended upon the phase relationship between the dark pulse and the rhythm. Dark pulses occurring either late in the subjective night or early in the subjective day had little effect on the activity rhythm. In contrast, dark pulses occurring either late in the subjective day or early in the subjective night consistently altered the rhythm in one of three ways: 1) The rhythm was phase advanced 0.5-2.0h (this should be contrasted to the phase delays occurring in response to light pulses administered at this circadian time during maintenance in DD); 2) A new component of activity, in phase with the dark pulse, was induced and lasted for up to 4 cycles before fusing with the main bout of activity; 3) The activity rhythm was split into two distinct components within a day after the pulse.

While only 2 of 22 hamsters showed splitting the day after a dark pulse, splitting did eventually occur in 12 other animals. As in other reports on splitting, the 2 components stabilized at a 180° antiphase relationship, and the steady-state period of the split condition was always shorter than the period of the rhythm prior to splitting. Once the activity rhythm was split, subsequent dark pulses had little effect. Hormonal manipulations (castration, pinealectomy, melatonin treatment) which have been found to alter rhythmicity under other experimental conditions, were also without effect on splitting.

The present study indicates that dark pulses on a background of LL perturb the circadian system in a different manner than light pulses on a background of DD. The use of dark pulses to perturb circadian rhythmicity may be a useful tool in examining the formal properties of circadian systems. (NIH-HD-09885).

1572 BABOON CORTICOSTERONE: SUBSTANTIAL BRAIN BINDING OF A 'MINOR' ADRENAL GLUCOCORTICOID. Barbara B. Turner, Elaine M. Smith*, and Bernard J. Carroll*. Mental Health Res. Inst., Univ. Mich., Ann Arbor, MI 48109

In primates the predominant glucocorticoid secreted by the adrenal is cortisol. The concentration ratio of corticosterone to cortisol in plasma is usually less than 0.10. The ratio of corticosterone to cortisol in post-mortem brains (tissue concentration) has been reported to be higher than that of plasma. We wished to examine the extent to which disproportionate brain concentrations are reflected regionally in cytosol binding and nuclear binding in the primate brain.

Subjects were 4 baboons (Papio cynocephalus) being used in acute experiments. Under pentobarbital anesthesia, following 6-8 hrs of surgical stress, the baboons had blood samples withdrawn via femoral catheters. They were then immediately perfused with cold dextran-saline via the internal carotid arteries. The brains were quickly removed and then dissected at 4°C. Tissues for cytosol preparations were homogenized in Tris-EDTA buffer (10ml/g); following centrifugation, free steroid was removed by dextran-charcoal adsorption. Tissues for nuclear binding were homogenized in the absence of detergent, and the crude pellet repeatedly washed prior to final centrifugation through dense sucrose. Dried extracts, both cytosol and nuclear, were chromatographed on LH-20 columns. Cortisol and corticosterone fractions were quantitated by radioimmunoassay, taking into account sample recovery values.

The mean plasma values for corticosterone (B) and cortisol (F) were 3.1 $\pm$ 0.7 and 41.1 $\pm$ 5.7 respectively, yielding a B to F ratio of .075. The tissue concentration of B + F in the brain regions studied (preoptic-septum, hypothalamus, amygdala, hippocampus and cortex) was found to be about 8 picomols/mg protein, with the pituitary being somewhat higher. The ratio of B to F in tissues ranged between .2 and .4 in all regions, a marked increase over the plasma ratio. The cytosol binding of both B and F was found to range between 250 and 500 femtomoles/mg protein in the 8 regions examined. The ratio of B to F bound in cytosol was 0.5 or greater in all regions, with the lowest ratio observed in the thalamus and the highest in the pituitary (1.2) and hippocampus (1.6). In brain nuclei, the ratio of B to F was relatively uniform in the pituitary and the 5 brain regions, ranging between .50 and .75.

For both cytosol and nuclear binding the ratio of corticosterone to cortisol is at least .50, and in the majority of brain regions examined, the principal glucocorticoid bound is not cortisol, but rather corticosterone.

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1573 DOES GLIAL CELL ENCLOSURE OF NEURAL LOBE NEUROSECRETORY ENDINGS INHIBIT HORMONE RELEASE? C.D. Tweedle, and G.I. Hatton, Depts. Anat. & Psychol. & the Neuroscience Program, M.S.U., E. Lansing, MI 48824.

It has been noted previously in electron micrographs that neurosecretory axons in the neural lobe of the rat are sometimes completely surrounded by the cytoplasm of pituicytes, specialized glial cells. In an earlier ultrastructural morphometric study (Anat. Rec. 193, 707), we found that the number of such "enclosed" neurosecretory axons significantly decreased with water deprivation (24 h) and reappeared with subsequent rehydration (24 h) in the rat. This indicates that pituicyte enclosure of the neurosecretory axons is greatest during periods of low hormone release. To further analyze the relationship between neurosecretory endings and pituicytes, serial thin section analysis was carried out on the rat neural lobe. The data obtained to date indicate that, while neurosecretory axons are often only transiently wrapped in pituicyte cytoplasm (for .5-2.5 μm), numerous examples could be found where the neurosecretory axons actually terminated within the pituicyte cytoplasm. In these latter cases, in serial cross sections, the neurosecretory axons gradually attenuated and disappeared. During this process, the contents of the neurosecretory endings only rarely appeared degenerative and no unusual appearance of the surrounding pituicyte cytoplasm was noted. Synaptoid contacts between enclosed axons and pituicytes were occasionally seen.

The present finding that neurosecretory axons can frequently be observed to actually terminate within pituicyte cytoplasm plus previous observations that the number of enclosed neurosecretory axons is highest with low hormone release suggest to us that pituicyte enclosure is involved in the inhibition of hormone release. One possible mechanism of this inhibition could be an alteration in the ionic milieu of the extracellular space around the enclosed neurosecretory axon by the pituicyte. (Supported by NIH BGRS funds and NIH grant NS 09140.)

1574 ANGIOTENSIN II SPECIFIC BINDING SITES IN RAT BRAIN. Mark van Houten*, Ernesto L. Schiffrin*, Johannes F.E. Mann*, Barry I. Posner* and Roger Boucher* (SPON: R. Hirsh). Depart. of Medicine, McGill University, and the Institut de Researches Cliniques de Montréal, H3A 2B2.

The existence of brain angiotensin II (AII) receptors is well established. In the present study quantitative light microscopic radioautography (LMR) was used to determine the location of specific binding sites for blood-borne AII in the rat brain.

Ile^5-AII was monoiodinated (specific activity of 1.5 mCi/μg) as described by Freedlander and Goodfriend (Handbook of Radioimmunoassay, 1979), and 1.5 X 10^8 cpm was injected intracardially into anaesthetized male rats. Three minutes later brains were perfused with Ringer's lactate followed by Bouin's fixative solution. Four micron sections of brain were prepared for LMR.

Radioautographic reactions occurred over the choroid plexus and all the circumventricular organs (CVO) deficient in blood-brain barrier: organum vasculosum of the lamina terminalis (OVLT), subfornical organ (SFO), median eminence (ME) and area postrema (AP). ^{125}I-AII binding sites were concentrated in the deep center of the SFO, the medial palisade zone (MPZ) of the ME, and as "hot spots" about cells in the vicinity of blood vessels in the AP. Radiolabeling by ^{125}I-AII in the center of the SFO was 2.5-3 times greater than labeling in the other CVOs, which exhibited similar overall reaction intensities. Grain densities over all other regions of the central nervous system did not differ from background.

Femoral vein infusion of sar^1-ala^8-AII (10nM/Kg/min) 30 minutes prior to and during the injection of ^{125}I-AII reduced by 72-94% the radioautographic reaction caused by ^{125}I-AII over the CVOs, but reactions over the choroid plexus were unchanged. In prior experiments sar^1-ala^8-AII infusion caused an initial rise in blood pressure that rapidly returned to normal for the duration of the infusion, and completely blocked AII-induced blood pressure elevation. Infusion of an isomolar dose of des-asp^1-ile^8-AII was somewhat less effective in blocking ^{125}I-AII binding, and $ACTH^{1-24}$ was non-inhibitory.

These results demonstrate the presence of AII specific binding sites in the SFO and OVLT, regions thought to mediate central effects of blood-borne AII on drinking, and in the AP, which has been implicated in AII stimulation of central pressor activity. AII specific binding sites in the MPZ of the ME implicate AII in the control of hypophysiotropic function, perhaps in the secretion of vasopressin and/or serotonin.

1575 PUTATIVE ANDROGEN AND ESTROGEN RECEPTORS IN EMBRYONIC RAT HYPOTHALAMUS. Christine C. Vito, Sarah E. Bates* and Thomas O. Fox. Dept. of Neuroscience, Children's Hospital Med. Ctr., and Dept. of Neuropathology, Harvard Medical School, Boston, MA 02115

Sexual differentiation of brain occurs during a critical period of development which includes late embryonic and early neonatal ages of mice and rats. The organizational influences of gonadal steroids upon a non-differentiated neural substrate occur during this period. We reported previously that adult-like estrogen receptors (Vito & Fox, Science 204:517-519, 1979) and androgen receptors (Fox, Vito & Wieland, Amer. Zool. 18:525-537, 1978) exist in late embryonic and early neonatal mouse hypothalamus (HPOA).

Embryonic and neonatal rat HPOA also contain adult-like androgen and estrogen receptors. By DNA-cellulose (DC) affinity chromatography, we detect macromolecular hormone-binding activities in cytosols of rat HPOA as early as embryonic-day 15 (E15; vaginal plug = E0, birth = E21,22). Androgen- and estrogen-binding activities, respectively, from embryonic, neonatal and prepubertal rat HPOA adhere to DC and exhibit elution maxima in the 140 and 220 mM range of a NaCl elution gradient. These elution characteristics are typical of receptors from mouse HPOA and kidney. Heat activation ($22^{o}C$-$24^{o}C$) improves the detection of these rat androgen- and estrogen-binding activities by DC over previous levels (Vito & Fox, Neurosci. Abstr. 3:359, 1977). Embryonic and neonatal rat estrogen-binding activities saturate at ^{3}H-estradiol concentrations < 10nM and are competed by 100-fold excess nonradioactive diethylstilbestrol; androgen-binding activities saturate at ^{3}H-dihydrotestosterone (purified by high-performance liquid chromatography) concentrations < 10nM and are reduced > 90% by 10-fold excess non-radioactive testosterone. These indicators of hormone-binding affinities are similar to those observed for perinatal mouse estrogen and androgen receptors.

We have measured the detectable concentrations of putative androgen and estrogen receptors in rat HPOA. At E17, E18 and E19, estrogen receptor increases from 1.2 to 5.1 x 10^{-17} moles/mg HPOA (4-fold) and ranges from 11.6 to 13.0 x 10^{-17} between postnatal-day 4 (P4) and P21; androgen receptor increases from 0.3 to 0.7 x 10^{-17} at E17-E19 (2-fold) and from 0.8 to 3.2 x 10^{-17} between P4 and P21 (4-fold). At E16, E17 and E18, mouse HPOA contains 4.5 x 10^{-17} moles estrogen receptor/mg HPOA which increases only 2-fold by P21; while it contains 0.3 x 10^{-17} moles androgen receptor which increases 2-fold by P4 and 4-fold more by P21.

1576 IN VIVO AND IN VITRO STUDIES OF PINEAL-THYROID RELATIONSHIPS. Jerry Vriend*, Karl M. Knigge and Shirley Joseph*. Department of Anatomy, University of Rochester School of Medicine, Rochester, New York 14642.

Experiments with the golden hamster have demonstrated a dramatic influence of the pineal gland on the hormones of reproduction. Equally dramatic effects of the pineal gland can be demonstrated on thyroid hormones, particularly thyroxin. Depriving the hamster of light by bilateral orbital enucleation resulted in reduction of plasma thyroxin and an elevation of pituitary TSH. These changes were reversed by pinealectomy. Melatonin injections in either male or female hamsters resulted in significant depression of thyroxin concentrations. The depression obtained by melatonin injections was impaired by pinealectomy or subcutaneous implants of melatonin. In vitro experiments were designed to test the effects of pineal extracts on TRH induced TSH release by dispersed pituitary cells. An extract of bovine pineals was subjected to G-25 sephadex separation and the resulting fractions tested for their effects on TRH induced TSH release. Up to 80% inhibition of TRH activity was demonstrated with one of the fractions. Equal or greater inhibition of TRH activity was obtained in experiments with synthetic somatostatin (10^{-9}M). Melatonin at high doses (10^{-6}M) resulted in only 24% inhibition of TRH activity. These results suggest that the pineal gland, together with hypothalamic releasing factors, is involved in control of pituitary TSH secretion.

1577 THE EFFECT OF SEROTONIN ON PROLACTIN RELEASE IN NORMAL AND PITUITARY STALK SECTIONED RHESUS MONKEYS (MACACA MULATTA). W.B.Wehrenberg*, D.E.McNicol*, A.G.Frantz*, and M. Ferin* (SPON: J.L. Antunes) Dept. Ob/Gyn and Medicine, College of Physicians and Surgeons, Columbia University, New York, New York 10032

Biogenic amines have an important role in the regulation of prolactin (PRL) secretion. While we have reported that L-dopa, a dopamine precursor, can suppress PRL release in rhesus monkeys by a direct action on the pituitary, the site at which serotonin acts to stimulate PRL release is generally considered to be hypothalamic. In the present experiment we compared the effects of serotonin on PRL release in monkeys in which the pituitary gland was isolated from direct hypothalamic influences by pituitary stalk section and in normal monkeys. Pituitary stalk section had been performed under direct visualization via a transorbital approach and a silastic barrier was placed between the cut ends of the pituitary stalk to prevent revascularization. Serotonin was administered i.v. at doses of 50, 500 and 5000 µg to 4 normal and 3 stalk sectioned female rhesus monkeys. PRL concentrations were determined by radioimmunoassay. Basal PRL concentrations were elevated in stalk sectioned monkeys as compared to normal animals (34 ± 5 vs 3 ± 1 ng/ml, mean ± SEM), as a result of the loss of inhibitory hypothalamic influences. In normal animals, a 50 µg dose of serotonin did not induce a change in PRL concentrations. Following the administration of 500 µg PRL increased within 5 min from 3 ± 1 to 22 ± 6 ng/ml; there was an even greater response to 5000 µg of serotonin as PRL increased from 4 ± 2 to 82 ± 27 ng/ml within the same time interval. In both groups, PRL concentrations decreased within 10 min and reached baseline 50 - 60 min after the injection. Interestingly, serotonin administration resulted in an increase in PRL in stalk sectioned animals as well. Although a dose of 50 µg was ineffective, 500 µg and 5000 µg increased PRL from 27 ± 6 to 57 ± 10 ng/ml and from 30 ± 6 to 75 ± 26 ng/ml, respectively. Peak concentrations were reached after 5 min with the 500 µg dose and after 20 min with the 5000 µg dose.

These results confirm that serotonin releases prolactin in the normal rhesus monkey. Since serotonin induces a similar prolactin release in stalk sectioned monkeys, the results also suggest that this neurotransmitter, like dopamine, may mediate release of PRL by an effect on the pituitary.

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1578 EFFECTS OF CERVICAL STIMULATION ON PITUITARY AND PLASMA PROLACTIN AND HYPOTHALAMIC CATECHOLAMINES IN PREPUBERAL RATS. D.W. Weiss and V.D. Ramirez. Dept. of Physiol. and Biophys., Univ. of Illinois, Urbana, IL 61801.

A single cervical stimulation (CS) induces a marked increase in prolactin (PRL) in the 24 but not in the 22 day old rat. Catecholamines (CA), particularly dopamine (DA) are involved in the control of PRL secretion. In this study, CA levels in the median eminence (ME) and the medial basal hypothalamus (MBH), together with plasma and pituitary PRL levels were measured in prepuberal rats.

Female rats 26, 28, and 32 days of age, (14 hr/10 hr light/dark cycle) were CSx1 min. The stimulation was done at 20:00, 1 hour after the lights were turned off and 2 days after artificial vaginal opening. A control (CTL) group was handled in the same manner but not CS. Immediately following, and at intervals of 2, 4, 6, and 12 h groups of 4 animals were sacrificed by decapitation. Plasma and pituitary PRL were measured by radioimmunoassay, while CA were measured with a radioenzymatic assay.

As the CTL rats matured from 26 to 32 days of age, the mean ME-DA content across the entire 24 hr period more than doubled, while the MBH-DA and MBH-NE values decreased 42 and 48% respectively. The mean ME-NE content in the 26 and 32 day old rat were higher than those in the 28 day old rat.

In the 32, but not in the 26 or 28 day old CS rat, a 4 fold increase in plasma PRL was observed after 24 hours. No changes in plasma PRL were seen in any of the CTL rats for the entire 24 hr period. An 87% decrease in the ME-DA content was also seen in the 32 day old rat 6 hrs after CS which recovered to the initial value 18 hrs later. This decline was not observed at any other ages in either the CS or CTL groups. The MBH-DA values did not significantly change in any of the animals during the 24 hr period and the ME-NE content did not change in any of the 26 or 28 day old groups. In the 32 day old CS animal, the ME-DA content significantly decreased, while the ME-NE content significantly increased. In the 32 CTL rat, the ME-NE content increased.

In the 26 day old animal a significant decrease in pituitary PRL content, reaching a nadir by 4 hrs and recovering by 24 hrs, was observed in both the CS and CTL rats. In the 28 day old animal, this decrease was only observed in the CTL rat. In the 32 day CTL rat, no changes in pituitary PRL were observed, but a 2 fold increase was seen in the CS animals beginning 4 hrs and peaking 20 hrs later. It is concluded that complex interactions among factors such as CA, maturation and circadian elements regulates the induced PRL surge after CS.

1579 EFFECT OF REPRODUCTIVE STATUS ON MEAL PATTERNS AND ACTIVITY OF MALE AND FEMALE RATS. Freya Weizenbaum, Suzanne Lochner* and Amelia Bland*. Dept. Psych., V.P.I. & S.U., VA 24061.

Meal patterns and activity levels of female and male rats were determined. The rats were videotaped for 24 hours and feeding behavior and cage crossings (activity) were quantitated. Analysis of the behavior revealed that there are striking parallels in the ingestive patterns of estrous females and mated males on the day after ejaculation. Females in estrus decreased 24-hour food intake and body weight, and increased the number of small meals and motor activity, compared to diestrus. Mated males decreased 24-hour food intake and body weight in the 24-hour period after ejaculation, and there was an increase in the number of short meals and in the number of approaches to the food cup. Novel-Cage Control males (which were placed in an empty arena for a brief period on the mating day) exhibited different behavioral patterns. There was no consistent effect of the Novel-Cage treatment on 24-hour food intake or meal pattern, and body weight tended to rise. Both Mated and Novel-Cage Control males showed a depression in gross motor activity, although there was some indication that the circadian distribution of the decrease may differ in the 2 groups. Thus the meal pattern (nibbling) and food intake of Mated males and Estrous females are similar. The nibbling pattern is known to be under the control of estrogen in the female rat (PB 17:20, 1976). The behavioral parallels between the Mated male and the Estrous female suggest that a common endocrine mechanism may be involved, possibly aromatization of testosterone to estrogen increases as a result of the mating-induced rise in testosterone(HB 6:277, 1975).

1580 AFFERENT CONNECTIONS OF THE MEDIAN EMINENCE IN THE RAT. S.J. Wiegand and J.L. Price. Dept. Anat. and Neurobiol., Washington Univ. Sch. Med., St. Louis, MO 63110.

Retrograde axonal transport of horseradish peroxidase (HRP) or ^{125}I-wheat germ agglutinin (WGA) was used to identify the cell bodies of origin of axons terminating in the median eminence (ME). The basal hypothalamus was exposed by a ventral approach through the soft palate and base of the skull. Pressure injections of HRP (10%, 0.02 to 0.04 µl) or WGA (1.9×10^8 CPM/ml, 0.1 µl) were made under direct visual control into the ME of male and female rats through glass micropipettes (tip diameter, 6 to 10 µm). After 6 to 24 hours animals were sacrificed. Brains were processed for HRP with tetramethyl benzidine and for WGA by autoradiography.

Following injections into the ME, retrogradely labeled cells are found in the arcuate nucleus (ARC) and the periventricular region, as well as in the paraventricular nucleus (PVN), to a lesser degree, the supraoptic nucleus (SON) and other magnocellular neurosecretory cell groups. In the periventricular region, labeled cells are restricted to an area extending from the rostral part of the ARC to the level of the anterior commissure, and are particularly concentrated in the zone medial to the PVN. In the PVN labeled cells are found in all parts of the nucleus, but are most densely aggregated in the smaller-celled dorsomedial part. A few cells are also found in the rostral preoptic area lateral to the organum vasculosum of the lamina terminalis (OVLT). Cells of the ventromedial nucleus of the hypothalamus are not labeled in cases where the injection of WGA or HRP was confined to the ME, but are labeled if the injection spread into the ARC.

In order to determine if a portion of the labeling observed after ME injections was due to the spread of HRP into the posterior pituitary control injections were made directly into this structure. Following these injections, the PVN, SON and all "accessory" magnocellular cell groups are very heavily labeled. Cells containing HRP reaction product are found in all parts of the PVN, but are most densely concentrated in the larger-celled anteroventromedial and posterodorsolateral parts of the nucleus. In these areas virtually every cell is labeled. Scattered cells in the periventricular region near the PVN also contain reaction product. However, cells in the ARC, the ventral part of the periventricular region near the ARC, and the preoptic region lateral to the OVLT are not labeled after injection of HRP into the pituitary.

1581 URINE EXCRETION FOLLOWING ADMINISTRATION OF NALOXONE, OXILORPHAN AND BUTORPHANOL. Guillermo A. Zeballos* and Alan B. Rothballer. Dept. Neurosurgery, New York Medical College, Valhalla, New York 10595.

The possibility that opioids play a role in the regulation of neurohypophysial peptide release is suggested by the presence of opiate receptors and enkephalinergic fibers in the neurohypophysis (Rossier et al, Soc. of Neuroscience, Abstracts 4: 414, 1978), and the well-known antidiuretic action of morphine. Moreover, the morphine analogues oxilorphan (OX) and butorphanol (BU) produce diuresis (Miller, Neuroendocrinology, 19: 241, 1975) and suppress the release of vasopressin which normally follows hypertonic stimulation (Basulto et al, Fed. Proc. 37: 815, 1978). To investigate the type of opiate receptor mechanism possible involved, we studied the interaction of those analogues with the known potent opiate antagonist, Naloxone (NAL) on water diuresis. Six groups of adult male rats were used: a. saline (SA)+SA. b. SA+OX. c. SA+BU. d. NAL+SA. e. NAL+OX. f. NAL+BU. Animals were kept in individual cages with water ad libitum. All drugs, dissolved in SA, or SA alone (0.2 ml./100 gbw.), were injected subcutaneously. 1.0 mg/100 gbw. of NAL or saline was given at the beginning of the experiment (time 0), and 30m. later, 0.9 mg/100 gbw. of OX or BU was administered. Urine was collected every 30m. and volume and osmolality were measured. At the end of the experiment, the rats were killed by decapitation. Blood was collected in cooled tubes to measure plasma osmolality and plasma AVP levels. Rats receiving OX or BU developed a higher diuresis and produced urine with osmolalities significantly lower than the ones receiving SA alone or NAL plus SA. At the end of the third hour following drug administration urine and plasma osmolalities (mOsm/Kg.) for each group were as shown below.

	SA.	OX.	BU.	
SA.	a. 1986±290	b. 679±24	c. 316±12	Urine
	284±1	294±2	290±2	Plasma
NAL.	d. 1540±670	e. 990±450	f. 439±240	Urine
	283±2	290±2	283±3	Plasma

These data confirm the diuretic effect of OX and BU, an effect not blocked by NAL which had no effect when given alone. Since the drugs BU and OX had effects opposite to that of morphine and not blocked by NAL they seem to be acting not as morphine agonists, but rather as antagonists. Moreover, they seem to be acting on antagonist receptors different from those with affinity for NAL. (OX and BU were kindly supplied by Bristol Lab. and NAL by Endo Lab.).

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NEUROETHOLOGY

1582 JAMMING AVOIDANCE BEHAVIOR IN A PULSE SPECIES OF ELECTRIC FISH: SENSORY ENCODING FOR A TEMPORAL PATTERN DISCRIMINATION. Curtis L. Baker, Jr. Dept. of Neurosciences, U.C.S.D., La Jolla, CA 92093.

South American pulse species of weakly electric fish change their frequency of electric organ discharges(EODs) to avoid jamming of their electrolocation sense by other fish at similar frequencies. If a "foreign" fish is at a lower frequency its pulses scan from negative to positive latencies with respect to the fish's EOD, resulting in a strong acceleration in EOD frequency. Pulses of another fish at a higher frequency scan from positive to negative latencies, evoking a weak acceleration or a deceleration.

Neurophysiological studies reveal four classes of burst duration coder (BDC) electroreceptors which respond to scanning stimuli. α-type cells respond to the inward current component of the EOD, βs to outward current, and γs and δs to both components. All of these types respond strongly to foreign pulses at near-zero latencies with respect to the EOD. In addition, the δ-type cells and a subclass, α_es, respond to foreign pulses at negative latencies.

A series of foreign pulses at fixed negative latencies evoke large EOD accelerations, while pulses at near-zero latencies result in decelerations or very small accelerations. If foreign pulses are briefly placed at a negative latency, then at a near-zero latency, a large acceleration results. Conversely, pulses at first a near-zero and then a negative latency result in decelerations or weak accelerations. These "latency-clamp" stimuli thus seem to mimic scanning situations, and furthermore suggest a theory for directional responses: pulses at negative latencies activate an excitatory (E) process (resulting in acceleration), while those at near-zero latencies activate an inhibitory (I) process (preventing response to the first, and/or causing deceleration); the process activated first then dominates the overall response.

There are two possible ways in which information from BDCs could be used to drive the E and I processes. One way is to exploit the differential sensitivity to different latencies: α_e and δ types could activate E, while α, β, and/or γ types activate I. Another mechanism would be to pool responses from all BDC types and allow weak responses to drive E, while very strong responses would activate I.

The latter hypothesis is supported by experiments in which pulses at fixed latencies are amplitude-modulated; small vs. large modulations result in directional responses very similar to those obtained from scans and latency-clamps.

1583 PATTERNS OF NEURONAL ACTIVITY CORRELATED WITH THE JAMMING AVOIDANCE RESPONSE IN EIGENMANNIA. Joseph Bastian and Walter Heiligenberg. Dept. of Zoology, Univ. of Oklahoma, Norman, Ok. 73019 and Scripps Institution of Oceanography, UCSD, La Jolla, Ca. 92093.

The Jamming Avoidance Response (JAR) of weakly electric fish consists of an alteration of an animal's electric organ discharge frequency due to the presence of an interfering electric signal having a frequency from 1 to 10 Hz different than the former. If the interfering frequency is the higher then the fish responds by lowering its frequency (-JAR) and the fish raises its frequency in the presence of a lower frequency foreign signal (+JAR). Recent behavioral studies have identified a simple set of stimulus conditions under which this behavior occurs. We have studied the responses of single electroreceptors and of neurons in the Posterior Lateral-line Lobe (PLL) and in the Torus Semicircularis (TS) using stimuli that cause the behavior as well as stimuli that are lacking key features necessary for the behavior.

Previously Scheich (J. Comp. Physiol. 113, 207-255, 1977), using more complex stimuli, described responses of a type of receptors, PLL cells and of TS cells which differed in response to stimuli which evoked opposite signed JARs. Our results, using sinusoidal stimuli, show that different response patterns in the same types of receptors and PLL cells are not needed for complete behaviors of either sign to occur, however certain TS cells always show patterns of activity contingent upon the sign of the JAR. These cells are shown to respond to phase differences in electrical stimuli impinging upon nearby regions of the body.

1584 DISCRIMINATION OF SIGNAL FROM NOISE IN THE ESCAPE BEHAVIOR OF COCKROACHES. Jeffrey M. Camhi and Mark R. Plummer*. Sect. Neurobiol. and Behav., Cornell University, Ithaca, NY 14853

The cockroach Periplaneta americana responds to gentle wind puffs by turning and running away from the wind source. The animal is sufficiently sensitive that it detects and escapes from the strike of a natural predator, the toad Bufo marinus, by sensing primarily or only the wind which the toad makes during its strike (Camhi et. al. J. Comp. Physiol. 128, 203-212, 1978). This sensitivity presents a problem for the cockroach: how is the signal (toad's wind) discriminated from noise (background wind)?

To complicate this problem further, we find that restrained cockroaches are most sensitive to wind puffs delivered during slow walking (mean threshold for a pause or startle response, 3 mm/sec; for a running response, 12 mm/sec). If such a cockroach had walked about freely, it would have generated a "relative wind" directed posteriorly whose speed would equal the forward walking speed. When walking slowly (3 steps/sec) this would be approximately 80 mm/sec. We re-introduced this relative wind to restrained cockroaches by creating a steady posteriorly-directed flow which, at the cerci, measured 80 mm/sec. The cockroach's mean threshold was unchanged in the presence of this large wind. Moreover, while recording the wind speed just behind one cercus, we noticed that every walking step of the ipsilateral rear leg produced a gust of wind of 1-30 mm/sec. In addition, in these experiments, we did not shield the cockroach from room air currents, in an attempt grossly to stimulate ambient winds of the native habitat (Camhi et. al. op. cit.). Room currents ranged generally up to 50 mm/sec, and could flow in any direction. Therefore, the cercal wind receptors of a walking cockroach are subjected to a large and complex wind field. This constitutes background noise which can be more than 25 times larger than the just-threshold signal.

Although the threshold wind puffs had low flow rates, their accelerations were higher than the range of accelerations contained within the noise (noise, 0-300 mm/sec^2; threshold for pause or startle, 300 mm/sec^2; threshold for running, 750 mm/sec^2). Using ramp wind stimuli of 40 mm/sec peak flow but variable acceleration, accelerations greater than about 800 mm/sec^2 consistently evoked escape running, whereas those of about 300-800 mm/sec^2 most often evoked a pause in walking. Also, the lower the acceleration, the greater the probability that the stimulus would not evoke any response. At the lowest acceleration so far tried, 120 mm/sec^2, no response was evoked on about 50% of the trials. These data, plus recordings in progress from giant interneurons of walking cockroaches, suggest that a wind accelerometer in the escape circuit assists the animal in discriminating signal from noise.

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1585 A ROLE FOR NUCLEUS INTERCOLLICULARIS IN THE REPRODUCTIVE BEHAVIOR OF THE FEMALE RING DOVE. Jeffrey Cohen and Mei-Fang Cheng. Inst. Anim. Behav., Rutgers Univ., Newark, NJ 07102.

Autoradiographic studies (Martinez-Vargas et al., JCN 167: 83, 1976) have identified several estrogen-concentrating sites in the ring dove brain, including the n. intercollicularis (ICo) region in the midbrain. Our previous radiofrequency lesion study implicated the involvement of this region in the mediation of females' nest-coo behavior pattern, a species-typical courtship response involving an oblique posture, wing-flipping and coos. The present studies further characterize the role of the ICo region in female courtship behavior.

The first study was designed to determine whether intracranial implants of estrogen in the ICo region could elicit female courtship behavior. A single cannula (30 gauge) containing crystalline estradiol-17β (E), estradiol benzoate (EB) or cholesterol (C) was placed unilaterally in the ICo region and the surrounding areas with the aid of a stereotaxic instrument in the females following bilateral ovariectomy. Behavioral results and histological verification of implant placement revealed that E and EB were most effective in eliciting nest-coo behavior when the implant was in the medial ICo region. Implants in other surrounding regions were not effective. None of these implants was effective in eliciting sexual crouch.

In the 2nd study, silver stain degeneration methods were used to determine efferent connections from the ICo region. Within the mesencephalon, dense degeneration was found in the ipsilateral ICo extending around Mld, and to the substantia griseas centralis. Degeneration was also observed in the contralateral ICo, with a few axons entering the Mld. Two sets of fibers may be discerned leaving the site of the lesions. One tract passes ventromedially from the ICo region, decussates under the medial longitudinal fasciculus and proceeds ventrally, staying medial to the locus coeruleus and lateral to n. decussationis brachium conjunctivorium. Some of these fibers appear to terminate in n. centralis superior, while others continue caudally and terminate in part in n. paramedianus. A second group of fibers leaves the lateral portion of the ipsilateral ICo, passing ventromedially around n. isthmi, pars parvocellularis and continues caudally on the tegmentum floor around n. pontis lateralis. Some of these fibers appear to decussate.

The estrogen implant study supports the implication of the ICo region and its connections in the mediation of the female's nest-coo behavior pattern. The degeneration study, however, did not provide a clear anatomical basis for the relationship between the ICo region and the known neural sites and pathways for vocalization.

1586 THE CENTRAL NERVOUS SYSTEM AND VOCALIZATION IN THE DOMESTIC CAT. N. C. de Lanerolle* (SPON: J. P. Flynn). Dept. Psychiatry, Univ. Yale Med. Sch., New Haven, CT 06508.

The vocalizations of the domestic cat are important in intraspecific communication. They are also expressions of emotion. Understanding the causation of vocalization can throw much light on these two aspects of species-typical behavior.

Studies by others on the domestic cat brain using either electrical brain stimulation or brain lesion techniques have implicated a number of brain regions in vocalization. However, the precise neuroanatomical pathways between these areas, or the inputs to or outputs from these areas remain unknown. The manner in which these areas influence vocalization has also not been investigated. With these questions in mind we have explored a region of the pons around the medial lemniscus using electrical brain stimulation combined with silver staining of degeneration from lesions placed at vocalization sites. Kanai & Wang (1962) had suggested that in the above region were sites on the final common path for all vocalization in the cat. The existence of such a common pathway for all vocalization has been disputed by workers on the Squirrel Monkey (Jurgens & Ploog, 1970). We found vocalization sites in and very near the medial lemniscus. The threshold current for evoked-vocalizations ranged from 0.05 mA to 0.2 mA, and in the majority of cats was below 0.1 mA.

The vocalizations evoked from these sites were similar to naturally produced (a) soft meows, (b) low frequency (2 KHz)calls of long duration (growls), (c) calls typically showing very dense and evenly distributed representations of resonant frequency elements which extend up to 8 KHz (protest meows). Hiss-like calls were evoked from some of these sites whereas purrs were never elicited. Very little overt behavior accompanied the vocalizations. Typically, the cat would be seated on its belly, tail drawn forward, ears up and facing forward to lateral, with pupils narrow rather than dilated. No non-vocal signs of escape or defensive threat (e.g., locomotion, piloerection, ear-flatten -ing or arching of the back) were associated with these sites. Such behavior without vocalization was observed at sites dorsal to the call site.

Descending projections from the call sites were followed to the lateral pontine nucleus; through the trapezoid area into the facial, ambiguus and inferior olivary nucleus. Ascending projections were traced to the ipsilateral inferior colliculus, the VPL nucleus of the thalamus, and in some instances to the periacqueductal central gray. It is argued that the vocalizations evoked from these sites are the result of the activation of a vocalization control mechanism, and not the indirect result of stimulating pain pathways.

1587 MUSCULAR CONTROL OF FREQUENCY AND DURATION OF ULTRASONIC VOCALIZATION IN THE LARYNX OF THE ECHOLOCATING BAT, EPTESICUS FUSCUS. Gary E. Durrant* and Roderick A. Suthers. Physiology Section, Med. Sci. Prog., Indiana Univ. School of Medicine, Bloomington, IN 47401

Eptesicus fuscus emits ultrasonic sonar pulses having a duration of one to several msec and repetition rates of up to 200 pulses per second. These downward sweeping FM vocalizations are emitted at intensities up to 110 dB SPL. We here report evidence regarding the mechanism by which echolocating bats are able to independently control the frequency and duration of an orientation sound, yet precisely coordinate these control mechanisms so that phonation occurs only when an appropriate tension exists on the vocal membranes, which are the source of the ultrasonic vocalizations. The frequency at which these membranes vibrate is primarily controlled by the three branches of the cricothyroid muscles. In short (5 msec) duration sounds, all three cricothyroid branches begin contracting prior to vocalization but stop contracting 10-12 msec before the start of phonation. The frequency of the downward FM sweep is controlled by the relaxation of these muscles. The cricothyroid muscle also adducts the vocal folds which are ventrocaudal to the vocal membranes and form the glottis. During the cricothyroid contraction prior to phonation, subglottic pressure (P_S) increases to a maximum of 40 cm H_2O due to the glottal constriction. When the cricothyroids relax, the P_S forces the vocal folds apart allowing air to flow between the vocal membranes and initiating phonation. The ventral thyroarytenoid muscle acts as a supplemental glottal constrictor which can operate independently from cricothyroid-generated constriction. This muscle starts to contract 55 msec prior to phonation and relaxes 16 msec before the start of phonation. When the duration of vocalization is increased, the relative timing of contractile activity in the three parts of the cricothyroid muscles vary. The ventral thyroarytenoid muscles also change the temporal relationship of their contraction relative to that of the cricothyroids. Since the ventral thyroarytenoid muscle has no effect on vocal membrane tension, it provides a means of controlling the duration of vocalization independently from its frequency. This supplemental glottal closure force can allow P_S to increase, resulting in more intense vocalizations, and it can prevent premature onset of phonation as P_S rises. Vocalizations appear to be terminated by the anterior and posterior cricoarytenoid muscles which are the arytenoid abductors and start to contract 5 msec before phonation starts. (Supported by NSF Grant BNS 76-01716).

1588 LEVELS OF FUNCTION IN RAT GROOMING BEHAVIOR. E. K. Freed* and H. J. Grill. (SPON: Steven E. Brauth). Dept. Psychol., Univ. Pennsylvania, Philadelphia, Pa. 19104.

Decerebrate rats initiate spontaneous bouts of effective grooming. Thalamic rats, with more brain intact, are much less effective in their grooming (Grill and Norgren '78). Thalamics perform grooming movements but do not correctly direct these to their fur. Our strategy was to define the components of grooming in intact rats and then to compare this normative data to that of chronic decorticate and decerebrate rats. Male rats, 8 intact, 4 2-stage supracollicular decerebrates (hand-held spatula transection) and 3 2-stage neodecorticates (aspiration) were used. Animals were observed in two conditions: spontaneous (no exogenous stimuli) and elicited (food; dirt on fur) grooming. Grooming bouts were timed and coded; half of the observations were videotaped for slow motion sequential analysis. Intact grooming consisted of 21 components, including 5 types of face-washing, differentiated on the basis of the forepaw's trajectory over the face; 13 categories for grooming various body areas with the mouth and/or forepaws; one category for hind leg scratching and 3 for shaking. Results show that both decerebrate (5.59) and decorticate (5.58) rats elicit fewer spontaneous bouts of grooming per a 45 min. interval than do intact animals (15.00). Decerebrate bouts last as long as 20 min. while those of intact rats rarely exceed 5 min., and and decorticates 1 min. Both decorticates and decerebrates display almost all components shown by intact rats; decerebrates show morphological differences in these components as well as components not displayed by the intact. Intact rats are likely to begin a sequence with face grooming, and to then alternate between the face and other body regions. Similarly, decorticate rats tend to begin with face grooming, but are only half as likely to move on to other body regions. Decerebrates, like intact rats, both begin at the face and show the alternating pattern. The basics of normative sequence remain in the decerebrate. These data suggest that neural mechanisms for sequencing grooming exist caudal to the diencephalon. Although decerebrates show postural anomalies and are often incapable of negotiating postural changes while maintaining a stable position, they do initiate postural transitions in grooming. Shorter decorticate bouts have been interpreted (Vanderwolf, Kolb, and Cooley '78) to indicate that the cortex may be important in selecting and initiating postural changes allowing grooming to continue. Since decerebrates attempt to make necessary postural transitions, and assemble grooming sequences like those of the intact, the deficit which decorticates show in combining components to form extended bouts appears not to be mediated by direct effects on the neurological substrates of either postural transition or grooming, but rather by some other deficit. (Supported by NIH AM 21397.)

1589 **EYE POSITIONS IN GOLDFISH SUGGEST CENTRAL MECHANISMS COUNTERACTING VISUALLY INDUCED TONUS ASYMMETRIES.** **Werner Graf* and Dietrich L. Meyer. Dept. of Histology and Neuroanatomy, University of Goettingen, D-3400 Goettingen, West Germany**

Freely swimming and restrained goldfish were exposed to different directions of light in a tube tank.Body and eye positions were determined by single frame videotape analysis.
When the tank is illuminated from the side a freely swimming fish tends to orient its dorsal side towards the light source (Dorsal Light Response,DLR). The amount of tilt depends on where visual and vestibular equilibrium sense balance each other out (v.Holst,Z.vergl.Physiol. 32, 60 - 120, 1950). Bilaterally labyrinthectomized fish tilt their bodies to an angle which corresponds to the angle of maximum light intensity.

A restrained animal turns its eyes towards the light source when a bodily response is prevented. Thus, the ipsilateral eye (w.r.t. direction of light) is deviated down, and the contralateral eye is deviated up.

This behaviour is observed in normal, as well as in bilaterally labyrinthectomized fish. Although lesioned animals display more pronounced eye deviations than normals, ocular displacements in lesioned fish never fully match the angle at which the light is directed towards them.

Ocular deviations observed in these experiments are less than the physiological range of eye movements.

Our data indicate that the DLR - model of v.Holst for bodily reactions cannot be employed for ocular responses. We conclude that mechanisms exist which counteract visually induced central asymmetries of tonus, and thus curb ocular counterrolls under the experimental circumstances described.

1590 STREAKING IN A STATE-PLANE: THE ANALYSIS OF STIMULUS PARAMETERS CONTROLLING THE JAMMING AVOIDANCE RESPONSE IN EIGENMANNIA. Walter Heiligenberg and Joseph Bastian . Scripps Institution of Oceanography,UCSD, La Jolla, Ca.92093 and Dept. of Zoology, University of Oklahoma, Norman, Okl. 73069.

Eigenmannia shifts its electric organ pacemaker frequency away from similar frequencies of other fish. This Jamming Avoidance Response (JAR) is driven by simultaneous modulations of phase and amplitude in electroreceptive afferences from the animal's sinusoidal electric organ discharges (EODs) beating against EODs of another fish. Modulations in phase and amplitude can be represented as graphs in a two-dimensional state-plane, and two classes of electroreceptors, T- and P-units, encode the two coordinates of this plane respectively. In addition to naturally occurring graphs, which characterize the beating of two sinusoidal signals, various computer generated artificial graphs were tested in order to determine the algorithm by which such graphs control the animal's electric organ pacemaker. It is demonstrated that short streaks in the state-plane alone can influence the pacemaker, and that the effect of a whole graph can be represented as the sum of the contributions of incremental streaks, i.e. line segments, which constitute this graph. The effect of a given graph upon the pacemaker can thus be written as its line integral in a vector field which characterizes the state-plane.

1591 NEURAL MODELLING OF PREY-PREDATOR INTERACTIONS IN FROG AND TOAD VISUOMOTOR COORDINATION. Rolando Lara*, Michael A. Arbib and Andrew Cromarty*. Center for Systems Neuroscience, Univ. of Massachusetts, Amherst, MA 01003.

Computer simulation is used to explore parameter-settings which will enable anatomically plausible neural networks to exhibit activity consonant with the behavioral and physiological findings of Ewert and Ingle. We model interaction between pretectum/thalamus and tectum, with the tectum represented as an array of tectal columns whose structure is abstracted from anatomical findings of Székely (Hdbk. Sens. Physiol., VII 3/B: 1-21, 1973). Simulation of a single tectal-column exhibits time-constant and synaptic settings which yield facilitation effects akin to those observed by Ingle (Science, 188: 1033-1035, 1975). These parameters extend to intercolumn connectivity to yield predictions on 'wormness'/velocity tradeoffs in the toad (Ewert, Naturwiss. Rundsch. 25: 1-11, 1972). Extending a model of Didday (Math. Biosci., 30: 169-180, 1976), we model thalamic-tectal interactions in offering an explanation of data of Ingle (Brain Behav. Evol. 1: 500-518, 1968) on choice between fly-like stimuli in frog, and of Ewert and von Wietersheim (J. Comp. Physiol. 92: 149-160, 1974) on thalamic modulation of tectal response to worm and 'antiworm' stimuli. [Research supported in part by NIH grant 1 R01 NS14971-01, Michael A. Arbib, P.I.]

1592 TEMPORAL ORGANIZATION OF SWIMMING ACTIVITY IN SARSIA TUBULOSA M. SARS (HYDROZOA). Janet L. Leonard* (SPON: Philippa Claude). Friday Harbor Laboratories, Friday Harbor, Washington 98250, and University of Wisconsin-Madison, Madison, Wisconsin 53706.

A quantitative ethological study of Sarsia medusae has been undertaken to establish what factors determine the timing of bouts of swimming in this animal. During the daytime undisturbed Sarsia in squaria alternate between periods of swimming by means of a series of bell contractions (swim bouts) and periods of quiet floating (pauses). Although the timing and duration of swim bouts and pauses can be very variable, the individual swimming contractions are initiated by an endogenous pacemaker. The behavior of Sarsia in aquaria was recorded over periods of six hours by a time-lapse videotape recorder. The videotapes were played back at normal speed and data were recorded onto a computer-compatible event recorder.

Three possible types of organization were considered in the analysis: 1) temporal organization, in which events occur with regular periodicity 2) serial organization, in which the duration of an event is a good predictor of the duration of some following event, and 3) no organization, in which both of the first two types are absent. Fourier analysis was used to detect temporal organization. Serial auto- and cross-correlation coefficients out to lags of 23 were calculated and plotted as correlograms to detect serial organization. Three parameters were used in the correlation analysis; swim bout duration, pause duration, and interonset interval (the length of time between starts of swim bouts).

For animals that were swimming less than 25% of the time, there was a remarkable similarity in shape between swim bout and interonset interval autocorrelograms. This means that swim bout duration determined the interonset interval. In more active animals pause durations were more important in determining when swim bouts would start, perhaps because of fatigue. The pauses in active animals may represent a necessary period of recovery before the animal can swim again.

Fourier analysis showed that the bulk of the power spectrum is always concentrated at frequencies lower than .030 cps. Some animals showed a strong rhythm with a frequency of .005-.025 cps. All other animals had their greatest peaks at frequencies very near zero. A given animal usually had a characteristic spectrogram shape for up to three days. This suggests that the way swim bouts and pauses are coupled may vary somewhat from individual to individual.

1593 MULTIMODAL DETECTION OF MOVING OBJECTS BY AN ELECTRIC FISH, STERNOPYGUS. Joanne A. Matsubara. Scripps Institution of Oceanography, UCSD, La Jolla, Calif. 92093.

Extracellular, single unit recordings from the posterior lateral line lobe (pLLL) were undertaken in a weakly electric fish, Sternopygus (Gymnotoidei). Sternopygus, like all other weakly electric fish, can electrolocate, i.e. they can detect objects which differ in impedance from the surrounding water. However, unlike all other wave-species tested, Sternopygus has no jamming avoidance response (JAR), yet can electrolocate even in the presence of electrical jamming signals.

Results indicate some pLLL units are bimodal; they respond to both electrical and mechanical stimulation. This is in accord with previous studies of the pLLL (Enger and Szabo, 1965). Evidence from this study suggests additionally that the behavioral relevance of such bimodal units is to encode movement of nearby objects. These units encode movement of objects by changes in their spike frequency, and such responses persist even after elimination of electrical and visual cues.

It is assumed that all fish receive information about moving objects in the water through the ordinary lateral line system (Dijkgraaf, 1967). In weakly electric fish, the lateral line lobes receive primary afference from both ordinary lateral line receptors (canal organs and free neuromasts) and specialized lateral line receptors (tuberous and ampullary electroreceptors). Hence, weakly electric fish possess two closely-related sensory systems with which they can monitor movement of objects in their environment. Since the presence of any object provides many different sensory cues, it is conceivable that the naturally-behaving animal will differencially rely upon the less ambiguous sensory aspects associated with its environment rather than "jammed" cues. This "selective attention" to mechanoreception may help Sternopygus to follow the motion of objects even in the presence of electrical jamming signals (Matsubara and Heiligenberg, 1978).

1594 PREOPTIC AND VMH LESIONS SEPARATE PROCEPTIVE AND RECEPTIVE COMPONENTS OF FEMALE SEX BEHAVIOR. Dennis A. Merkle* and Owen R. Floody. Bucknell Univ., Lewisburg, PA 17837.

Female sexual motivation can be expressed in distinct proceptive and receptive behaviors which function to initiate heterosexual contact and to facilitate male copulatory attempts, respectively (Beach, Horm. Behav., 7, 105, 1976). For example, ultrasonic vocalizations by estrous female hamsters are attractive to males and are produced most often before and after, but not during, male-female contact. In contrast, female lordosis clearly facilitates successful copulation and normally appears in response to tactile cues provided by a male just during close social contact.

The different roles played by hamster ultrasounds and lordosis in reproduction suggest that these behaviors depend on neural circuits that are at least partially distinct. To test this hypothesis, we studied the effects of preoptic area (POA) and ventromedial hypothalamic (VMH) lesions on female ultrasound production and lordosis. Whereas destruction of either area was expected to affect lordosis (e.g., Nance et al., Brain Res. Bull., 2, 307, 1977), previous studies implicate the POA, but not the VMH, in the control of reproductive vocalizations (Wada et al., Horm. Behav., 9, 141, 1977).

Ultrasound rates of ovariectomized, hormone-primed female hamsters were observed in tests with stimulus males and ultrasounds. The postoperative call rates of females with bilateral electrolytic lesions of the POA or VMH were compared with preop rates for the same females, and with postop rates of females subjected to sham lesions. POA lesions consistently caused decreases in rates of ultrasound production. In contrast, females with VMH or sham lesions showed no consistent postop change in call rate.

Lordosis responses were observed in tests with stud males. Females with VMH lesions showed consistent decrements in lordosis. Females with POA lesions were more similar to sham-operated controls, but tended to show increased lordosis durations in postop tests.

These results show that brain lesions can dissociate the proceptive and receptive behaviors of female hamsters. In particular, VMH lesions affected receptivity (lordosis), but not proceptivity (ultrasound production). POA lesions tended to affect both proceptive and receptive behaviors, but in opposite directions. These results, then, emphasize differences between the brain mechanisms for proceptive and receptive components of female sexual behavior.

1595 COPULATION ELEVATES PLASMA β-ENDORPHIN IN THE MALE HAMSTER. Michael R. Murphy, Donald L. Bowie*, & Candace B. Pert. NIMH, Bethesda, MD 20205.

In men, use of opiate drugs can cause depressed libido, delayed ejaculation and impotence. In opiate addicts, withdrawal can cause increased libido, premature ejaculation and spontaneous erection. We investigated the role of the endogenous opiates in sexual function by using a convenient animal model to (1) assay for changes in plasma β-endorphin during copulation, and (2) analyze the effects of opiate agonists and antagonists on mating behavior.

Male hamsters in one group were decapitated 30 sec. after their 5th ejaculation, during the refractory period. Hamsters in a 2nd group were decapitated 30 sec. after a non-ejaculatory intromission following their 5th ejaculation. Members of a 3rd group were decapitated after removal from their home cage. Blood was collected in EDTA (5ml) and bacitracin (500μg/ml), plasma obtained and β-endorphin was extracted by alumina absorption. Radioimmunoassay of plasma extracts resulted in non-detectable levels of β-endorphin ($<5x10^{-13}$M) in the unmated group. β-endorphin levels were at least 86 times higher ($4.3x10^{-11}$M, $p<.01$) in plasma collected following ejaculation. Levels in plasma collected after a non-ejaculatory intromission were slightly, but significantly, elevated ($1.6x10^{-12}$M, $p<.01$).

In other experiments the mating behavior of male hamsters was tested after injections of saline; 1, 2, 4, 8, or 16mg/kg of methadone; 2, 20, or 200mg/kg of naltrexone; or a combination of both drugs. Sixteen behavior patterns seen during mating were recorded on a computerized event recorder and analyzed with respect to frequency, total duration, latency and average duration. Methadone and naltrexone both caused a dose dependent reduction in libido, with the methadone effect being the most severe. Potency (ability to achieve erection and intromission) was greatly reduced by methadone (2mg/kg and higher) but was significantly increased by naltrexone (20mg/kg). Pretreatment with naltrexone blocked the effects of methadone; posttreatment reversed the effects.

These results show that β-endorphin is elevated following ejaculation, during the post-ejaculatory refractory period, and could be involved in the inhibition of mating during this period. The behavioral results further suggest that endogenous opiates may be involved in the control of sexual potency and libido in the male. Our findings with the hamsters are consistent with recent reports that in rats opiate peptides inhibit the mating of sexually vigorous males (Meyerson & Terenius; Gessa, et al.) and naltrexone induces mating in sexually inactive males (Gessa, et al.).

1596 BEHAVIORAL AND PHYSIOLOGICAL ASPECTS OF SOUND RECEPTION IN THE COCKROACH, GROMPHADORHINA PORTENTOSA. Margaret C. Nelson, Dept. of Neurobiol., Harvard Med. Sch., Boston, MA 02115

The giant Madagascar cockroach produces audible hisses in association with its courtship behavior. Two types of hisses, differing in amplitude modulation and temporal characteristics, occur in conjunction with 2 stereotyped behaviors performed by the male (Nelson, in press, J.C.P.&(1). These observations raise several questions: are these hisses used as communicative signals, affecting the female's behavior? If so, what is the sensory basis of reception of such signals? How closely is reception matched to the acoustical characteristics of the sound?

All hisses produced by this species contain a broad range of frequencies extending into the ultrasonic, but most of the sound energy is concentrated in 2 broad peaks at about 4-8 and 12-16 kHz. To determine the behavioral consequences of courtship hissing, I first silenced males by blocking the specialized spiracles, and then restored the acoustical component of hissing by playing recorded courtship hisses to mute males and females paired with them at the appropriate moments of courtship. The timing and frequency of several courtship displays were compared across 4 groups of animals: normal, sham-operated, mute (silenced, no playback) and playback (silenced, sound playback provided). The playback signal had a frequency cut-off of 16 kHz.

Both muting and playback had significant effects on the outcome of courtship. Silenced males spent significantly more time in the early phases of courtship, and without playback achieved significantly fewer copulations than males in control groups; the receptive behavior of females paired with them was significantly reduced from normal levels. In contrast, when silenced males did receive playback, they achieved near-normal levels of copulation, and females paired with them showed normal levels of receptive behavior. Thus I conclude that acoustic stimulation is a necessary feature of G. portentosa's social behavior.

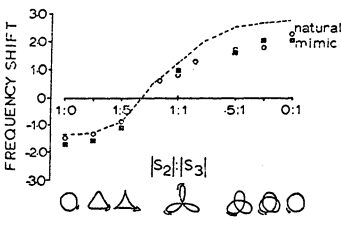
To investigate the physiological basis of sound communication I recorded from the thoracic connectives of minimally dissected animals while playing both pure-tone and filtered noise stimuli. This revealed up to 4 interneurons which were driven by farfield sound stimulation, with the best frequencies ranging from 4 to 7 kHz. In the natural hiss this is the frequency range that contains the most energy. These preliminary data suggest that some neural elements involved in sound reception are tuned to the high-energy portion of the hiss's sound spectrum, in agreement with the observation that a playback signal lacking the higher-frequency components of the hiss was very effective in eliciting normal courtship behavior from females.

(1) Nelson and Fraser, Soc. Neurosci. Abstracts 3, 1977.

1597 THREE'S A CROWD: THE RESPONSES OF EIGENMANNIA TO MULTIPLE JAMMING ARE PREDICTABLE. Brian Partridge and Walter Heiligenberg Scripps Institution of Oceanography, UCSD, La Jolla, Ca. 92093

Most species of electric fish shift their electric organ pacemaker frequency away from potentially jamming signals, and a number of studies have investigated the behavior and its neuronal basis (e.g. Bullock et al., J.C.P. 77:1-48, 1972). With the exception of a brief mention by Bullock et al., however previous studies have examined the effect of only a single jamming stimulus whereas Eigenmannia normally live in groups and must constantly be subjected to the electric discharges of several neighbors.

We have extended the model proposed by Heiligenberg and Bastian (abstr., this vol.) to predict Eigenmannia's response to multiple jamming stimuli. Close matching between predicted and observed responses provides independent confirmation of the model since it was originally developed to explain results from experiments with only single jamming stimuli. In the model, we represent the fish's EOD and jamming stimuli as vectors in the complex plane and draw resulting graphs of modulation of phase (H) and amplitude (S) of the combined signal in a 2-D state-plane. Shape of the graphs and their direction of rotation provide quantitative predictions for fishes' behavior during multiple jamming. Behavior of Eigenmannia in both intact and curarized conditions is in close agreement with that predicted (see fig. left: dashed line shows predicted values.)



Previous work has shown that Eigenmannia does not require an internal representation of its own EOD to produce correct JARs. Contrary to earlier assumptions, our results demonstrate that fish also do not need to determine the separate DFs of jamming stimuli since attributes of the combined signal provide sufficient information for correct JARs.

1598 DIRECT HEATING OF MOTHER RATS CURTAILS MATERNAL NEST BOUTS. Rodney J. Pelchat, Barbara Woodside*, and Michael Leon*. Dept. Psych., McMaster Univ., Hamilton, Ontario, Canada.

Croskerry, Smith and Leon (Nature, 273: 299, 1978) have suggested that thermal cues limit nest bout duration in rats. We tested this hypothesis by directly heating either the ventrum, core or preoptic area of dams soon after nest bout initiation and measuring the time to nest bout termination. Ventral and core heating significantly reduced nest bout duration. However, termination of the bout was not concurrent with an acute, localized rise in temperature in either area but seemed to require time for temperature elevation in other areas as well. Diathermic heating of the preoptic area terminated bouts very rapidly. Therefore, thermal factors are sufficient to terminate nest bouts. Continuous recording of maternal temperature also suggests that thermal factors play a greater role in nest bout termination late in lactation (Day 10 after parturition) than early in lactation (Day 4). These data support a model that proposes the additivity of acute (eg. litter size and age, dam size, nest insulation, ambient temperature) and chronic (eg. elevated body temperature during lactation) factors in the control of maternal nesting.

1599 INHIBITION OF SHOCK-FACILITATED AGGRESSION IN FEMALE HAMSTERS BY SEPTAL STIMULATION. Michael Potegal, Alan Blau*, Rosemary Sweeney* and Murray Glusman. Dept. Behavioral Physiology, N.Y. State Psychiatric Institute, New York, NY 10032.

In order to evaluate the full potency of septal stimulation to inhibit intraspecific aggression in the hamster (Potegal et al, Soc. Neurosci. 4:364, 1978) we have modified our experimental procedure to maximize the frequency and intensity of aggression. We have found that septal stimulation will inhibit attacks by female Syrian golden hamsters (which are more aggressive than males in this species) even when these attacks are facilitated by foot shock.

Our subjects were retired breeders which were ovariectomized to eliminate the variability in aggressiveness accompanying the estrous cycle. Under our test conditions aggression levels of ovariectomized females are not different from those of intact, non-estrous, animals (cf. Floody and Pfaff, JCPP 91:443, 1977). Subjects were then screened for high aggressiveness and implanted with bipolar septal electrodes. After recovery from implantation they were presented in their home cages with non-aggressive female target hamsters prepared in standard fashion (Potegal et al, Soc. Neurosci. 4:364, 1978). Current values of 100 pps, 0.1 msec stimulation (82-370μA) that were sufficient to prevent the initiation of "spontaneous" (unshocked) aggression also proved sufficient to immediately stop already ongoing "spontaneous" aggression.

In subsequent daily sessions subjects were placed in a small shock chamber with a target animal and given ten 2 mA, 0.5 sec foot shocks at 30 sec intershock intervals (cf. Shipley & Kolb, JCPP 91:1056, 1977). Control sessions, C, (with no septal stimulation) preceded and followed experimental sessions, X, (with continuous septal stimulation at current values sufficient to inhibit "spontaneous" aggression) in the sequence: CCCXXC. To date, subjects have attacked following 50% of C session shocks for a mean of 18.6 sec of attack/session; in X sessions subjects have attacked following only 7.5% of the shocks for a mean of 1 sec of attack/session.

Subsequent flinch-jump tests of foot-shock pain thresholds show that septal stimulation at aggression-inhibiting current levels has had no effect to date on pain thresholds (mean control jump thresholds = 0.91 mA, mean stimulation-accompanied threshold = 0.90 mA). As in our earlier study, septal stimulation did not produce electrographic seizure activity in the septum and did not affect sunflower seed acceptance. It thus appears that septal stimulation is a potent and relatively specific inhibitor of intraspecific aggression in hamsters. (Supported by an H.F. Guggenheim Foundation Grant)

1600 A CRUSTACEAN STATOCYST: STRUCTURAL SIMPLICITY AND POSSIBLE FUNCTION IN BEHAVIOR. R. D. Rose*, R. Tawil* and D. R. Stokes* (SPON: J. W. Manning). Dept. Biol., Emory Univ., Atlanta, GA 30322

Paired statocysts occur in the telson of Cyathura polita (Crustacea, Isopoda, Anthuridae). Each statocyst is a hollow chitinous sphere (~140μm), containing a single concretion. Three pits, arranged to form the corners of a triangle, occur in the ventro-medial floor of the interior cyst wall. A single hair protrudes from each of these pits and penetrates the concretion. Each of the pits on the internal floor of the cyst corresponds with a nodule on the external wall of the cyst. Each nodule contains a central pore from which a process emerges. A branch of nerve 1, a posterior extension of the ventral nerve cord, contacts this nodular region, and presumably carries sensory input from the cyst to higher centers. The small number of elements in each cyst as well as the way in which they are morphologically coupled may be functionally significant. Penetration of the hairs into the solitary concretion insures that contact will be maintained, and the triangular placement of the hairs may, by vector addition of the shearing forces produced on each hair, provide information on both magnitude and direction of the shearing force generated by displacement of the concretion.

Functional static input may be important to the burrowing habit of this benthic animal. Lesion studies involving ablation of one or both cysts as well as several different control lesions have been performed and the effects of these lesions on behavior have been examined. Two separate paradigms have been used: one tested the ability of the animal to maintain an upright posture, the other tested burrowing efficiency. Cyathura is metastable, the center of gravity being above the dorso-ventral midline of the animal. Postural control, therefore, is an active process. Bilaterally lesioned animals show the greatest deficits in both experimental situations, unilateral lesions produce intermediate results, and control lesions produce only slight deficits.

Animals lacking one or both statocysts have difficulty in maintenance of posture, periodically falling to one side or onto their back; a few show no behavioral deficiencies. In some cases all burrowing activity is abolished; in others the animals discontinue burrowing after the head and anterior thoracic segments penetrate the substrate. These results suggest that other sensory structures are also involved in these behaviors. Vision may play a role since some bilaterally lesioned animals cease burrowing once their eyes are beneath the substrate surface.

1601 EVIDENCE FOR FEATURE DETECTION IN CORTICAL AUDITORY NEURONS OF SQUIRREL MONKEY. David Symmes, John D. Newman, and Shozo Kojima.* Lab. Dev. Neurobiol., NICHD, Bethesda, MD 20205.

Previous reports on response characteristics of cortical cells selective for one or a few natural calls in a test series have left at least two explanatory hypotheses untested. One hypothesis ("call detector") suggests that an array of unique acoustical properties activates the cell through temporal facilitation, giving rise to a response only after all elements have been added sequentially. A second and less pontifical idea is that cortical cells respond to unique, short-lasting acoustic fragments wherever and whenever such fragments occur in natural vocalizations ("feature detector"). We have tested these hypotheses by examining the role of temporal facilitation in well-timed, stable responses to vocalizations presented under optimal conditions. Active short fragments (usually less than 100 msec.) were identified within intact vocalizations and presented in an isolated manner by deletion of remaining parts. No evidence of temporal facilitation was found, and in fact many cells exhibited stronger responses to isolated fragments. This finding suggests that temporal inhibition may be common and serve to sharpen feature boundaries. Our results do not favor the view that individual "grandperson" cells encode the meaning of species-specific vocalizations, at least in primary auditory cortex.

1602 NEUROETHOLOGICAL STUDIES OF SWIMMING IN APLYSIA BRASILIANA.
K. von der Porten and H. Pinsker. Marine Biomedical Institute, and Dept. Physiol. & Biophys. and Psychiat. & Behav. Sci., UTMB, Galveston, TX 77550.

Aplysia brasiliana swim by bilateral parapodial flapping with a rostro-caudal metachronal wave. Video tape records of freely swimming animals provide a noninvasive monitor of two components of this behavior, the period and metachronal offset. Changes in temperature affect the period (1.7 sec at 21°C to 3.1 sec at 12°C) but not the metachronal offset (0.5 sec at both temperatures). This results in a change in phase relationships between anterior and posterior oscillations at different temperatures and suggests that the period and metachronal wave have different underlying mechanisms.

Peripheral nerve lesions established that the anterior, middle and posterior parapodial nerves are necessary and sufficient for normal flapping with the anterior nerve playing the major role. Synchrony of flapping in the two parapodia is abolished by lesions of the pedal commissure, but bilateral removal of the pleural ganglia has no effect. Bilateral lesions of the cerebro-pedal connectives totally abolish swimming, but tonic stimulation of this pathway via a cuff electrode produces normal parapodial flapping for as long as the stimulus is maintained. Thus the command to swim originates in the cerebral ganglia but a neuronal oscillator for parapodial flapping is located within each pedal hemiganglion.

Phasic bursts of efferent activity in large units are recorded by implanted cuff electrodes during swimming in unrestrained animals. These bursts are synchronous in the anterior, middle and posterior nerves, despite the metachronal offset in the parapodial oscillations. Reducing the temperature produces a significant decrease in conduction velocity of large efferent units which will increase the offset in time of arrival of the efferent volley at the anterior and posterior parapodia. Therefore the lack of any effect of cooling on the metachronal offset suggests that neuronal conduction time distal to the cuff does not contribute substantially to this component. Phasic bursts of descending activity in large units are also recorded from the cerebro-pedal connectives during swimming.

Isolated brain studies support the hypothesis of a central pattern generator. Although difficult to trigger, phasic bursts of large efferent spikes alternating with bursts of smaller efferent spikes can be seen in some preparations, due to an overall reduction in number of contributing units. The increase in signal-to-noise ratio in isolated brains should make it possible to analyze single unit activity with existing spike separation techniques (Camp and Pinsker, 1979). (This research was supported by NSF grants BNS 76-17480 and 77-25584 to H.P.).

NEUROMUSCULAR JUNCTION

1603 REVERSIBLE ACCUMULATION OF ACETYLCHOLINE RECEPTORS DURING THE DEVELOPMENT OF AN AMPHIBIAN NEUROMUSCULAR JUNCTION IN CELL CULTURE. M. J. Anderson* and F. G. Klier* (SPON. A. Selverston) Salk Institute, San Diego, CA. 92112

Cells from the myotomal muscle and neural tube of the embryos of Xenopus laevis were grown together in culture. Over a period of 1-2 days after adding the nerve cells to established muscle cultures, discrete acetylcholine receptor accumulations could be detected along the path of nerve-muscle contact after staining with tetramethylrhodamine-labelled α-bungarotoxin. Sequential observations on individual muscle cells over a period of 16-20 hours revealed a progressive formation and growth of discrete acetylcholine receptor aggregates at sites of nerve-muscle contact. Examination of identified nerve-contacted cells in the electron microscope further revealed that the larger regions of receptor accumulation corresponded to sites of extensive synaptic differentiation. These regions of cell-contact contained clusters of synaptic vessicles, post-synaptic membrane thickening and an intercellular specialization of the basal lamina. When such cells became denervated, either spontaneously or as a result of severing the developing neurite, the dense receptor accumulations along the nerve decreased in area or disappeared entirely within 16-20 hours. On the basis of these observations it is concluded that aggregates of junctional acetylcholine receptors remain dependant upon some continuing action of the nerve at these early stages of synaptogenesis.

1604 CALCIUM DEPENDENCE OF EVOKED TRANSMITTER RELEASE AT VERY LOW QUANTAL CONTENTS AT THE FROG NEUROMUSCULAR JUNCTION. Roberto Andreu* and Ellen F. Barrett. Dept. Physiol. & Biophys., Sch. Med., Univ. of Miami, Miami, FLA 33101.

At the frog neuromuscular junction a double logarithmic plot of evoked transmitter release vs. extracellular $[Ca^{2+}]$ has an average slope of nearly 4 at low release rates, suggesting that 4 Ca^{2+} might be necessary to activate a release site (Dodge & Rahamimoff, 1967). However, Crawford (1974) reported that when evoked release rates are lowered still further, the relationship between release and extracellular $[Ca^{2+}]$ changes rather suddenly from fourth power to linear. We reexamined the Ca dependence of evoked transmitter release at very low release rates using surface neuromuscular junctions of the frog cutaneous pectoris muscle. The average quantal content (m) of the end-plate potential was reduced to very low levels (between 0.002 and 1) by reducing bath $[Ca^{2+}]$ and adding 2 mM Mn^{2+}, 4 mM Co^{2+} or 10 mM Mg^{2+}. We found that when the motor nerve was stimulated at low frequencies (0.5 - 2 Hz) in Mn^{2+} or Co^{2+} m was proportional to the fourth power of extracellular $[Ca^{2+}]$ down to the lowest m values we could measure, 0.002 to 0.003. Together with Dodge and Rahamimoff's earlier results, this result suggests that some step in the transmitter release pathway has a steep, nonlinear dependence on bath $[Ca^{2+}]$ over more than 3 orders of magnitude of evoked release rate. When 10 mM Mg^{2+} was added to the bath, the lowest m values were markedly higher than the fourth power prediction. Increasing the stimulus frequency to 5 - 20 Hz progressively increased both m and the rate of 'background' release during the interstimulus interval. In the range of low quantal contents studied here, frequency enhancement of both evoked and background release was more pronounced in 10 mM Mg^{2+} or 4 mM Co^{2+} than in 2 mM Mn^{2+}. These results suggest that Mg^{2+} and possibly Co^{2+} are weak activators of transmitter release, so weak that their activating abilities are evident only when normal Ca^{2+}-activated release is greatly reduced. This frequency-dependent enhancement of low release rates in Mg and Co probably accounts for the difference between our and Crawford's results. Supported by NIH grant NS 12404.

1605 THE PARTIAL PURIFICATION OF A NEURONAL FACTOR WHICH AGGREGATES MUSCLE ACETYLCHOLINE RECEPTORS. Hans-Christian Bauer*, Mathew P. Daniels, Sandra Fitzgerald*, Paul Pudimat*, Joav Prives* and Clifford N. Christian*. (SPON: John D. Newman). NIH, Bethesda, MD 20205.

Medium conditioned by contact with NG108-15 clonal neuroblastoma x glioma hybrid cells contains a factor which increases the number of acetylcholine receptor (AChR) aggregates on cultured myotubes (Christian et al. 1978, PNAS 75: 4011-4015). A factor with this activity is also found in the cytoplasmic fraction of the hybrid cells and in the cytoplasmic fraction of embryonic rat brain. AChR aggregation activity was not found in the cytoplasmic fraction of adult rat brain. The AChR aggregation activity of hybrid cell conditioned medium or the cytoplasmic fraction of hybrid cells was concentrated by ultrafiltration, loaded on a Sephacryl S-300 column and eluted in 50mM Tris HCl buffer as a fraction with a molecular weight of from 150,000 to 200,000 daltons. At neutral pH, 80% of the protein in this active fraction bound to a DEAE cellulose column, and AChR aggregation activity was eluted in a linear NaCl gradient at an approximate salt concentration of 300 mM. Further separation on a Sephacryl S-200 column resulted in detectable aggregation activity at 4 μg of protein per ml, indicating purification of the starting material of over 10 fold. In addition, a factor which depressed the number of AChR aggregates on cultured myotubes was separated from the aggregation activity. Part of the receptor aggregation activity in conditioned medium bound to concanavalin-A sepharose and was eluted by α-methylmannoside. Hence a neuronal factor which aggregates myotube AChR appears to be a large, negatively charged glycoprotein.

1606 THE EFFECTS OF PARTIAL DENERVATION AT BIRTH ON THE DEVELOPMENT OF MOTOR UNITS AND MUSCLE FIBERS IN RAT LUMBRICAL MUSCLE. W.J. Betz, J.H. Caldwell*, and R.R. Ribchester*. Dept. Physiol., Univ. Colo. Med. Sch., Denver, CO 80262.

At birth, rat lumbrical muscle fibers are polyneuronally innervated, but by three weeks of age the adult pattern is reached, in which each muscle fiber is innervated by a single motor axon. The loss of polyneuronal innervation through elimination of some synapses results in a reduction in the size of individual motor units innervating the muscle (from about 120 at birth to about 85 in mature muscles). We were interested in knowing to what extent this normal reduction in motor unit size depends upon competition between motor neurons. The lumbrical muscle can be partially denervated at birth in such a way that (in about 1/4 of the muscles) a single motor axon is left innervating the muscle. Partial denervation was accomplished by cutting the lateral plantar nerve; the remaining motor axons to the muscle traveled in the sural nerve. After the animals matured, the number of remaining motor units was determined by in vitro twitch tension recordings. The muscles were then frozen, sectioned, stained, and muscle fiber diameters were measured with a camera lucida attachment. In single motor unit muscles, the number of large diameter muscle fibers provided an estimate of motor unit size. In such cases, the remaining motor unit matured in the absence of competing influences from other motor axons. We measured the size of the single remaining motor unit in 23 such muscles. The mean size (117) was nearly the same as in newborn muscles, which suggests that the normal reduction in motor unit size is the result of competitive interactions between motor axons.

Since the lumbrical muscle at birth contains only 50-60% of the adult number of muscle fibers, we were also interested in the extent to which postnatal production of muscle fibers depends upon the presence of motor axons. To measure this, we counted the total number of muscle fibers in muscles with different numbers of remaining motor units. The results showed the following: 1) In muscles totally denervated at birth, few or no additional muscle fibers were produced. 2) In muscles with only 8 remaining motor units (normal is about 12) mature muscles contained the normal adult number of muscle fibers. 3) The overall relationship between the number of muscle fibers and the number of remaining motor units could be described with reasonable quantitative accuracy by a simple model.

1607 TESTOSTERONE EFFECTS ON CHOLINE ACETYLASE AND ACETYLCHOLINESTERASE IN THE XIIth CRANIAL NERVE AND SYRINGEAL MUSCLES OF THE ZEBRA FINCH. W. Bleisch, V. Luine, F. Nottebohm and B. McEwen. Rockefeller University, New York NY 10021.

The syrinx is the organ of song production in passerine birds and is innervated by the tracheosyringealis branch of the XIIth cranial nerve. In many passerines, song is an important component of male sexual behavior, and it disappears following castration. Recent work shows that the syringeal muscles and the motor neurons which innervate them contain protein receptors for testosterone (T) which are specific and show high affinity (Arnold et al., J. Comp. Neurology 165:487, 1976; Lieberburg & Nottebohm, J. Gen. & Comp. Endo., in press). That these receptors may regulate function in the male Zebra Finch (ZF) is suggested by changes in syringeal weight and acetylcholinesterase (AChE) activity which occur when circulating levels of T are changed (Luine et al., Soc. Neuro. Sci. Abstracts 4:371, 1978).

In this study, we have examined the effects of altered circulating T levels on the tracheosyringealis nerve by measuring nerve choline acetylase (ChAc) and AChE. We have further explored the effects of T on syringeal muscle AChE using sucrose density gradient analysis. Adult male ZFs were castrated and implanted one week later with Silastic capsules containing cholesterol (C) or T. Four to 5 weeks after castration, nerve ChAc levels declined by 34% (from 3678 ± 229 μmoles/gm protein/hr. in intacts to 2444 ± 434 in C treated castrates). AChE levels decreased by 60% (1817 ± 207 μmoles/gm protein/hr. to 734 ± 93). T replacement for 3 to 4 weeks restored AChE activity (1741 ± 323) and partially restored ChAc (3299 ± 176). A similar pattern was observed for AChE and ChAc activities in syringeal muscle.

As in other vertebrates, sucrose density gradient centrifugation of ZF muscle homogenates revealed three peaks of AChE activity. In ZF, these occur at approximately 16 S (the H form) 10 S (M) and 4.5 S (L). Syrinx, larynx and hyoid muscles were homogenized in 1 M NaCl, .5% Triton X-100, .2mM EDTA, 50 mM sodium phosphate, pH 7.2, and applied to a 5 ml. 5-20% sucrose density gradient and centrifuged at 45,000 rpm (189000 x g) for 9 hours. In the syrinx, the L peak predominated strikingly, whereas the activities in the H and L peaks were more similar in the larynx and hyoids. Syringeal AChE was analyzed in intact birds and in castrates treated with T or C in Silastic capsules, as above. In contrast to the marked effects of T depletion on both total and specific AChE, no gross changes in the distribution of AChE activity between the three peaks was observed after castration. (Supported by PHS Grants HD12011 and MH18343, and by RF70095 from the Rockefeller Foundation.)

1608 The Effects of Ca^{++}-Deprivation on Accumulations of Acetylcholine Receptor at the Developing Neuromusclar Junction. Robert J. Bloch* and Joe Henry Steinbach. Neurobiology Laboratory, The Salk Institute, San Diego, Ca. 92112.

One of the earliest events in the formation of the neuromuscular junction is the accumulation of a high density of acetylcholine receptor (AChR) in the postsynaptic membrane. In order to determine what forces are involved in maintaining and maturing these primitive synaptic structures, we have subjected intact muscles excised from rats at different stages of development to disruptive treatments, and, in particular, to removal of Ca^{++}. Sternomastoid muscles were excised and pinned out on plastic dishes. AChR was visualized using a tetramethyl-rhodamine derivative of α-bungarotoxin (R-αBt). Removal of Ca^{++} was accomplished either by culturing in Dulbecco-Vogt modified Eagle's medium prepared free of Ca^{++}, or by using medium buffered with the Ca^{++} chelator, EGTA. When muscles from 16 day embryonic rats were treated for 6 hours at 37° in medium containing decreased concentrations of free Ca^{++} (<200 μM), extensive loss of AChR accumulations was observed. This loss was independent of whether staining with R-αBt was performed before or after incubation in Ca^{++} depleted medium. Muscles deprived of Ca^{++} carried on control levels of protein synthesis. Physically damaging muscles did not cause loss of AChR accumulations. Thus, Ca^{++} deprivation, while it disperses AChR accumulations at the newly formed neuromuscular junction, does not appear to do so by damaging muscle. As a function of developmental time, junctional AChR accumulations become increasingly resistant to the effects of Ca^{++} deprivation. This was already observable with neonatal sternomastoid muscle and was complete by 3 weeks after birth. Our results suggest that newly formed AChR accumulations at the developing neuromuscular junction are less stable than accumulations at mature junctions, and that stabilization occurs relatively rapidly after formation of the junction.

1609 PERTURBATION OF CONFORMATIONAL EQUILIBRIA OF THE MEMBRANE-BOUND CHOLINERGIC RECEPTOR OF TORPEDO INDUCED BY NONCOMPETITIVE ANTAGONISTS. Norman D. Boyd* and Jonathan B. Cohen. Dept. of Pharmacol., Harvard Med. Sch., Boston, MA 02115

The nicotinic cholinergic receptor in postsynaptic membranes isolated from Torpedo electric tissue has been shown to exist in two interconvertible conformations, one binding acetylcholine (AcCh) with low affinity and associated with channel activation (R_{Lo}) and a second binding AcCh with high affinity (R_{Hi}, desensitized conformation). In order to determine the mechanism by which noncompetitive antagonists cause receptor desensitization, we have measured their effects on receptor conformational equilibria in the absence and presence of [^{3}H]-AcCh. In the absence of ligands the fraction (F) of AcCh binding sites existing in the high affinity conformation was equal to 0.17 ± .02, while noncompetitive antagonists caused a concentration dependent stabilization of R_{Hi}. The maximal perturbation (F_{max}) and the ligand concentration (C_{50}) producing a half-maximal perturbation was dependent on the ligand employed. For proadifen C_{50} = 10 μM, F_{max} = 0.78; for histrionicotoxin (HTX), C_{50} = 15 μM, F_{max} = 0.31; and for 2-propanol, C_{50} = 0.8 M, F_{max} = 0.91. Since HTX produced only a small conformational perturbation, its effects on the actions of proadifen and 2-propanol were examined. HTX antagonized the conformational perturbation caused by proadifen alone, while the effects of HTX and 2-propanol were additive. The apparent competition between proadifen and HTX indicate that the receptor perturbation induced by the aromatic amine in the absence of cholinergic ligands results from ligand binding to a specific site, presumably the site identified by equilibrium binding of [^{14}C]-meproadifen (E. Krodel et al., Mol. Pharmacol. 15: 294, 1979). The additivity observed with HTX and 2-propanol indicate a different mode of action for 2-propanol.

The high affinity receptor conformations stabilized by the different noncompetitive antagonists and by AcCh were characterized by: the rate constant for receptor reisomerization upon removal of the stabilizing ligand (k_{rec} = 2.7 x 10^{-3} sec^{-1}); the dissociation constant for [^{3}H]-AcCh (K_{Hi} = 1 nM); the rate constant for the dissociation of [^{3}H]-AcCh-receptor complexes (k_{dis} = 4.3 x 10^{-2} sec^{-1}). On the basis of these criteria a unique high affinity receptor conformation is defined for all stabilizing ligands that is independent of the mechanism of stabilization. (Supported in part by USPHS grants NS 12408, NS00155 and MDAA fellowship to NDB.)

1610 A CORRELATION BETWEEN THE APPEARANCE OF 11 - 19 NM INTERMEMBRANE PARTICLES AND ACETYLCHOLINE SENSITIVITY IN XENOPUS EMBRYONIC MUSCLE. Paul C. Bridgman* and Allan S. Greenberg* (SPON: Y. Nakajima). Dept. Biol. Sci., Purdue Univ., W. Lafayette, IN 47907

The onset of acetylcholine (ACh) sensitivity can occur with surprising speed in Xenopus embryonic muscle (Blackshaw and Warner, Nature 262: 217, 1976). To establish whether ACh receptors (AChRs) in Xenopus are incorporated into the sarcolemma in a physiologically active state or "activated" after incorporation, we correlated ACh sensitivity with the initial appearance of putative AChR (11 - 19 nm) intermembrane particles (IMPs). These studies were conducted both in vivo and in vitro.

In vivo electrophysiological studies confirm previous work (Blackshaw and Warner, 1976; Kullberg, et al, Dev. Biol. 60; 101, 1977) which indicates that the onset of ACh sensitivity in Xenopus embryos occurs between Nieuwkoop and Faber stages 19 and 24. Embryos from which intracellular recordings had been taken were freeze-fractured. We have been able to observe a direct temporal correlation between the appearance of 11 - 19 nm IMPs and ACh sensitivity. Embryos (st. 19 - 24) which did not exhibit ACh sensitivity had particle sizes distributed normally, around a mean value of 8.2 ± 0.3 nm (n = 7). Embryos with sensitivity (st. 20 - 27) had particle size distributions that were skewed to the right because of the increase in number of 11 - 19 nm IMPs. This had the effect of shifting the mean IMP size towards a larger average value (9.7 ± 0.2 nm; n = 13).

We have performed similar experiments on cultured myotome cells, dissociated from state 13 - 17 embryos. Cells were impaled with microelectrodes and tested iontophoretically for ACh sensitivity. Cells gained sensitivity between the corresponding stages 20 - 24 similar to that observed in vivo. Cells tested were photographed for later identification and then processed for freeze-fracturing. Preliminary results from these identified cells indicate that only cells with ACh sensitivity contain large numbers of 11 - 19 nm IMPs. We tentatively conclude that the appearance of 11 - 19 nm IMPs may occur almost simultaneously with the appearance of ACh sensitivity. This lends further support to the present notion that 11 - 19 nm IMPs represent AChRs. In addition, these results suggest that AChRs are physiologically active soon after, if not immediately following, incorporation into the sarcolemma.

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1611 MORPHOLOGY AND ELECTROPHYSIOLOGY OF DYSTROPHIC CHICKEN MUSCLE. Joan S. Bryan* and Michael S. Letinsky. Dept. Physiol., Sch. Med. UCLA, Los Angeles, CA 90024.

Alterations in the morphological and electrophysiological properties of a small wing muscle, the extensor digitorum II, (EDII), have been examined in chickens with inherited muscular dystrophy ranging in age from 5 to 48 wks ex ovo (dystrophic line 455 and control line 454 obtained from Dept. of Avian Sciences, U. of Cal., Davis). These are new dystrophic and control lines with high hatchability and early onset of dystrophy.

We have shown that the EDII is a homogeneously fast twitch muscle having extensive pathological changes at the earliest age examined histologically (7 wks) including: extensive variation in muscle fiber diameter, muscle fiber hypertrophy, increased numbers of muscle fiber nuclei, many of which are centrally located, muscle fiber splitting and increased muscle connective tissue.

For electrophysiology the EDII was removed and continuously perfused with oxygenated chicken ringer,Hepes buffered to pH 7.2 at 21±1 deg.C. Muscle fiber resting potentials from control birds aged 6 to 34 wks were normally distributed with a mean of -77mV and did not change with age. Resting potentials from dystrophic muscle fibers (5 to 48 wks) were bimodally distributed with one mode at -78 mV and another mode at -58 mV. In dystrophic muscle resting potentials began diverging markedly from normal levels at 9 to 11 wks of age. In control chickens from 6 to 13 wks there was a decrease of about 30% in mean input resistance (from 1.3 to 0.8 Mohm) which correlated with about a 40% increase in mean muscle fiber diameter (from about 26 to 35 microns). Fiber diameter increased more rapidly in dystrophic muscles during this period; but in spite of this relative hypertrophy, dystrophic muscle fiber input resistance increased to 225% to 280% of control. A large jump in input resistance occurred in dystrophic muscle at about 7 to 9 wks (from .85 to 2.5 Mohm). Muscle fiber membrane time constants in normal chickens aged 6 to 14 wks averaged about 8 ms and were shorter than time constants in dystrophic fibers of this age range which increased from 18 to 28 ms. Miniature endplate potentials (Mepps) were recorded using intracellular electrodes placed close to endplates located using Hoffman modulation contrast optics. At all ages examined Mepp frequency was lower at dystrophic than at normal endplates. At 5 wks ex ovo Mepp frequency at dystrophic endplates (2.4 per min) was only 45% of the control frequency (5.4 per min); and although Mepp frequency increased with age in dystrophic muscles, it was still only 72% of control by 13 wks (11 per min in dystrophics vs 15 per min in normals). It is significant that this neuronal difference existed prior to functional muscle pathology observed here. Supported by grants from PHS (NS 14417) and from the Muscular Dystrophy Association of America.

1612 SURFACE SPECIALIZATIONS ASSOCIATED WITH HIGH DENSITY ACCUMULATIONS OF ACETYLCHOLINE RECEPTORS IN EMBRYONIC CHICK MUSCLE. Thomas G. Burrage* and Thomas L. Lentz. Section of Cell Biology, Yale Univ. School of Medicine, New Haven, CT 06510.

Staining with horseradish peroxidase-labeled α-bungarotoxin (HRP-α-Btx) reveals small patches of high density accumulations of acetylcholine receptors (AChR) on uninnervated surfaces and at sites of early nerve contact in myogenic cells of embryonic chick latissimus dorsi muscle in vivo (Jacob and Lentz, J. Cell Biol., in press). This study was undertaken to characterize the morphological specializations of these regions of high receptor density using different heavy metal stains. Latissimus dorsi muscles from 10 to 19 day old chick embryos were viewed by electron microscopy after glutaraldehyde and/or osmium fixation and combinations of heavy metal stains. The surface morphology of myogenic cells is demonstrated by osmium tetroxide fixation, uranyl acetate (UA) staining en bloc, and UA and lead citrate poststaining. A sparse, discontinuous external coating representing the precursor of the basement membrane is present on some of the myoblasts and early myotubes. Also, discrete, local specializations similar in location and extent to the HRP-α-Btx stained patches are present on the surfaces of the myogenic cells. These regions usually appear as small elevated ridges 0.1 to 0.5μm in width. The external surface of the patches is coated with a layer of amorphous to very fine textured material sometimes separated from the plasmalemma by a thin clear space. On the internal surface and coextensive with the external layer, dense material is applied immediately adjacent to the plasmalemma. A network of 60A filaments is contiguous with the dense material. The extraneous coating of these patches as well as the submembranous density is stained with ethanolic phosphotungstic acid (E-PTA) and bismuth iodide. E-PTA also stains a prominent band beneath the submembranous density and separated from it by a space. The specialized regions are usually widely separated on uninnervated surfaces of myogenic cells. At sites of early nerve-muscle contact, patches are more numerous and larger. Often the external coating extends a greater distance into the extracellular space forming bridges with a sparse coating on the surface of growing nerve fibers. In more highly developed junctions, the external layer and submembranous density occupy the entire postsynaptic region. This study reveals the presence of external and internal specializations at regions of high AChR density and at sites of initial nerve-muscle contact. These specializations may be involved in the localization or positioning of AChR in the membrane or contain components which establish these areas as preferential sites of nerve contact. (Supported by the Osserman Fellowship of the Myasthenia Gravis Foundation and NSF Grant BNS 78-13729).

1613 COATED VESICLES IN CULTURED MYOTUBES CONTAIN ACETYLCHOLINE RECEPTORS. S. Bursztajn and G.D. Fischbach. Dept. of Pharmacology, Harvard Medical School, Boston, Mass. 02115.

Chick myotubes and spinal cord explants were cocultured on glass coverslips. After myoblasts had fused to form myotubes, spinal cord slices cut from 12 day embryonic cords or isolated neurons dissociated from 8 day ciliary ganglia were added to the muscle cultures. Two to four days later, nerve-muscle synapses were located (within 3 to 5μm) by focal extracellular recording. Electrophysiologically identified sites of transmitter release were photographed at high magnification (X900) and the surrounding area was photographed at low power (X150). Synapses were relocated after the cells were fixed and embedded in Epon 812. They were circled with a diamond object marker (50μm in diameter) and this region was cut out and remounted. A striking feature of the identified synapses was the accumulation of coated vesicles in the muscle cytoplasm near the postsynaptic membrane. In several instances coated vesicles opened on the cell surface and appeared to contribute to the postsynaptic density. In some sections coated vesicles appeared to fuse with or bud off from dilated ampula of the "T" system. Serial sections showed that the postsynaptic membrane was dense and that small clear synaptic vesicles were clustered in the opposed nerve process. Morphometric analysis revealed that there was a 5.5 fold increase in the number of coated vesicles at functional synapses, compared to nonsynaptic nerve-muscle contacts. Coated vesicles did not appear to fill with HRP and ferritin even if these extracellular tracers were present for 3 hrs prior to fixation. This suggests that at least some coated vesicles in myotubes are involved in exocytosis.

The possibility that coated vesicles contain acetylcholine (ACh) receptors was investigated with HRP-αBTX conjugates, after the cell membranes were "permeabilized" with saponin according to the procedures described by Ohtsuki et al. (Biol. Cellul., 31:119-126, 1978) and Fambrough and Devreotes (J. Cell Biol. 237-244, 1978). The histochemical HRP reaction yields a fine precipitate that can be localized with a high degree of resolution. Treated cells were not stained with uranyl acetate. Reaction product was seen over some but not all of the coated vesicles. Reaction product was also found in segments of Golgi cisternae. No staining was evident in control cultures preincubated in unconjugated αBTX or curare. These results suggest that ACh receptors are inserted into the plasma membrane via coated vesicles.

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1614 THE SIZE OF MOTOR UNITS DURING POSTNATAL DEVELOPMENT OF LUMBRICAL MUSCLE. J.H. Caldwell*, W.J. Betz, and R.R. Ribchester* (SPON: A.R. Martin). Dept. Physiol., Univ. Colo. Med. Sch., Denver, CO 80262.

The number of muscle fibers innervated by individual motor neurons (motor unit size) was measured in lumbrical muscles of rats aged 0-28 days, during the period of elimination of polyneuronal innervation. Motor unit sizes were determined from twitch tension measurements combined with muscle fiber counts made from histological sections of the muscles. The relative tensions contributed by individual motor units declined from about 25% of the total tension at birth, to about 9% at 28 days of age. Intracellular recordings showed that part of this decrease reflected the elimination of synapses from polyneuronally innervated muscle fibers.

During the same period, however, new muscle fibers were produced. The total number of muscle fibers present increased from about 550 fibers at birth to about 950 fibers in mature muscles. These two processes were offsetting: some synapses were eliminated (from polyneuronally innervated fibers) while simultaneously others were formed de novo (on newly produced muscle fibers). Quantitative measurements showed that for the first ten days after birth, there was little change in motor unit size. Thereafter production of new muscle fibers ceased, and with the final elimination of synapses from polyneuronally-innervated muscle fibers, motor unit size decreased to the adult level. It is concluded that during early postnatal development, a lumbrical motor neuron maintains a nearly constant number of synapses, but extensively reorganizes its synaptic field, retracting synapses from some muscle fibers, while forming new synapses with other fibers.

1615 THE EFFECT OF TEMPERATURE ON MEPP AMPLITUDE DISTRIBUTIONS AT THE MOUSE DIAPHRAGM. C.G. Carlson*, C.G. Muniak*, and F. Llados* (SPON: C. Edwards). Dept. of Physiology, Upstate Medical Center, Syracuse, NY 13210

Kriebel, Llados, and Matteson (J. Physiol. 262, 1976) observed a class of small spontaneous potentials (subminiature endplate potentials, s-MEPPs) in muscle fibers of the mouse diaphragm. These s-MEPPs are generally 1/10 to 1/15 the amplitude of normal amplitude MEPPs (major mode MEPPs, m-mode), and are relatively resistant to the depressive effects of Botulinum toxin on spontaneous frequency. In these experiments we determined the effect of low temperature on the relative frequencies of s-MEPPs and m-mode MEPPs.

Between 11 and 30°C, the overall MEPP frequency (s-MEPPs and m-mode MEPPs combined) showed a positive temperature dependence (c.f., Liley, J. Physiol. 132, 1956). The MEPP frequency increased from about 0.5 sec^{-1} at 15°C to 1.4 sec^{-1} at 25°C. At temperatures above 20°C, the MEPP distributions were similar to those observed at higher temperatures (32-40°C). MEPP amplitudes 1 to 4 times the s-MEPP showed distributions that skewed towards the smaller MEPP amplitudes (the "skew class"). MEPP amplitudes 5 to 20 times the s-MEPP showed distributions that were bell-shaped (the "bell-shaped" or m-mode class). Below 16 to 17°C, the s-MEPPs and corresponding "skew class" of MEPPs virtually disappeared in relation to the number of MEPPs in the bell-shaped part of the distribution. This indicates that in the range of temperatures from 11-25°C, the frequencies of s-MEPPs and "skew class" MEPPs are more sensitive to a reduction in temperature than the frequency of the larger MEPPs in the "bell-shaped" class. Although the MEPPs at lower temperatures (11-18°C) were slower in time course than MEPPs at higher temperatures (23-27°C), there was no obvious effect of temperature on either the s-MEPP or m-mode MEPP amplitude.

In some cells an optimal temperature for the release of s-MEPPs was seen between 25 and 30°C. This is consistent with the observation that the percentage of s-MEPPs at 32-34° was greater than at 38°C. These results provide further evidence that s-MEPPs and m-mode MEPPs are released by relatively independent mechanisms. (Supported by NIH Grant #11-1524D)

1616 THE EFFECT OF A SODIUM IONOPHORE, MONENSIN, ON NEURO-MUSCULAR TRANSMISSION. Milton P. Charlton, Brian J. Farnell* and Harold L. Atwood. Zoology Dept., University of Toronto, Toronto, Canada, M5S 1A1.

Mounting evidence indicates that intracellular Na^+ may play a role in modulating the secretion of transmitters at synapses. Increases in intracellular Na^+ caused by Na^+ pump inhibitors (Proc.Roy.Soc.B, 170,381-399), prolonged stimulation (Br.Res., 100, 198-204), direct injection (Br. Res., 134, 367-371) or loading by liposomes (P.N.A.S.USA, 75, 5214-5216) can result in increases in both spontaneous and evoked release. In this study Na^+ loading was attempted by the use of a carboxylic Na^+ ionophore, Monensin (Mon), which shows moderate selectivity against K^+ and very little complexation with Ca^{++} (Ann.Rev. Biochem., 45, 501-530). Mon (3-7uM) caused an increase in excitatory post synaptic potentials (EPSP) of up to 250% within 15 min of application to crayfish opener, stretcher and abdominal extensor muscles (12-15°C). EPSP's were not increased by Mon in saline containing only 1/3 [Na^+]. It is therefore likely that Mon is effective by increasing intracellular [Na^+]. The effect of the ionophore was reversible following washout but return to normal transmission took longer than the initial facilitation.

The effect of Mon on spontaneous release of transmitter was tested in the frog cutaneous pectoralis muscle by recording miniature endplate potentials (mepps). The frequency of mepps was increased by over 2000% during an 8 min application of Mon (6 uM, 21°C). Mepp frequency was still increased 1000% 13 min after washout of Mon. The membrane potential of muscle cells in either animal was not affected by Mon.

Compared to other techniques for Na^+ loading, the application of Mon is simple, rapid, reversible, does not involve poisons, does not require stimulation and can be used on cells of all sizes. The results confirm the observation that intracellular Na^+ can modulate transmitter release and indicate that Mon may be a valuable tool in the study of the mechanism of this phenomenon.

1617 SHIFTS IN THE MULTIPLE FORMS OF ACETYLCHOLINESTERASE IN CHICK EMBRYONIC SKELETAL MUSCLE IN VIVO AND IN VITRO. C. Michael Cisson, Carole H. McQuarrie*, Mark G. McNamee, and Barry W. Wilson. University of California, Davis, CA 95616

Experiments continue in our laboratory to understand the progression of development of multiple forms of acetylcholinesterase (AChE). The multiple forms in 11- and 18-day-old chick embryonic pectoral muscle and cultured muscle grown aneurally for 10 days were determined by centrifugation on a linear 5-20% sucrose gradient. Three forms were found in embryonic muscle in vivo: 7S (6.8±0.3), 11S (11.2±0.8), and 20S (20.4±0.6). The specific activity of AChE did not differ between 11- and 18-day-old muscle, but there was a shift in distribution of AChE activity among the three forms:

Embryo Age	% Total Activity 7S	11S	20S	N
11D	60.4±3.1	31.6±4.5	8.0±4.2	4
18D	46.9±4.4	19.7±2.7	33.5±3.9	4

Only the 7S and 11S forms were found in muscle cultured from 11-day embryos. 76.2±3.8% of the AChE activity was found in the 7S form and 23.8±3.8% in the 11S (N = 6). Newly synthesized AChE in DFP-treated cells initially appeared as a 7S form; a distinct 11S form did not appear until 2 hours after treatment.

The addition of two proteolytic enzyme inhibitors, pepstatin and phenylmethylsulfonyl fluoride, to the homogenization buffer (1 M NaCl, 0.5% Triton X-100, 50 mM Tris, 0.2 mM EDTA) did not alter the distribution of these forms.

AChE activity found in embryo plasma sedimented at 11S and that released into the muscle culture medium sedimented at 9S. In one experiment, a small amount of non-specific ChE appeared in 18-day-old muscle sedimenting at approximately 5.5S, 7.5S and 10S, and comprised 24% of the total ChE activity. Non-specific ChE was not detected in either 11-day embryo or cultured muscle.

The results reinforce the idea that there is a shift from smaller to larger molecular forms of AChE during development and synthesis and that functional innervation is required to maintain a 20S form in embryonic skeletal muscle. These findings will be compared to previous work from this lab examining electrophoretic multiple forms of AChE in chick muscle and plasma. (Supported in part by NIH grant ES 00202).

1618 DEVELOPMENT OF SYNAPTIC SPECIALIZATIONS AT SITES OF NERVE-MUSCLE CONTACT IN CULTURE: DEPENDENCE ON NERVE TYPE. M.W. Cohen and P.R. Weldon*. Dept. Physiol., McGill Univ., Montreal, Quebec.

In cultures of myotomal muscle and spinal cord (SC) derived from Xenopus embryos SC neurites can trigger the development of a high density of ACh receptors along the paths of nerve-muscle contact (1,2) and electron microscopy reveals the presence of typical synaptic specializations at many of the neuromuscular contacts (3,4). We have now examined whether neurites of dorsal root ganglia (DRG) and sympathetic ganglia (SG) interact with the cultured muscle cells in a similar fashion.

Muscle cells were plated at the same time as the nerve or shortly after the onset of neuritic outgrowth. After 2-4 days, when nerve-muscle contacts were apparent, ACh receptors were stained with fluorescent α-bungarotoxin in order to be able to visualize sites of high receptor density. Over 70% of SC-contacted muscle cells displayed some stain along the paths of contact whereas the corresponding values of DRG- and SG-contacted muscle cells were 9% and 5% respectively. Similar differences between SC- and SG-contacted muscle cells were also seen when both types of nerve were present together in the same culture chamber, thereby indicating that the SG explants did not adversely alter the culture medium. The few examples of stain associated with DRG- and SG-muscle contacts resembled the characteristic patches of stain seen on non-contacted muscle cells rather than the unique long bands of stain seen along some of the SC-muscle contacts. It is concluded that the accumulation of ACh receptors at SC-muscle contacts is triggered by a specific neural property which is lacking in the DRG and SG neurites.

Cultures containing muscle and DRG or SG explants were also examined by electron microscopy. Although the neurites approached to within 100 Å of the muscle cells synaptic specializations, particularly a thickened sarcolemma and an overlying basal lamina, were rarely observed at these nerve-muscle contacts. The much more frequent occurrence of these synaptic specializations at SC-muscle contacts (4) indicates that the SC neurites possess a specific property which is important for the establishment of synaptic contacts with muscle. This property is presumably the same as that which triggers ACh receptor accumulation at SC-muscle contacts.

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1619 SYNAPSE FORMATION AT OLD AND DE NOVO ENDPLATE SITES. Ronald Ding* (SPON: A. D. Grinnell). Dept. Physiology, Sch. Med., UCLA, Los Angeles, CA 90024.

Re-innervation of old endplate sites and formation of de novo junctions at ectopic sites were studied in the cutaneous pectoris of Rana pipiens using electrophysiology and light microscopy. Each muscle fiber studied electrophysiologically was marked intracellularly with Chicago Blue 6B so that it could be identified later after staining the entire muscle for nerve terminals using nitroblue tetrazolium and for cholinesterase by the method of Karnovsky (J. Cell Biol. 23:217-232, 1964). Seven days or more after nerve crush, nerve terminal could be seen regrowing into and quickly filling old synaptic gutters. The nerve terminal had its fastest growth phase at postoperative times from 10 to 15 days. Up to 30 days after nerve crush, miniature endplate potential (mepp) frequency per unit length of nerve terminal was appreciably lower than normal. Preliminary evidence showed an initial increase in quantal content per unit length of terminal, followed by a decrease and then another increase. Data obtained at postoperative delays of 30 days or more in general showed that the staining pattern of nerve terminal, mepp frequency and quantal content per unit length of terminal were all roughly equivalent to normal.

In other animals when the nerve was transposed to an endplate-free region of muscle and the endplate-containing portion discarded, de novo synapse formation occurred in ectopic regions. Compared to normal of re-innervated junctions these de novo junctions were more branched, were not as consistently aligned with the long axis of the muscle fibers, and were associated with more but smaller cholinesterase spots per muscle fiber. This distinct morphology persists for as long as 2 years. Mepps, endplate potentials, and contractions were all first seen at 4 weeks postoperative. Physiological similarities and differences between these synapses and those observed at re-innervated endplates will be discussed.

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1620 Differential Effects of Acetylcholine and Edrophonium on the Motor Nerve Ending. Kenneth L. Dretchen, R. Anthony Howard*, Frank G. Standaert, and Carolyn S. Rabe*. Dept. of Pharmacology, Sch. Med., Georgetown Univ., Washington, D.C. 20007.

The effects of acetylcholine and edrophonium on motor nerve endings were studied in the in vivo cat soleus nerve muscle preparation. Administration of edrophonium, 1.0 ug/kg i.a., resulted in the appearance of stimulus-bound repetitive activity (SBR) and druginduced activity recorded from the nerve. These neural responses were transmitted to the muscle; the former resulted in enhanced force of muscle contraction, the latter resulted in muscle fasciculations. The SBR persisted for 96 seconds and resulted in potentiation of contractile strength to 260% of control. Acetylcholine administered at 100 ug/kg i.a. also resulted in SBR and drug-induced activity though accompanied by a brief decrease in the force of muscle contraction. The SBR following acetylcholine persisted for only 15 sec. Pretreatment with tetrodotoxin, 30 ng/kg i.a., of 30 to 60 seconds duration, resulted in complete elimination of SBR and drug-induced activity following acetylcholine but only partial decreases is SBR, druginduced activity, and the enhanced contractile strength following edrophonium. Similar pretreatment with the calcium antagonists verapamil (10-100 ug/kg), D-600 (10-100 ug/kg) and $LaCl_3$ (0.1-10 ug/kg) resulted in dose-releated decreases in neural and muscle responses to edrophonium but had no effect on responses to acetylcholine. In order to study the interaction of these two compounds with cAMP, they were tested following administration of theophylline, a phosphodiesterase inhibitor. Theophylline (10-100 ug/kg) produced a dose-related increase in the neural and muscle response to edrophonium while it had no effect on responses to acetylcholine. It is concluded that the observed acetylcholine response probably results from a sodium depolarization of the motor nerve terminal while the edrophonium responses probably result from both a sodium depolarization and a cyclic AMP-mediated influx of calcium into the nerve ending.

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1621 COMPARISONS OF THE IMMUNOLOGICAL PROPERTIES OF JUNCTIONAL AND EXTRAJUNCTIONAL RECEPTOR. D. S. Dwyer, R. J. Bradley and G. E. Kemp*. Neurosciences Program, University of Alabama in Birmingham, Birmingham, Alabama 35294.

Differences have been detected between junctional and extrajunctional acetylcholine receptor (AChR) from rats using serum from myasthenic patients (Weinberg and Hall (1979) PNAS 76:504). Titers against extrajunctional AChR were an average of 1.5 times higher than titers against junctional AChR. We have investigated this problem using three separate assays: inhibition of ^{125}I-α-bungarotoxin binding, conventional immunoprecipitation and a modified immunoprecipitation developed in this laboratory (for details see: Dwyer et al., Clin. Exp. Imm., in press). Characterization with serum from 10 patients has been conducted. Our data indicate no systematic differences between the two types of receptor when titers are compared. Several patients show slightly higher titers against junctional AChR; the reverse is also found.

Inhibition assays detect differences between receptor. Unlike previous reports (Almon and Appel (1975) Biochim. Biophys. Acta 393:66), we can measure inhibition with junctional receptor in 50% of patients tested compared to 90% able to inhibit against denervated AChR. Maximum inhibition of toxin binding has been measured as high as 75-80% with some sera. When saturation curves are plotted with percent inhibition vs. amount of serum, both the slope of the line and the maximal inhibition is in most cases greater against extrajunctional receptor. This suggests that there is antibody in most myasthenic sera that recognizes a determinant on extrajunctional AChR close to the toxin binding site.

We have also observed that a certain fraction, approximately 10-15%, of either junctional or extrajunctional AChR is not precipitated by myasthenic IgG even at saturating antibody concentrations. This phenomenon occurs even when the modified procedure is used, which corrects for antibody precluded from binding AChR by the toxin label. This appears to be a consequence of microheterogeneity of AChR itself. Whether this heterogeneity exists in intact membranes or whether it occurs as a consequence of tissue disruption is not known.

1622 MULTIPLE FORMS OF ACETYLCHOLINESTERASE IN QUAIL MUSCLE CELL CULTURE. M.R. Emmerling*, C.D. Johnson and B.H. Lipton*. (Spon: M.D. Bownds) Neuroscience Program, Depts. of Anatomy and Zoology, University of Wisconsin, Madison, WI 53706.

The presence of multiple molecular forms of acetylcholinesterase (AChE) was examined in muscle cell cultures derived from pectoral muscle of 9 day quail (Coturnix coturnix japonica) embryos. Muscle cells were scraped from tissue culture plates, homogenized in a 0.02 M borate buffer (pH 8.8) containing 1M NaCl and 0.5% Triton X-100. The homogenate was centrifuged for 30 min. at 45,000g, the soluble AChE in the supernatant collected and analyzed by velocity sedimentation on a linear sucrose gradient containing 0.02 M borate (pH 8.8), 1M NaCl and 0.5% Triton X-100. Assay of gradient fractions for AChE activity revealed three major peaks with sedimentation values of 21, 13 and 6.5 S. The 21S form made up to 15% of total AChE activity on the gradient. When fractions containing peaks of activity were re-run, they sedimented to their original position and gave rise to no new enzyme forms. Both culture medium and chick muscle cell cultures did not possess a large form of AChE or peaks of activity coincident with the quail forms.

AChE isolated from quail muscle in vivo was found to possess three forms which correspond to the forms present in cultured quail muscle cells. Experiments were performed to compare the in vivo and in vitro 21S forms. When the two enzymes were sedimented in gradients containing reduced NaCl concentrations (0, 0.2, and 0.4M), both showed a tendency towards aggregation as reflected in broader peaks and higher S values. In addition both 21S forms were collagenase sensitive and acquired an increased sedimentation value of 22S when treated with the enzyme. These observations indicate that the in vivo and in vitro 21S AChE are similar and suggest the presence of a collagen-like tail on the enzyme.

Studies are underway to ascertain the relationship of the 21S form to the neuromuscular synapse and synaptogenesis both in vivo and in vitro

1623 MECHANISM OF NEUROMUSCULAR BLOCK BY STREPTOMYCIN. Jerry M. Farley*, Chau H. Wu and T. Narahashi. Dept. of Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611.

Aminoglycoside antibiotics are known to block neuromuscular transmission, but the mechanism of action is not clearly understood. Both pre- and postsynaptic blocking mechanisms have been implicated, but their relative contributions to the neuromuscular block have not been quantitatively determined. The effects of streptomycin on end-plate currents (EPC) were studied using the two microelectrode voltage clamp technique. Frog cutaneous pectoris muscles were used in all experiments. The EPC was decreased in amplitude by streptomycin at a concentration of 3×10^{-5} to 3×10^{-4}M. The linearity of the current-voltage relationship was not appreciably altered by streptomycin, although slight curvature might occur especially at large negative potentials. The EPC was slightly prolonged in time course; for example at -70 mV, the time constant of decay was increased from 1.31 to 1.54 msec by 3×10^{-4} streptomycin. No frequency dependent block was observed. The ED_{50} value for the block of EPC's under conditions of high quantal content was estimated to be 8.5×10^{-5}M. Under conditions of low quantal content in the presence of high magnesium and low calcium, 3×10^{-5}M streptomycin decreased the quantal content to 0.51 ± 0.03 (Mean ± SEM) of the control. At this concentration the miniature EPC was also decreased to 0.80 ± 0.04 of the control. We conclude that streptomycin blocks neuromuscular transmission by both pre- and postsynaptic effects. Under conditions of low quantal content the presynaptic block predominates. The characteristics of the presynaptic block resemble those of magnesium. The characteristics of the postsynaptic block resemble those of curare, and either the receptor or the ionic channel, or both are blocked. This study was supported by NIH grant NS14145.

1624 TRYPTAMINE-INDUCED ALTERATIONS OF ACETYLCHOLINE RELEASE AT A NEUROMUSCULAR JUNCTION. Richard N. Friedman* (SPON: R. Rahamimoff). Dept. Physiol. Hebrew Univ.-Hadassah Med. Sch., Jerusalem, Israel.

Tryptamine, which is endogenous in several invertebrate and vertebrate nervous systems, has a concentration-dependent, biphasic effect on excitatory endplate potentials (EPPs) evoked at a lobster neuromuscular junction where glutamate is the putative excitatory neurotransmitter. Tryptamine also reduced GABA mediated inhibitory postsynaptic potential amplitudes (Friedman, et al.).

In the present study, I used conventional electrophysiological techniques to examine the effects of tryptamine on cholinergic transmission in the frog sartorius nerve-muscle preparation bathed in a modified (0.4mM Ca^{++}) Ringer's solution. Tryptamine (0.1-0.5μM) caused an increase in EPP amplitudes. Since the average miniature EPP amplitude was virtually unchanged, it seems that the action of tryptamine is a presynaptic effect which increases the mean number of quanta liberated by the nerve impulse. Higher tryptamine concentrations (≥10μM) depressed average EPP amplitudes. Both effects were reversible upon washing. These results indicate that the effects of tryptamine at a cholinergic synapse are similar to those observed at presumably glutamatergic synapses of the lobster neuromuscular junction.

Friedman, R.N., Shank, R.P., and Freeman, A.R. Brain Res. (submitted).

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1625 ULTRASTRUCTURE OF LOBSTER NEUROMUSCULAR JUNCTIONS TREATED WITH BLACK WIDOW SPIDER VENOM. L.C. Fritz*, H.L. Atwood and S.S. Jahromi*. Rockefeller University, New York, NY 10021 and Dept. of Zoology, University of Toronto, Toronto, M5S 1A1 Ontario, Canada.

Lobster stretcher muscles were treated with black widow spider venom (BWSV) and the resulting physiological and morphological effects were examined. Application of BWSV causes potentiation of EPSP's and IPSP's and a massive increase in the frequency of spontaneous miniature EPSP's. With time, evoked potentials are abolished, miniature EPSP frequency decreases to zero, and the synapse becomes electrically quiet except for large slow spontaneous events ("giant minis") [Kawai et al, J. Gen. Physiol. (1972) 650-664]. The effects of BWSV on miniature EPSP frequency are abolished if Ca^{++} is replaced by Co^{++} in the bathing solution.

Lobster preparations were fixed for electron microscopy after intermediate (~ 15 min) and long (~ 1 hr) periods of BWSV exposure. Terminals treated for intermediate periods (while miniature EPSP frequency was still high) contained vesicles, but these vesicles were not localized to release zones as they are in control preparations. Furthermore, many instances of vesicle clumping and vesicle-vesicle fusion were seen. After long exposure to BWSV (after miniature EPSP frequency fell to zero), most terminals were depleted of vesicles, and large infoldings of the plasmalemma were evident. In addition, terminal mitochondria were grossly swollen.

The results suggest that BWSV causes an influx of calcium into the nerve terminal leading to vesicle dispersion, vesicle-vesicle fusion, vesicle exocytosis and mitochondrial swelling. "Giant minis" may be caused by the release of clumped or prefused vesicles. The morphological results are similar to those seen following BWSV treatment of vertebrate neuromuscular junctions, even though the effects in crustaceans are mediated by a different venom component than are the effects in vertebrates [Fritz et al, Soc. for Neurosci. Abs. (1978) 4, 369].

1626 β-Bungarotoxin Stimulates Acetylcholine Synthesis in Rat Diaphragm. Cameron B. Gundersen, Michael W. Newton*, Donald J. Jenden. Dept. Pharmacology, UCLA School of Medicine, Los Angeles CA 90024

β-Bungarotoxin (β-Btx) is a presynaptically acting polypeptide neurotoxin with Ca^{++}-dependent phospholipase A activity. We have observed that β-Btx (140 ng/ml) caused a large increase in tissue levels of acetylcholine (ACh) in rat hemidiaphragms. Relative to control values (1.3 pmol/mg wet wgt), a 100% increase in tissue ACh was measured within 45 min of toxin exposure of an unstimulated muscle in the absence of a cholinesterase inhibitor. Subsequent studies indicate that uptake of extracellular choline (Ch) and Ca^{++} are both necessary to produce the rise in tissue ACh content. Fan-shaped segments of hemidiaphragm (wet wgt: 50-90 mg) of 90-150 g male rats were treated for 30 min with β-Btx (140 ng/ml) in an eserinized (15 μM) Krebs-bicarbonate medium at 37°C. After three 10 min periods of indirect (10 Hz) stimulation and a final 15 min rest period the released ACh and the tissue ACh were assayed by gas chromatography mass spectrometry. When $[^2H_4]$-Ch (1 μM) was added to the Krebs medium, very little label was incorporated into ACh in either toxin treated or control diaphragms. This may be due to the dilution of labelled precursor by endogenous Ch, which is released by diaphragm at rates in excess of 1.0 pmol min^{-1}-mg wet wgt^{-1}. The average Ch concentration of diaphragm is 50 pmol/mg wet wgt. Using $[^2H_4]$-Ch (10 μM), 55 pmol of $[^2H_4]$-ACh (0.74 pmol per mg wet wgt) was retained by control preparations, while 249 pmol of $[^2H_4]$-ACh (4.1 pmol per mg wet wgt) was present in toxin treated samples. Total ACh levels (pmol/mg wet wgt) were 9.4 in toxin treated vs 2.3 in controls. Hemicholinium-3 (10 μM) blocked the increase in tissue levels of ACh caused by β-Btx and reduced stores to 0.7 pmol per mg wet wgt. When Ca^{++}-free Krebs supplemented with $SrCl_2$ (2 mM) or EGTA (1 mM) was used, β-Btx did not cause an increase in tissue ACh levels. Evoked ACh release in both the $SrCl_2$ and EGTA containing media was inhibited. We conclude that external sources of Ch and Ca^{++}, of which the latter may be necessary for the toxin phospholipase activity, are required for the increased ACh levels following β-Btx treatment of the rat diaphragm. (Supported by USPHS grants NS-05753 and MH-17691).

1627 ENHANCEMENT OF TRANSMISSION AT CHRONICALLY DISUSED NEUROMUSCULAR JUNCTIONS IN RATS: EVIDENCE FOR FUNCTIONAL END-PLATE ENLARGEMENT. G.L. Harris* and W.D. Snider* (SPON: J.N. Weakly). Dept. Physiol. Sch. Med., U. of N.C., Chapel Hill, N.C. 27514.

Recent histological studies have shown that chronic conduction block of a muscle nerve produces sprouting of the motor nerve terminal and an increase in end-plate size (Brown, M.C. and Ironton, R., Nature 265: 459, 1977., Pestronk, A., and Drachman, D.B., Science 199: 1223, 1978). If the junctional enlargement were functional, then one might expect a corresponding increase in the mean quantum content (m) and the frequency of spontaneous miniature end-plate potentials (m.e.p.p.s) both of which have been shown in the frog to be positively correlated with end-plate area (Kuno, M. et al., J. Physiol., Lond. 213: 545, 1971.). To test whether these parameters are increased following the elimination of muscle activity (disuse), we made comparisons of synaptic function between disused and normal (contralateral) soleus muscles in rats.

Muscle disuse was effected by implanting tetrodotoxin (TTX) impregnated silicone cuffs around the sciatic nerve. Conduction block was confirmed by the absence of the toe-spreading reflex on the cuff side. A quantal analysis and determination of m.e.p.p. frequency were made in excised muscle-nerve preparations after 8-9 days of conduction block. The muscles were pinned side by side in a chamber and superfused with an oxygenated, low calcium and high magnesium buffered saline solution. Records of 200 evoked end-plate potentials (0.5/sec) and approximately 50 seconds of spontaneous m.e.p.p.s were obtained alternately from disused and control fibers. For each muscle, 5-7 junctions were examined.

In the seven animals studied, the mean m value from the disused side was significantly greater than that from the control side ($P < 0.01$, by t-test for matched pairs), the average difference being 40%. In five animals from which m.e.p.p. data were obtained, the mean frequency was also significantly greater on the disused side ($P < 0.05$), the average difference being 80%. In five additional animals, the effect of a control cuff (no TTX) was similarly determined. No significant differences in m or m.e.p.p. frequency between the two sides were noted.

We conclude that chronic disuse leads to an increased evoked release of transmitter as well as an increased spontaneous release of quanta. This is consistent with the hypothesis that under such circumstances, the functional end-plate area becomes enlarged.

(This work was supported by USPHS grants NS 11132 and NS 10319)

1628 ANALYSIS OF MINIATURE ENDPLATE CURRENTS IN THE FROG NEUROMUSCULAR JUNCTION UNDER PHYSIOLOGICAL CONDITIONS. Edward G. Henderson and Linda S. Reynolds*, Dept. of Pharmacol., U. Conn. Health Center, Farmington, CT 06032.

Frog sartorius and cutaneous pectoris endplate membranes were voltage clamped by standard techniques in normal phosphate buffered Ringer solution at room temperature (20-22°C). Under these conditions, peak miniature endplate current (mepc) amplitude as a function of holding potential (V_m) was found to be non-linear and discontinuous between negative and positive V_m's. In fact, average mepc amplitudes as a function of V_m (-20 to -100mV) could be fit by a straight line with an average slope of $1x10^{-8}$mho, while those between +10 and +60mV were fit by a different linear regression line of average slope $2.5x10^{-8}$mho. Extrapolation of the regression line for net outward current to negative V_m's predicted a reversal potential (V_r) between -15 to -30mV and an intersection with the net inward current curve at potentials more negative than -60mV. Extrapolation of the regression line for net inward current predicted intersection with the voltage axis at about +60mV. The mepc decay under these conditions was found to be voltage dependent in a fashion similar to that shown by others (i.e. $\alpha(-90mV)=0.40\pm.02msec^{-1}$; $\alpha(+40mV)=.92\pm.12msec^{-1}$). The addition of neostigmine ($3x10^{-6}$M) to the Ringer solution or prior exposure of the muscles to glycerol (400-500mM) produced linear mepc-Vm (-60 to -40mV) relationships with slopes between 3.2 and $4.7x10^{-8}$mho's with a typical reduced slope at more hyperpolarized V_m's. V_r's in neostigmine or glycerol treated preparations obtained by the intercept of regression lines were between -5 and -3mV. Endplate currents (EPC's) in Mg^{++}-treated muscles also provided linear EPC-V_m (-80 to +40mV) relationships with slopes of the order of $20x10^{-8}$mho's and an average E_R of -5mV. These results suggest for MEPC's under physiological conditions (i.e. when cleft acetylcholine concentration and frequency of release are low) that a discrete E_R is unobtainable and that inward and outward conductances are distinguishable. (Supported by NS12563.)

1629 SYNAPTIC EFFECTIVENESS AND MOTOR UNIT SIZE: EVIDENCE FOR PHYSIOLOGICAL PLASTICITY IN TRANSMITTER RELEASE AT NEUROMUSCULAR JUNCTIONS. Albert A. Herrera* and Alan D. Grinnell (SPON: Alison J. Longley). Dept. Biol., UCLA, Los Angeles, CA 90024.

An important factor in synapse elimination at the neuromuscular junction during development may be the extent to which a particular nerve terminal is supported by its soma. This level of support may affect both the ability of a terminal to withstand elimination and the efficacy of transmission by that terminal. The support each terminal receives may in turn depend on the total number of terminals supported by that soma, i.e., motor unit size. We have partially tested these hypotheses by assessing the effects of changing motor unit size on synaptic effectiveness.

The left sartorius muscle of anesthetized adult Rana pipiens was exposed, the sartorius nerve crushed, and a longitudinal cut made through the sartorius so as to remove the fibers in the lateral half of the muscle. When the motor axons regenerated, they would encounter only the intact medial half of the muscle, containing approximately half the normal number of fibers. Control operations, where the nerve was crushed but the muscle left intact, were also performed. After 8 - 11 weeks synaptic strength was tested in two ways.

First, we measured the tension of nerve-evoked twitches while lowering $[Ca^{++}]$ in the Ringer from normal (1.8 mM) to 1.0 or 0.6 mM. Reduction of tension occurs when all the junctions on some fibers fall below threshold. In a normal sartorius muscle, reducing Ca to 1.0 mM causes tension to drop to 42% of tension in 1.8 mM. Lowering Ca to 0.6 mM causes a drop to 5%. Control muscles in which the nerve was crushed followed by reinnervation of the whole muscle behaved similarly. However, the experimental muscles with reduced motor unit size showed enhanced synaptic strength (75% and 19% in 1.0 and 0.6 mM, respectively).

Secondly, to test if the release properties of these stronger terminals were inherently different, we measured quantal content in Ringer containing 0.3 mM Ca and 1 mM Mg. The junctions were marked by intracellular dye injection for identification and analysis of junctional morphology in the light and electron microscope. The experimental muscles showed an approximately 5-fold increase in the number of quanta released per 100 µm of terminal length as compared to normal muscles.

We conclude that there can be pronounced physiological plasticity in the release of transmitter at neuromuscular junctions, with motor unit size an important regulating factor. It is possible that competition between synapses on the same fiber, or other variables, will also prove to be important.

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1630 ASYMMETRY IN THE ACETYLCHOLINE CHANNEL. Richard Horn, Malcolm S. Brodwick* and W. Daryl Dickey*. Dept. Physiol. and Biophys., UTMB, Galveston, TX 77550.

The quaternary lidocaine derivative QX-314 blocks open ACh channels in a voltage-dependent manner from the external membrane surface of frog muscle (Neher and Steinback, 1978). We have applied QX-314 to both internal and external membrane surfaces in order to determine if its blocking action is equivalent from each side. The thighs of neonatal rats were dissociated into myoblasts with 0.025% trypsin and grown under tissue culture conditions. The myoblasts fused to form multinucleate myotubes, which were treated with 10^{-8}M vinblastine to form 60-130 µm spherical myoballs. The myoballs were sucked onto the tip of a glass pipette, the membrane was ruptured, and the cell was internally dialyzed (Lee, K., et al., 1978). The internal and external solutions were approximately symmetrical, each containing 85mM Na aspartate, 150mM sucrose, 5mM HEPES. The external solution had 1.5mM $CaCl_2$, the internal 2mM EGTA. The adequacy of the internal dialysis was confirmed by the fact that the resting potential, Na channel reversal potential, and ACh reversal potential were all approximately 0 mV. In many cells the series resistance through the suction pipette was greater than the membrane resistance. Therefore a microelectrode was used to record membrane potential, V_m. The cells were voltage-clamped and ACh was applied iontophoretically. Both V_m ramps and V_m jumps were applied to the cell during the action of ACh. External QX-314 (0.1mM) produced a V_m and time-dependent block of the open ACh channel. Internal QX-314 (1.0mM) produced a frequency-dependent block of the Na channel without affecting current in the ACh channel. This result suggests that a barrier prevents internal QX-314 from reaching the same site in the ACh channel which QX-314 can reach from the external surface. Further work will determine if this barrier affects the movement of smaller cations through the channel. Supported by NS-13778 and CA-19017.

1631 SINGLE-CHANNEL CURRENTS OF ACETYLCHOLINE RECEPTORS IN CULTURED RAT MUSCLE. M. B. Jackson*, C. N. Christian* and H. Lecar* (SPON: R. E. Taylor). NIH, Bethesda, MD 20205.

Single-channel current fluctuations were observed for acetylcholine (ACh)-sensitive channels in cultured embryonic rat myotubes. The current measurements employed a 1-2 μ diameter extracellular electrode containing 1 or 10 μM carbamyl choline which was pressed against the cell membrane. Rectangular current-fluctuations caused by the agonist-activation of individual ionic channels were observed with a pipette-to-membrane seal resistance of 20 MΩ or greater. For 10-day old cultures, cells held at a membrane potential of -90 mV, gave current jumps of 4.4 pA at 22°C. Assuming a reversal potential of 0 mV, this leads to a value for the unit channel conductance of 49 pS. Open-channel durations are exponentially distributed, with a mean open time of 3.3 msec. As a convenient means of analyzing channel properties, the fluctuation records were processed by a Fourier transform program. The resulting power spectra were Lorentzian, and the open-channel durations could be extracted even for records so dense that individual-channel events are unresolvable. The surfaces of the myotubes were probed for variations in acetylcholine sensitivity. The regions of intense channel activity were correlated with regions of high receptor density as measured by fluorescence microscopy with rhodamine labelled α-bungarotoxin. The direct recording of ACh-sensitive single-channels together with the analysis of membrane noise, permits the quantitative mapping of the spatial distribution of ACh receptors and the characterization of the parameters of the ACh sensitive channels.

1632 MIGRATION OF SCHWANN CELLS AND WRAPPING OF NEURITES IN VITRO: A FUNCTION OF PROTEASE ACTIVITY IN THE GROWTH MEDIUM. Nurit Kalderon. The Rockefeller University, New York, N.Y. 10021.

Proteolytic enzymes activities associated with chick embryo spinal cord cells are studied in culture, in relation to the ability of these cells to invade, migrate and extend during the development of both the peripheral nervous system and the neuromuscular junction. It was found that dissociated spinal cord cells from 7-9 day old chick embryos produce a proteolytic enzyme, as determined by their fibrinolytic activity in culture. The proteolytic enzyme was identified as plasminogen activator. The relative spatial organization of the dissociated spinal cord cells was found to be directly related to the expression of their plasminogen activator activity. The neuron cell bodies aggregated in ganglia-like clusters, from these aggregates there was an extensive outgrowth of bundles of axons, and these bundles were wrapped by Schwann cells. This was determined both by EM ultrastructural analysis, and by tritiated thymidine labeling of cell nuclei. Neurons at this stage of differentiation for the most part do not divide whereas the Schwann cells do. When [^{3}H] thymidine was added to the cultures the nuclei of the wrapping cells were radiolabeled whereas the clusters of neurons did not incorporate any label. In cases in which the spinal cord cells were grown either in regular medium (supplemented with horse serum and chick embryo extract),or in medium in which plasminogen was eliminated, no plasminogen activator activity could be detected, and the cells displayed a random growth pattern. In these cultures the neurons were individually spread on a carpet of non-neuronal cells as previously described (Fischbach and Dichter, 1974; Dev. Biol. 37:100). It is possible to conclude from the data described above that Schwann cells migrate and wrap the growing neurites as a result of the presence of the proteolytic activity of plasmin. The plasminogen probably is activated by plasminogen activator produced by one of the cell types in the culture. Thus, to achieve wrapping of neurons by Schwann cells in culture, it is essential to have the expression of plasminogen activator activity.

It was suggested that the elimination of multiple innervation of the skeletal muscle is carried out by proteolytic activity (O'Brien et al., 1978; J. Physiol. 282:571). The role of the Schwann cell and the plasminogen activator activity in the formation of the neuromuscular junction will be discussed with respect to this observation.

1633 IMMUNOHISTOCHEMICAL LOCALIZATION OF CHOLINE ACETYLTRANSFERASE AT THE NEUROMUSCULAR JUNCTIONS OF RABBIT DIAPHRAM. K.-S. K. Kan and L.-P. Chao. Dept. Neuro., Reed Center, Sch. Med., UCLA, Los Angeles, CA 90024.

The innervation of skeletal muscle at the neuromuscular junction is known to be cholinergic. Since choline acetyltransferase (ChAc) catalyzes the synthesis of acetylcholine, high ChAc activity has been detected in the ventral horn, the ventral root and the nerves that innervate skeletal muscle. Until recently we have visualized ChAc in motor neurons of bovine spinal cord with an immunofluorescent method using guinea pig antiserum to purified bovine ChAc (Nature 250: 243, 1974). This antiserum cross-reacts with ChAc from rabbit brain and we have localized ChAc in the motor neurons of rabbit spinal cord (Brain Res. 146: 221, 1978) by the newer and more sensitive immunoperoxidase method. The present communication reports the localization of ChAc at the neuromuscular junction. Frozen sections of rabbit diaphram were stained histochemically for cholinesterase (ChE) using 5-bromoindoxyl acetate as the substrate. This yielded a blue deposit at the motor end plate. After this histochemical staining of ChE, the immunoperoxidase staining for ChAc was performed on the same section. The immunoperoxidase staining involved the sequential incubation of the sections with guinea pig antiserum to bovine ChAc, rabbit anti-guinea pig IgG and guinea pig peroxidase-antiperoxidase (PAP) (Brain Res. 146: 221, 1978). The brown reaction deposits of the complex of ChAc, antibody and peroxidase was visualized by incubation with diaminobenzidine in the presence of H_2O_2. This combination of histochemical staining for ChE and immunohistochemical staining for ChAc revealed blue motor end plates and brown nerve terminals. The ChE at the neuromuscular junction was localized mainly in post-synaptic structures while the ChAc was localized at the presynaptic nerve terminals. Similar presynaptic nerve terminals were observed when a silver stain was substituted for the immunoperoxidase stain. Supported by USPH Service Grant No. NS-11087 and BRSG from NPI of UCLA.

1634 DEPRESSANT EFFECTS OF MORPHINE AND MEPERIDINE ON NEUROMUSCULAR TRANSMISSION IN RAT AND HUMAN MYASTHENIC MUSCLES. Yong I. Kim,* James F. Howard* and Donald B. Sanders. Dept. of Neurology, Univ. of Virginia Sch. of Med., Charlottesville, VA 22908

The depressant actions of narcotic analgesics, morphine (MO) and meperidine (ME), on neuromuscular transmission were investigated in vitro in forelimb flexor digitorum longus muscles of normal rats and those with experimental autoimmune myasthenia gravis (EAMG) and in intercostal muscle biopsies from patients with myasthenia gravis (MG).

In the rat muscles MO and ME at concentrations from 8 to 80μM reduced the amplitudes of miniature end-plate potentials (MEPPs) and end-plate potentials (EPPs) in a dose-dependent manner. At equimolar concentrations (24 and 80μM), MO and ME produced a similar reduction in MEPP and EPP amplitudes. At maximum therapeutic concentrations (MO:0.03μM, ME: 0.7μM), both drugs showed little effect on neuromuscular transmission, but at 10 to 40 times the therapeutic concentration only ME produced an apparent neuromuscular depression. At very high concentrations (160μM), both drugs decreased the amplitude and maximum rates of rise and fall of APs, suggesting an effect on the ionic conductances of muscle membranes. Isometric twitch tension was significantly reduced by both drugs in muscles in which neuromuscular transmission was partially blocked by d-tubocurarine. The concentration of MO required to produce a given reduction of twitch tension was greater than that of ME. Similar depressant effects of MO and ME on neuromuscular transmission were found in muscles from rats with EAMG.

In intercostal muscles from patients with MG, both MO and ME depressed MEPP and EPP amplitudes in a dose dependent manner. Reductions in MEPP and EPP amplitudes were observed with 30μM MO and 70μM ME but the quantum content of evoked transmitter release was not altered, indicating that the major depressant action of MO and ME occurs postsynaptically. As in rat muscle, ME produced a greater neuromuscular depression than did MO. At 10 times the maximum therapeutic concentration, both drugs had no inhibitory effect. At 100 times the therapeutic concentration, MO (3μM) still had no inhibitory effect, but ME (70μM) significantly impaired the neuromuscular transmission.

These results demonstrate that meperidine has a greater depressant effect on neuromuscular transmission in vitro than does morphine, but that these effects are seen only at concentrations significantly greater than are achieved clinically. (Supported by NIH Grant NS-12905 and a center grant from the Muscular Dystrophy Association)

1635 DENERVATION CHANGES IN FROG NEUROMUSCULAR JUNCTIONS: FREEZE-FRACTURE STUDIES. Chien-Ping Ko. Lab. Neuropath. Neuroanat. Sci., NINCDS, Natl. Inst. Health, Bethesda, MD 20205.

Neuromuscular junctions of frog cutaneous pectoris muscle were freeze-fractured 1-14 days after cutting the nerve 3-5 mm from the muscle. The results confirmed earlier findings that structural changes in the presynaptic elements occur abruptly. In addition, it was found that Schwann cells after denervation possessed membrane ridges opposed to the junctional folds of the muscle.

During the first three days after denervation, neuromuscular junctions showed the normal 3-cell arrangement: muscle with junctional folds, axon and Schwann cell. No abnormalities were detected at the active zones or elsewhere in the presynaptic membrane (36 terminals with 880 active zones from 4 frogs). At 4-7 days, neuromuscular junctions had either (1) the usual 3-cell arrangement with normal appearing nerve terminals or (2) complete replacement of the nerve terminal by the Schwann cell. Occasionally, a few transitional stages were seen, in which processes interpreted to be portions of nerve terminals were surrounded by Schwann cells already occupying most of the junctional gutter. The scarcity of such transitional stages implies a rapid engulfment of nerve terminals by Schwann cells and abrupt changes in the membrane structures of nerve terminals if they occur at all before engulfment.

At 1-2 weeks after denervation, nerve terminals were no longer seen and the muscle did not contract when the nerve stump was stimulated. At this stage, Schwann cells still occupied the synaptic gutters, but now ridges appeared on the cytoplasmic leaflets of the Schwann cell membrane. Like the active zones of nerve terminals, these ridges lay directly over the junctional folds. However, no rows of particles marked these sites on the Schwann cell membrane. Examination of thin sections from paired muscles confirmed that Schwann cell processes form these ridges and that these ridges are over the junctional folds. However, no other specialized cytoplasmic structures or clustering of organelles in these regions of Schwann cells were found. Molding of the Schwann cell membrane to the contours of the junctional folds suggests attachment of the Schwann cell to the muscle or its basement membrane in the location to which nerve terminals are also thought to be attached.

1636 ACh RECEPTOR CHANNELS BEGIN TO OPEN WITHIN 10 μsec AFTER AGONIST IS APPLIED. Mauri E. Krouse*, Henry A. Lester, Menasche M. Nass, Jeanne M. Nerbonne*, Norbert H. Wassermann* and Bernard F. Erlanger* (SPON: W. F. Agnew). Division of Biology, California Institute of Technology, Pasadena, CA 91125 and College of Physicians and Surgeons, Columbia University, New York, NY 10032.

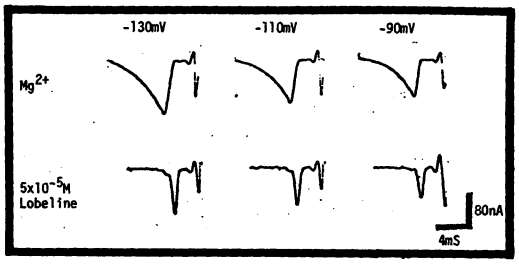
We have improved (by a factor of 10) our estimate of how rapidly acetylcholine (ACh) receptor channels begin to open after agonist appears nearby. Isolated Electrophorus electroplaques are arranged for transcellular recording. The innervated face is bathed in a solution of cis-Bis-Q (3,3'-Bis-[α-(trimethylammonium) methyl] azobenzene) which has no effect on receptors. Trans-Bis-Q is a potent agonist; a concentration of 350 nM activates half the receptors at the resting potential. Roughly half the cis-Bis-Q molecules are photoisomerized to the trans configuration within 1 μsec by a pulse of 440-nm light from a dye laser. Our methods can detect a depolarization caused by activation of 0.5-1% of the receptor channels. At 23° C and at a trans-Bis-Q concentration of 1 μM, this level of activation occurs within 10 μsec after the flash. This latency has a Q_{10} close to 3 and at lower [trans-Bis-Q], the latency increases as the inverse of the concentration. Thus, the minimum latency has the same concentration and temperature dependence on a μsec time scale as the channel opening rate, measured in relaxation experiments, has on a millisecond time scale. The data show that if there is an absolute delay involved in activation of receptor channels by agonist, this delay is less than 10 μsec at 23°C. Our agonist concentration jumps are still much smaller than the local ones that occur when the presynaptic nerve terminal releases a quantum of ACh into the synaptic cleft.

Further information is obtained from a second flash, which photoisomerizes trans-Bis-Q molecules bound to receptors (as well as Bis-Q molecules in solution). Since the cis configuration is a much weaker agonist, channels close rapidly and the electroplaque repolarizes. The observable latency for this repolarization is even less than for the depolarization due to the first flash.

This work was supported by the NIH (NS-11756, NS-06216, NS-242) by the Muscular Dystrophy Association and by the NSF (PCM 74-02140).

1637 THE ACTION OF LOBELINE ON THE ACETYLCHOLINE-ACTIVATED IONIC CHANNEL. Jeremy J. Lambert*, Linda S. Reynolds*, Robert L. Volle and Edward G. Henderson. Dept. Pharmacol., U Conn. Health Center Farmington, CT 06032.

Frog sartorius and cutaneous pectoris muscles were studied using conventional endplate voltage clamp techniques. Lobeline has previously been shown to cause neuromuscular blockade characterized by a non-depolarizing postsynaptic action (N.-Schmied. Arch. Pharmacol. 272:16, 1972). Unlike tubocurarine, lobeline enhances receptor desensitization (N.-Schmied. Arch. Pharmacol. 272:307, 1972). The half time of decay ($t_{½}$) of the endplate current (e.p.c.) in Mg^{2+} or tubocurarine-pretreated preparations was logarithmically related to holding potential (Vm) in the range -30mV to -130mV, being slower at more hyperpolarized potentials. In preparations pretreated with lobeline ($5x10^{-5}$M) the $t_{½}$ of the e.p.c. was shown to be independent of Vm (at -90mV, $t_{½}$ = 0.46± 0.03mS; at -130mV, $t_{½}$ = 0.45±0.02mS). When lobeline ($5x10^{-5}$M) was added to Mg^{2+} (11-14mM) pretreated preparations the amplitude of the e.p.c. at -90mV was 18.1±4.9% of control and $t_{½}$ was decreased in the range -90mV to -130mV. The dependence of $t_{½}$ on Vm was still evident but modified from that in Mg^{2+} alone (at -90mV, $t_{½}$ = .94±0.14mS; at -130mV, $t_{½}$ = 1.14±0.22mS). After a 15 min. wash with Mg^{2+}-Ringer (lobeline-free) the amplitude of the e.p.c. remained depressed but $t_{½}$ returned to control values. The reversal potential measured in the presence of lobeline alone was not different from values obtained in Mg^{2+} alone (-5 to 0 mV) however, the amplitudes of the e.p.c.'s for Vm in the range 0 to +40mV were more depressed than in the range 0 to -90mV. This data is consistent with an action of lobeline on the acetylcholine-activated ionic channel, as has been suggested for several other drugs. Lobeline may serve as a useful tool for elucidating the properties of the endplate channel and its interaction with the membrane electric field. (Supported by NS-07540 and NS-12563.)



1638 REGENERATION FOLLOWING MICRO-CAUTERY OF NEUROMUSCULAR JUNCTIONS OF NORMAL AND DYSTROPHIC MOUSE MUSCLES. Peter K. Law. Dept. Neurol., Jerry Lewis Neuromus. Ctr., Vanderbilt Univ. Sch. Med., Nashville, TN 37232.

Recovery of neuromuscular function after micro-cautery of the motor end-plate zone was studied in normal and dystrophic littermates of the 129/ReJ-dy mice. A fine platinum wire (5 mm long, 0.005" diameter) heated to about 400C by a needle-tipped soldering iron was applied to the middle third of the right soleus, which was cooled immediately with a flush of equilibrated Krebs-Henseleit solution at 22C. This procedure caused immediate neuromuscular dysfunction without disrupting the physical continuity of nerve and blood vessels into the muscle. The soleus was used because of its discrete, central end-plate zone and because its fibers ran longitudinally from tendon to tendon. Unoperated solei in the left legs served as controls. Mice were cauterized at 6 to 7 weeks of age and examined 3 months post-operatively.

Results were obtained from 10 dystrophic and 9 normal mice. Measurements were made in vivo at 37C. Cauterized and unoperated normal solei did not differ in indirectly evoked twitch and tetanus tensions. However, cauterized dystrophic solei produced only 62.8% of twitch, and 66% of tetanus tensions of their controls. Motor unit counts did not differ between cauterized and unoperated solei, regardless whether the preparation was normal or dystrophic. Cauterized normal myofibers were more depolarized (68 ± 5 mV, X ± SD) than unoperated normals (73 ± 4 mV). There is no difference in the mean resting membrane potentials between cauterized dystrophic (64 ± 8 mV), and unoperated dystrophic myofibers (64 ± 7 mV). There is no difference in contraction time and half-relaxation time between cauterized and unoperated solei. The results indicated that the dystrophic mouse exhibited less-than-normal neuromuscular regenerative capability after an acute lesion. (Supported by MDA, MDAC).

1639 AGONIST INDUCED MYOPATHY IS MEDIATED BY CALCIUM. John P. Leonard* and Miriam M. Salpeter. Section of Neurobiology and Behavior, Cornell University, Ithaca, New York 14853.

Inactivation of cholinesterases at mammalian neuromuscular junctions (nmj) produces extensive muscle "necrosis", which was first reported by Ariens et al. (Experientia 25:57-59, 1969). Fine-structurally this myopathy begins at the nmj with an increase in large diameter (>130nm) vesicles in the soleplasm, the dissolution of Z-disks, dilation of mitochondria, dilation of sarcoplasmic reticulum and often a highly specific contracture of the muscle under the endplate.

Biochemical studies have shown that a Ca^{++}-activated protease, which specifically removes Z-disks from isolated myofibrils in vitro, is present in mammalian skeletal muscle. (Busch et al. J. Cell Biol. 52:367-380, 1972). Since agonist causes specific Ca^{++} influx at motor endplates, (e.g. Evans J. Physiol, 240: 517-533, 1974; and Miledi, et al. J. Physiol. 268:32-33p, 1977) it was suggested that the myopathy after esterase inactivation may be due to Ca^{++} influx during the prolonged Ach lifetime (Salpeter et al. J. Neurocytol. 8:95-115, 1979). To test this hypothesis an isolated nerve muscle preparation of the mouse extensor digitorum longus (EDL) was used. The myopathy near endplates was first produced by esterase inactivation with diisopropylfluorophosphate (DFP) followed by nerve stimulation, and later mimicked by bath application of 10^{-4}M carbamylcholine (CCh) without esterase inhibitors. The extent of damage was assessed from the two most consistent morphological changes: vesiculation of the soleplasm and Z-disk dissolution. The damage produced in a muscle preparation maintained in vitro for 3 hours, depended on the relative time that CCh was present in the bath. The myopathy was prevented by inactivating the acetylcholine receptors (AChR) with 1μM α-bungarotoxin or with 5μM d-tubocurarine. The myopathy was also completely prevented by removing Ca^{++} from the CCh bath with 5mM EGTA, and partially prevented by adding .1mM D-600 (a specific Ca^{++} flux inhibitor) to the CCh bath. The myopathy was unaffected when Na^{+} was replaced by either Choline or Tris HCl.

These results favor the hypothesis that prolonged exposure to agonist produces a myopathy which mimicks one produced by esterase inactivation, is mediated by Ca^{++} and requires an unblocked AChR.

Supported by NIH Grant NS 09315

1640 A COVALENTLY BOUND PHOTOISOMERIZABLE AGONIST AT ELECTROPHORUS ELECTROPLAQUES: EQUILIBRIA, KINETICS, AND STOICHIOMETRY. H. A. Lester, M. E. Krouse# and M. M. Nass, Division of Biology, California Institute of Technology, Pasadena, CA 91125; N. H. Wassermann# and B. F. Erlanger#, Department of Microbiology, Columbia University, New York, NY 10032.

After disulphide bonds are reduced with dithiothreitol, trans-3-(α-bromomethyl)-3'-[α-(trimethylammonium)methyl] azobenzene (trans-QBr) alkylates a sulfhydryl group on receptors. The membrane conductance induced by this "tethered agonist" shares many properties with that induced by reversible agonists. Equilibrium conductance increases as the membrane potential is made more negative; the voltage sensitivity resembles that seen with 50 μM carbachol. Voltage-jump relaxations follow an exponential time course; the rate constants are about twice as large as those seen with 50 μM carbachol and have the same voltage and temperature sensitivity. With reversible agonists, the rate of channel opening increases with the frequency of agonist-receptor collisions; but with tethered trans-QBr, this rate depends only on intramolecular events. In comparison to the conductance induced by reversible agonists, the QBr-induced conductance is at least tenfold less sensitive to competitive blockade by tubocurarine and roughly as sensitive to "open-channel blockade" by QX-222.

Light-flash experiments with tethered QBr resemble those with the reversible photoisomerizable agonist, Bis-Q: the conductance is increased by cis → trans photoisomerizations and decreased by trans → cis photoisomerizations. As with Bis-Q, light-flash relaxations have the same rate constant as voltage-jump relaxations. Receptors with tethered cis-QBr have a channel duration severalfold briefer than with the tethered trans isomer. By comparing the agonist-induced conductance with the cis/trans ratio, we conclude that each channel's activation is determined by the configuration of a single tethered QBr molecule. The QBr-induced conductance shows slow decreases (time constant several hundred msec) which can be partially reversed by flashes.

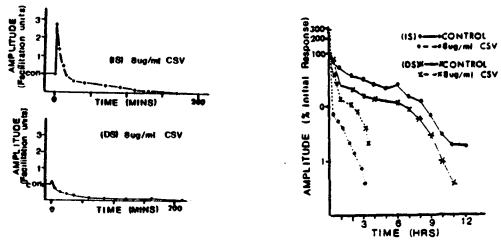
The similarities suggest that the same rate-limiting step governs the opening and closing of channels for both reversible and tethered agonists. Therefore, this step is probably not the initial encounter between agonist and receptor molecules.

Supported by the Muscular Dystrophy Association, the NIH (RCDA NS-272 and Grant NS-11756) and the NSF (Grant PCM 74-2140).

1641 COMPARISON OF THE EFFECTS OF CENTRUROIDES SCULPTURATUS VENOM ON FROG MUSCLE TWITCH TENSION DEVELOPMENT VIA INDIRECT (IS) (NEUROTRANSMISSION) OR DIRECT (DS) ELECTRICAL STIMULATION OF THE MUSCLE. Gesina L. Longenecker, Herbert E. Longenecker, Jr., and Barbara Beyers, Department of Pharmacology, College of Medicine, University of South Alabama, Mobile, AL 36688.

In preliminary twitch tension studies (Soc. for Neuroscience Abstracts, Vol. 4) with C. sculpturatus venom (CSV) a gradual rundown of the IS frog preparation over a period of many hours occurred. No systematic study of this observation was pursued, nor a directly stimulated (DS) control preparation examined. We have now extended our earlier observations in these directions. Isolated frog sciatic/sartorious nerve muscle preparations, dissected and mounted in Ringer's (2.5 mMK^{+}, 2.0 mM Ca^{++}, 114.5 mM Na^{+}) were used. CSV solutions (0.1 to 50 ug/ml) were made in the Ringer's. Either indirect (electrodes placed on sciatic nerve) or direct (electrodes placed bilaterally on the muscle at the plevic end) stimuli were adjusted to 125% of that voltage giving maximal twitch tension.

Twitch tension in both IS and DS preparations declined irreversibly to zero amplitude over a period of approximately 12 hours although after "initial rundown" both preparations exhibited an 'apparent' plateau of 3-5 hours. CSV added to either preparation substantially accelerated eventual twitch decline at all concentrations of venom studied. With IS, an initial several fold facilitation was followed by twitch block, whereas with DS, following barely perceptible facilitation (with lower concentrations only) twitch tension rapidly decreased. However, twitch tension declined considerably more rapidly with IS as opposed to the DS preparation at any dose of venom. Thus, CSV effect on neurotransmission contributes significantly to the eventual twitch block. Block of twitch in both preparations may be accounted for by known effects of CSV on active sodium permeability. Indeed, this effect alone probably accounts for the failure of the DS preparation.



1642 AMANTADINE AND ITS ANALOGS ON ENDPLATE CONDUCTANCE AND LIGAND BINDING AT THE NEUROMUSCULAR JUNCTION. M. A. Maleque*, J. E. Warnick, M. E. Eldefrawi* and E. X. Albuquerque, Dept. of Pharmacol. & Exper. Therap., University of MD, Balto. MD. 21201.

Amantadine (1-adamantamine) blocks neuromuscular transmission in a voltage-dependent manner, by reacting directly with the ionic channel of the acetylcholine (ACh) receptor. In this study amantadine and its N-alkyl substituted analogs: N-methyl- (ID 2), N-ethyl- (ID 3), N-(1-propyl)- (ID 4), N-(1-butyl)- (ID 6), and N,N-diethyl-amantadine (ID 27) and six bicyclo-octane compounds: 4-methylbicyclo [2.2.2] oct-2-ene-1-carboxylic acid (ID IIIb), 4-methyl-bicyclo [2.2.2] octane-1-carboxylic acid (ID Vb), 1-amino-4(1-propyl)-1,4-bicyclo [2.2.2] octane (ID 11), 1-amino-4(1-hexyl)-1, 4-bicyclo [2.2.2] octane (ID 16), 1-(amino-methyl)-4-methyl- (ID 33) and 1(N,N-dipropyl-aminomethyl)-4-methyl (1,4-bicyclo [2.2.2] octane (ID 36) were investigated on frog sciatic sartorius preparations. Amantadine and ID 2, 4, 6, 11, 27, 33 and 36 blocked indirectly elicited twitches while ID 4, 6 and 27 also potentiated directly elicited twitches and prolonged the muscle action potential. ID IIIb, Vb and 16 had no effect on twitches. Fifty percent blockade of the indirect twitch with amantadine occurred at 130 μM; at 12 - 15 μM for ID 2, 3, 4, 11 and 27; 50-55 μM for ID 6 and 36; and 100 μM for ID 33. Miniature endplate potential (m.e.p.p.) amplitude was reduced by amantadine (by 80% in 60 min) and by ID 2, 3, 4 and 27. Neither amantadine nor its analogs affected m.e.p.p. frequency or membrane potential. Amantadine and ID 2, 3, 4, 6, 11, 27, 33 and 36 caused a marked departure from linearity in the voltage-current relationship of the peak endplate current (EPC). This effect was clearly apparent at very negative membrane potentials. The voltage dependent peak EPC amplitude for amantadine and ID 2, 4 and 33 remained unattenuated at depolarized membrane potentials. Amantadine and ID 2, 3, 27 and 33 altered the voltage dependency of the falling phase of EPC and reduced the slope of the relationship between τ (of the EPC) and the membrane potential with subsequent reversal of the slope. Peak EPC amplitude and shortening of the falling phase of the EPC were concentration dependent. ID 4, 6, 11 and 36 decreased the voltage dependency of the falling phase at a low concentration (20-50 μM) but was voltage independent at a higher concentration (100 μM). Neither amantadine nor its N-alkyl substituted analogs and bicyclo-octane derivatives inhibited the binding of ^{3}H-ACh to its receptors but did inhibit competitively the binding of ^{3}H-perhydrohistrionicotoxin to the ionic channel of the ACh receptor with Ki for each compound equal to: amantadine, 60 μM; ID 2, 30 μM; ID 3, 15 μM; ID4, 40 μM; ID 6, 40 μM; ID 27, 15 μM; ID IIIb > 1mM; ID Vb, > 1 mM; ID 11, 200 μM; ID 16, 30 μM; ID 33, 600 μM; ID 36, 50 μM. It is suggested that amantadine and its analogs react with the ionic channel of the ACh receptor causing the appearance of two species of conductance, one where the channel is partially blocked (hyperpolarized state) and the other where the channel is in an open conformation (depolarized state). (Supported in part by USPHS Grant NS-12063 and the Dept. of the Army.)

1643 DISTRIBUTION OF ACHR IN DEVELOPING AND DYSTROPHIC MOUSE NEUROMUSCULAR JUNCTIONS. Julia Matthews-Bellinger* and Miriam M. Salpeter. (SPON: Lee Rubin). Neurobiology and Behavior, Cornell University, Ithaca, NY, 14853. Supported by NIH Grant NS 09315.

The microdistribution of the acetylcholine receptor at extensor digitorium longus (EDL) muscle endplates during normal postnatal development of mice (inbred strain 129/ReJ) was studied by EM autoradiography after ^{125}I-α-bungarotoxin (BTX) labeling. In adult mouse endplates the ACh receptive surface corresponds to a thickened region of the post-junctional membrane (pjm) at the crests of the junctional folds (Fertuck and Salpeter, J. Cell Biol. 69:144, 1976). The morphological maturation of the pjm during development was quantified by the ratios of the lengths of total pjm and thickened pjm to the length of the primary cleft midline (1°cleft). Approximately 50% of the 1°cleft is covered by Schwann cell extensions between day 1 and 5, whereas by 2 weeks axon terminals have expanded to occupy most of the cleft. Results are given in the Table:

age (#animals)	Genotype	Morophology: total pjm/1° cleft	Morophology: thickened pjm/1° cleft	^{125}I-α-BTX site density†: per μm^2 1°cleft	^{125}I-α-BTX site density†: per μm^2 thickened pjm
	normal (+/+)	$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD
1d(3)		1.14±.01	0.58±.08	0.63±.12	1.05±.13
5d(3)		1.23±.07	0.66±.03	0.67±.10	1.01±.12
2wk(2)		2.03±.13	0.73±.10	0.82±.11	1.13±.15
>4mo(3)		4.45±.79	1.34±.11	1.34±.11	1.00±.04
>4mo(2)	+/dy	4.32±.65	1.33±.12	1.76±.11	1.27±.08
>4mo(2)	dy/dy	1.42±.32	0.83±.23	0.96±.34	1.13±.17

The ratio of total pjm/1°cleft shows that junctional folds, absent at birth, develop over a period of several weeks. The length ratio of thickened pjm to 1°cleft also increases very gradually. ^{125}I-α-BTX site density per unit area of 1°cleft increases but per unit area of thickened pjm is constant throughout postnatal development. Furthermore, we found that these post-junctional specializations are comparable under axon and Schwann cell.

A single autosomal recessive mutation for "dystrophy" (dy) occurs in strain 129/ReJ. The endplate in adult dystrophics (dy/dy) is enlarged with minimal junctional folds, generally patchy distribution of thickened pjm and extensive subneural regions lacking recognizable thickened pjm and having greatly reduced ^{125}I-α-BTX binding. However per total area of thickened pjm whenever it occurs the ^{125}I-α-BTX binding density is that of normal adults in this strain.

†normalized to site density per thickened pjm in normal adult.

1644 EFFECTS OF PURIFIED CONUS GEOGRAPHICUS TOXINS G_1 AND G_{11} ON NEUROMUSCULAR ELECTROPHYSIOLOGY. Owen McManus* and James Musick (SPON: S. Stensaas). Dept. Physiology, Col. of Med., U. of Utah, Salt Lake City, UT 84108.

The electrophysiological effects of two purified peptide toxins (G_1 and G_{11}) isolated from the venom of the marine snail Conus Geographicus were investigated. The composition of the two peptides has been previously reported as: G_1 = Glu (Arg, His, Asn, Ser, Pro, Cys_4, Gly, Ala, Try) and G_{11} = Glu (Lys, His_2, Ser, Pro, Cys_4, Gly, Ala, Phe), and the LD_{50} in mice reported as 12 μg/KG (Gray, Luque, Olivera, Fed. Proc., 61: 2171, 1977; Cruz Gray, Olivera, Arch. Bioch. Biophys., 190: 2, 1978). The toxin was applied in all cases as an equal mixture of G_1 and G_{11}.

The toxin was applied to a 5 mm segment of ventral root from the bullfrog sciatic nerve in concentrations ranging from 5 μg/l to 1 mg/l. The nerve was stimulated distal to the site of toxin application and recording leads were placed on the ventral root proximal to the site of toxin application. After 25 min. exposure to toxin, the amplitude of the extracellularly recorded action potential was within 5% of the value predicted from a baseline extrapolation. The twitch tension developed by directly stimulated frog sartorius muscle was similar to control values after 25 min. exposure to toxin at 300 μg/l. These results indicate that even in high concentrations, G_1 and G_{11} have a negligible effect on action potential propogation in nerve or muscle.

The effects of G_1 and G_{11} on neuromuscular transmission were investigated in the mouse hemidiaphragm using intracellular recording techniques. While recording miniature endplate potentials (m.e.p.p.s.) the toxin was added to a static, directly oxygenated bath. Exposure to toxin concentrations between 220 μg/l and 475 μg/l caused a dimunition of m.e.p.p. amplitude until m.e.p.p.s. became indistinguishable from noise. Upon superfusion with control saline, m.e.p.p. amplitude increased, but this return to near control levels was significantly slower than the onset of effects. These results suggest that G_1 and G_{11} may act as receptor antagonists at the neuromuscular junction. The results do not rule out the possibility that G_1 and G_{11} act by altering the conductance of the subsynaptic membrane or by affecting presynaptic function.

Supported by NIH grant NS07938 from the U.S. Public Health Service, intramural funds from the U. Utah College of Medicine, and a U. Utah Graduate Research Fellowship.

1645 WHAT IS THE SITE OF ACTION OF MAGNESIUM ION AT THE MOTOR NERVE TERMINAL? STUDIES WITH ION-CONTAINING LIPOSOMES. A.M. Mellow, E.M. Silinsky and A.F. Boyne. Dept. Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611.

It has long been known that magnesium ion (Mg^{++}) is an effective inhibitor of the evoked release of transmitter from presynaptic nerve terminals. Mg^{++} is thought to act as a competitive antagonist of calcium ion (Ca^{++}), the physiological mediator of depolarization-secretion coupling. Both intracellular and extracellular sites for this antagonism have been proposed, but definitive evidence for either site remains lacking. The recent observations of Rahamimoff et. al. (PNAS, 75:5214, 1978) suggest that liposomes can be used to introduce ions directly into the cytoplasm of nerve terminals. We have utilized this technique to test the hypothesis that Mg^{++} has an intracellular site of action.

Sonicated liposomes containing either 80 mM $CaCl_2$ or 80 mM $MgCl_2$ were prepared from egg phosphatidylcholine according to standard procedures and were dialyzed in an Amicon ultrafiltration unit against a control Ringers solution which contained 0.3 - 0.4 mM $CaCl_2$ and 1 - 2 mM $MgCl_2$. The integrity of the liposomes and the extent of dialysis were assessed by the addition of $^{45}CaCl_2$ to the lipid dispersions prior to sonication. Liposome suspensions were perfused over isolated cutaneous pectoris nerve-muscle preparations of the frog and quantal content was measured at single end-plates using conventional electrophysiological techniques. Liposomes containing $CaCl_2$ produced a three to fourfold increase in quantal content over that seen in control Ringers solution. This effect is thought to result from the activation of the release mechanism by Ca^{++} which has been introduced directly into the nerve terminal (presumably due to an interaction between liposomes and the nerve terminal membrane). When liposomes containing $MgCl_2$ were applied to the preparations, no change in quantal content could be detected. These results suggest that when Mg^{++} is introduced directly into the nerve terminal cytoplasm, it is ineffective in antagonizing transmitter release. This study therefore supports the notion that the primary site of action of Mg^{++} is extracellular, presumably as a blocker of depolarization-induced Ca^{++} entry through the presynaptic membrane.

1646 MINIATURE ENDPLATE POTENTIALS DO NOT APPEAR TO BE COMPOSED OF SEVERAL SUBUNITS. Douglas C. Miller* and Karl L. Magleby. Dept. of Physiol. and Biophys., U. Miami Sch. Med., Miami, FL. 33101

It has been suggested that quanta of transmitter release, as represented by miniature endplate potentials (mepps), are made up of about 7 smaller subunits. Support for this hypothesis comes from the existence of a distinct class of small mepps usually about 1/7 the amplitude of the mean mepp amplitude, and from the presence in histograms of mepp amplitudes of multiple peaks, which often appear to occur at regular intervals (Kriebel et al. 1976 J. Physiol. 262:553; Wernig and Stirner 1977, Nature 269:820.

The autocorrelation function is a powerful test for regularly repeating events in sets of data. We have obtained the autocorrelations of histograms of mepp amplitudes from 27 cells and found no evidence for regularly recurring peaks, suggesting the absence of subunits. Mepps were recorded intracellulary from frog and mouse neuromuscular junctions; usually 2000-3000 mepps were recorded per cell. In data analyzed, resting potentials were usually stable within 2%, and mean mepp amplitude usually varied less than 5% throughout the course of the experiment. Since the autocorrelations of the mepp amplitude histograms indicated no evidence for a repeating pattern, the observed peaks in the histograms did not fall at regular intervals, as expected for the subunit hypothesis. We have previously suggested that random variation in the data could give rise to peaks on histograms (Miller et al. 1978, Nature 274:388).

To determine whether the autocorrelation technique could detect subunits we did correlations of histograms of simulated mepp amplitudes in which the mepps were composed of a mean of 7 subunits. Provided that the baseline noise did not exceed about ± 1/2 the mean subunit amplitude and that the standard deviation of the subunits did not exceed about 10% of the mean subunit amplitude, the autocorrelations of the simulated data clearly indicated the presence of regularly spaced peaks in the amplitude histograms. These results are consistent with these alternatives: 1) mepps are not composed of subunits; 2) mepps are composed of subunits but the standard deviation of the subunits is greater than about 10% of the mean subunit amplitude so they would not be detected by our method; 3) mepps are composed of subunits but the subunits are much smaller than previously proposed so they would be obscured by the noise in the recording system.

Simulated histograms constructed using the assumption that mepp amplitudes fall into a bimodal distribution, the small mode representing the class of small mepps and the larger mode representing the classical mepps, provided a good fit to experimental data. Supported by a Muscular Dystrophy Association fellowship to DCM and NIH grant NS10277 to KLM.

1647 MONOCLONAL ANTIBODIES TO THE NICOTINIC ACETYLCHOLINE RECEPTOR FROM RAT MUSCLE. Jeffrey Boone Miller* and Zach W. Hall. (SPON: M. Stryker). Department of Physiology, University of California, San Francisco, CA 94143

We have used the hybridoma technique to prepare monoclonal antibodies to acetylcholine (ACh) receptor purified from denervated rat skeletal muscle. Both immunized rats and mice were used as sources of antibody-producing cells. BALB/c mice were immunized by two injections of either 0.5 μg ACh receptor in complete Freund's adjuvant or of 10-50 ng of receptor that was bound to cobrotoxin-Sepharose and treated with glutaraldehyde. Rats were immunized with four 1 μg injections of receptor over two months. In all cases, animals produced detectable serum antibodies to the ACh receptor. Spleen cells from immunized animals were fused with cells from a mouse myeloma line, NS-1, and cultured in a medium that selected for hybrids. Anti-ACh receptor antibodies that were secreted into the culture medium by the hybrid cells were detected with a solid phase radioimmune assay that required only 1 ng of muscle receptor as a target antigen.

Anti-receptor producing hybridomas were detected at a low frequency (less than 1% of the hybrid cells), and six hybridoma lines that secrete anti-receptor were isolated and have been continuously cultured. Of these, three are derived from mouse spleen/NS-1 hybrids and secrete IgM, while three are rat/mouse hybrids that secrete IgG. Antibodies from all six lines did not distinguish junctional and extra-junctional forms of the rat muscle receptor, and did not cross-react with purified, solubilized *Torpedo* receptor in the solid phase assay.

Tumors that grew from mouse hybrid cells injected into host mice produced anti-receptor antibody in the sera of host animals, but symptoms of experimental autoimmune myasthenia gravis were not observed.

This work was supported by grants from the Muscular Dystrophy Association and the N.I.H., and by a post-doctoral fellowship from the Muscular Dystrophy Association to J.B.M.

1648 MECHANISM OF MAGNESIUM INHIBITION OF TRANSMITTER RELEASE: IMPLICATIONS IN DEPOLARIZATION-SECRETION (D-S) COUPLING. M.D. Miyamoto and R.A. Previti*. Dept. Pharmacol., E. Tenn. St. Univ. Col. Med., Johnson City, TN 37601 and Dept. Physiol. Biophys., Univ. Vermont Sch. Med., Burlington, VT 05401.

At frog motor nerve terminals, endplate potential (e.p.p.) size is related to $[Ca]^4$, suggesting a co-operativity, with competitive antagonism by Mg(Dodge & Rahamimoff, J. Physiol. 193: 419, 1967). Cooke et al. (J. Physiol. 228: 459, 1973) indicate that the role of Ca in D-S coupling can be described by two steps, Mg competing with Ca at both steps. The present results reveal further details of the actions of Mg and Ca in D-S coupling.

Miniature e.p.p. frequency (F) was continuously recorded from single endplates of frog cutaneous pectoris, during eight changes in [Mg] from 0.03 to 15 mM (Ca constant at 1.8 mM). Results from 5 to 10 of these experiments were normalized and pooled for $[K]_o$'s of 2.5, 5, 8, 11 and 14 mM. The family of curves of F vs. $[Mg]^o$ showed a transition from little effect at 2.5 mM K to a hyperbolic relationship at 14 mM K. Since a similar hyperbolic relationship is found for e.p.p.s. vs. [Mg] (Dodge & Rahamimoff, 1967), this indicates that the mechanisms for spontaneous and nerve-evoked transmitter release are equivalent.

Plots of F vs. Ca/(1+Ca+Mg/3) for each $[K]_o$ revealed a family of curves with parallel slopes at low Mg. However, with high Mg there was a reversal in curvature, indicating that high Mg could also increase F. Curve fitting was based on the assumption that 4th power co-operativity for Ca held for all levels of K depolarization (as indicated by the parallel slopes at low Mg for all levels of $[K]_o$). Improvement was made using a two stage model (Cooke et al., 1973), whereby Ca and Mg compete for a voltage-dependent (ν) Ca channel (binding but no co-operativity),as well as at the intracellular releasing site (possibly vesicular and/or active site membrane). Thus,

$$F = A\left[\frac{Ca}{1/\nu+Ca+Mg/3}\right]^4 + A\left[\frac{\beta Mg/9}{1/\nu+Ca+Mg/3}\right]^2$$

A,β=constants
ν=f(K^+),
$0 \leq \nu \leq 1$.

According to this model, 1) 4th power co-operativity may hold, at the releasing site, regardless of the extent of membrane depolarization (ν); 2) the 'inhibitory effect' of Mg may be attributed to competition at intra- and extracellular sites and need not invoke a charge screening mechanism (Muller & Finkelstein, PNAS 71: 923, 1974); and 3) the increased F with high Mg may be explained by either a Mg action at the release site or at the mitochondria to inhibit re-uptake of residual Ca. (Supported by USPHS NS 12270 & 15089).

1649 DEVELOPMENT OF SYNAPTIC SPECIALIZATIONS ON CULTURED, NON-INNERVATED, MUSCLE CELLS AT SITES OF CONTACT WITH THE CULTURE DISH. F. Moody-Corbett* and M.W. Cohen (SPON: R. Chase). Dept. Physiol., McGill Univ., Montreal, Quebec.

Cultured muscle cells derived from the myotomes of 1-day-old Xenopus embryos develop discrete patches of ACh receptors which can be readily visualized by labelling the receptors with fluorescent α-bungarotoxin. Many of the patches form on the bottom surface of the muscle cells, facing the collagen-coated surface of the culture dish (1,2). In the present study we have found that some 80% of these receptor patches are also sites of cholinesterase activity as revealed histochemically.

Another characteristic feature of the neuromuscular junction is the strong adhesiveness between the pre- and postsynaptic membranes. Interestingly many of the ACh receptor patches on the bottom surface of the cultured muscle cells appear to be located at sites of adhesion with the culture dish. When the cultures are treated with high concentrations of dibucaine (0.5-2.5 mM) the muscle cells round up but often remain attached to the culture dish in a region which contains one or more ACh receptor patches. Furthermore in cases where the muscle cells have a number of processes, those processes without receptor patches tend to withdraw whereas those with receptor patches tend to remain attached to the dish. That the regions which remain attached are relatively adhesive is further emphasized by the observation that subsequent agitation can tear the rounded cells away, leaving behind only small muscle fragments and associated ACh receptor patches.

These results taken together with those of previous studies (3,4) emphasize that several synaptic specializations can develop on cultured amphibian muscle cells in the absence of innervation. The finding that ACh receptor patches on non-innervated muscle cells are often located at sites of contact with the culture dish also raises the possibility that surface interactions may be important in triggering, and in acting as a locus for, the generation of synaptic specializations.

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1650 EFFECT OF AN EXOPEPTIDASE INHIBITOR ON ACETYLCHOLINE RELEASE FROM MOUSE MOTOR NERVE TERMINALS. James R. Musick. Dept. Physiol., Sch. Med., University of Utah, Salt Lake City, UT 84108.

Acetylcholine (ACh) release at the neuromuscular junction is accompanied by release of other molecules that react in assays for proteins and their derivatives. The proteinaceous molecules may be released as a consequence of secretion of exo- and endopeptidase enzymes from motor nerve terminals (Musick, J.R., Am. J. Physiol: Cell Physiol. 236 (5), 1979). Since it may be possible to infer a functional role for this phenomenon by observing the effect of its inhibition, an amino acid derivative known to inhibit cathepsin C was applied to the phrenic nerve-mouse diaphragm preparation and the effect on spontaneous ACh release was determined by electrophysiological methods. Exposure of preparations to 1.5 mM L-trytophanamide (Try-NH_2), which was 2 x K_i for inhibition of cathepsin C, produced the following reversible effects on signals, intracellularly recorded from the endplate region of diaphragm muscle fibers: 1. A 20 mV depolarization of the muscle fiber resting membrane potential (RMP) (-80 to -60 mV). 2. A more slowly developing 50% drop in miniature endplate potential (MEPP) amplitude (2.0 to 1.0 mV), which was coincident with, 3. A 2.9-fold increase in the frequency of MEPPs (1.8 to 5.2 sec^{-1}).

The effects of the amino acid, L-Tryptophan (Try) were then compared to Try-NH_2 because the former compound is not known to inhibit cathepsin-C, but is a structural analogue of Try-NH_2. Try mimicked the effects of Try-NH_2 on the muscle cell RMP and MEPP amplitude but had an opposite effect on MEPP frequency. While Try-NH_2 increased MEPP frequency ca. 2-fold, the same concentration of Try (1.5 mM) reversibly reduced MEPP frequency by 26% (from 1.15 to 0.85 sec^{-1}). It is therefore concluded that the Try-NH_2-induced increase in MEPP frequency is due to its additional amide linkage. Hence, this effect may be due to inhibition of exopeptidase activity at the neuromuscular junction. These results suggest a regulatory function for the proteinaceous molecules released in association with ACh from motor nerve terminals. Assuming that some of these molecules are formed as a consequence of exopeptidase activity, inhibiting the production of a small protein derivative by Try-NH_2 could block feedback inhibition of spontaneous ACh release, and thus imply that these molecules normally regulate ACh release from motor nerve terminals.

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1651 PROPERTIES OF SINGLE CHANNELS IN TISSUE CULTURED SKELETAL MUSCLE. D. J. Nelson* and F. Sachs* (SPON: B. Bishop). Dept. of Pharmacology, SUNY at Buffalo, Buffalo, NY 14214.

Using the technique of Neher et al. (Pflug. Arch. 375: 219, 1978) we have recorded the currents produced by single nicotinic channels in tissue cultured chick skeletal muscle cells. In myotubes, the single channel conductance, when activated by suberyldicholine in Hanks' solution, had a positive temperature coefficient, so that the conductance increased from approximately 50 pS at 17°C to 115 pS at 36°C. We saw no evidence of a levelling off of conductance with increasing temperature as reported by Fischbach and Lass (J. Physiol. 280: 527, 1978). The conductance values are consistent with the values obtained by noise analysis on normal and vinblastine treated cultures (Sachs and Lecar, Biophys. J. 17: 129, 1977; Nature 246: 214, 1973).

Histograms of the open channel durations were exponential implying a Poisson process of channel closing. The time constants with suberyldicholine activation were about 25 msec at 17°C and 6 msec at 36°C. The activation enthalpy, based on Arrhenius plots was about 10 kcal/mole. Confirming the results on frog endplate, different agonists appear to open the channels for different periods of time. However, the enthalpy of activation decreases with decreasing closing rate, implying a significant entropy increase between the open state and the transition state. (Supported by HL 21294, NS 13194 and the Muscular Dystrophy Association.)

1652 EFFECTS OF Ca^{++}, Ba^{++}, AND Sr^{++} ON THE KINETICS OF TRANSMITTER RELEASE AT THE FROG NEUROMUSCULAR JUNCTION UNDER CONDITIONS OF NORMAL QUANTAL CONTENT. Barry S. Pallotta and Karl L. Magleby. Dept. Physiol. & Biophysics, U. Miami Sch. Med., Miami, FL 33101.

Under conditions of low quantal content repetitive stimulation leads to an increase in the amount of transmitter released with each nerve impulse. Following repetitive stimulation, evoked transmitter release decays towards pre-stimulation levels with a multiexponential time course. Four individual components of the decay have been identified on the basis of time constants: two components of facilitation (time constants approximately 50 and 300 msec), augmentation (7 sec), and potentiation (tens of seconds to minutes). Under conditions of low quantal content the second component of facilitation and augmentation have been identified pharmacologically with Sr^{++} and Ba^{++}. Sr^{++} increases the magnitude and time constant of decay of the second component of facilitation, while Ba^{++} selectively increases the magnitude of augmentation. The purpose of our experiments was to determine whether facilitation, augmentation and potentiation can be observed under conditions of normal and increased quantal contents, and whether Sr^{++} and Ba^{++} still retain their selective effects under these conditions.

End-plate potentials (EPPs) were recorded extracellularly from the frog sartorius nerve-muscle preparation. Quantal content was changed by altering $[Ca^{++}]_o$, and curare was used as required to prevent contraction.

Using a variety of stimulation patterns, four components of the EPP amplitude decay were observed with time constants similar to those observed at low quantal content. For a train of 10 impulses at 10 Hz the time course of the change of EPP amplitudes during and following stimulation was similar in 0.3 mM Ca^{++} and 1.8 mM Ca^{++}. However, at 7.2 mM Ca^{++}, EPP amplitudes first increased, then decreased during the conditioning train. The depression of release then recovered with a 4-6 sec time constant. Although increasing $[Ca^{++}]_o$ greatly increased quantal content, the magnitudes and time constants of the two components of facilitation, after correcting for depression, were little affected over this 24-fold range of Ca^{++} concentrations.

The selective effects of Sr^{++}, although present, appeared to decrease with increasing quantal contents, while the magnitude of augmentation was still increased by Ba^{++}.

These results suggest that the processes which affect transmitter release at low quantal content are also present and have similar kinetic properties under conditions of higher quantal contents. The differential effects of Sr^{++} and Ba^{++} suggest that some of the underlying factors affecting these processes are different.

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1653 TWO CLASSES OF ACETYLCHOLINE RECEPTORS ON CULTURED MUSCLE CELLS DISTINGUISHED BY DETERGENT EXTRACTION. J. Prives*, M. P. Daniels, F.M. Neal*, H-C Bauer*, S. Penman*, C.N. Christian*. (SPON: B.K. Schrier). NIH, Bethesda, MD 20205 and Dept. of Biology, M.I.T., Cambridge, MA 02139.

Cultures of embryonic myotubes were extracted with 0.5% Triton-X 100 under conditions that removed membrane lipids and soluble proteins. The intact myotube cytoskeleton remained attached to the substrate. Two populations of AChR, labelled with (^{125}I) α-bungarotoxin (α-btx) were distinguished by different rates of detergent extraction.

In newly formed muscle cells, AChR are diffusely distributed on the myotube surface, and totally extracted at a rapid rate by detergent (T 1/2 <1 min.). Further differentiation of cultured myotubes is marked by aggregation of surface AChR and the appearance of a subpopulation of AChR resistant to rapid detergent extraction. Quantitative fluorescence microscopy of rhodamine α-btx labelled muscle cells indicated that diffusely distributed AChR were rapidly extracted by detergent whereas a major proportion of AChR in aggregates was retained with the myotube cytoskeleton. Medium conditioned by the neuronal cells NG108-15 increased the number of AChR aggregates per myotube, and increased the proportion of AChR retained after brief detergent extraction.

These observations suggest that the decreased lateral mobility of AChR found in aggregates may be associated with the anchorage of AChR to the myotube cytoskeleton. In marked contrast, the freely mobile AChR which are diffusely distributed on the cell surface at the initial stages of postfusional differentiation, show no comparable anchorage.

We propose that anchorage of AChR to the muscle cytoskeleton underlies the spatial organization of AChR during development and innervation.

1654 CHANGES IN PROPERTIES OF THE ACETYLCHOLINE RECEPTOR DURING DEVELOPMENT OF RAT SKELETAL MUSCLE. C. Gary Reiness* and Zach W. Hall, Dept. of Physiology, UCSF, San Francisco CA 94143.

Acetylcholine receptors (AChRs) at the neuromuscular junction of adult skeletal muscles differ from the receptors that occur on the extrajunctional surface of embryonic muscle in having a faster metabolic turnover time, a higher density in the membrane and distinct immunological properties. High density clusters of AChRs appear at sites of neuromuscular contact in rat muscle by embryonic day 16 (ED 16) while the extrajunctional receptors (EJRs) initially present persist until after birth (ED 22) and disappear during the first two weeks postnatally (for review, see Fambrough, Physiol. Rev. 59, 165, 1979). As a first step in identifying the molecular changes in AChRs that underlie physiological changes, we have followed the changes in turnover time and immunological properties that occur during development.

AChR turnover was measured by following the loss of ^{125}I-α-bungarotoxin bound to AChR in situ. We compared by autoradiography the turnover of clustered AChR and EJR in embryonic diaphragm. At ED 17 AChR in clusters found at sites of acetylcholinesterase staining have a turnover rate that is close to that of EJR ($t_{\frac{1}{2}}$=32h v 24h), but by ED 20 turnover in the clusters is significantly slower. At birth the half-time of turnover of clustered receptors is nearly the same as for receptors in adult muscle, while the turnover time for EJR is unchanged.

The immunological properties of receptors in developing muscle were analyzed using a serum from a patient with myasthenia gravis that contained antibodies that recognize unique determinants on EJR (Weinberg and Hall, PNAS 76, 504, 1979). Under appropriate conditions the extent of reaction with this serum was directly proportional to the fraction of receptor in muscle extracts with the immunological properties of embryonic EJR. Analysis of both diaphragm and total carcass muscle showed that adult AChR was first detectable at postnatal day 5 (PD 5), and became an increasing fraction of the total receptor number over the next 10 d. Whether receptors with the immunological properties of adult AChR were present at earlier times could not be determined because of the large amount of EJR in the muscles.

We conclude that ACh receptors clustered at sites of neuromuscular contact in rat become stable at about the time of birth. The immunologically adult form of the receptor can first be detected at PD 5 but may be present earlier. (Supported by grants from NIH, the Muscular Dystrophy Association, and a fellowship to C.G.R. from the Muscular Dystrophy Association).

1655 MEMBRANE STRUCTURE AND PHYSIOLOGY OF AN IMMATURE SYNAPSE. Mary B. Rheuben and Ann E. Kammer. Dept. of Biology, The Pennsylvania State Univ., University Park, PA 16802 and Div. of Biology, Kansas State Univ., Manhattan, KS 66506.

During development of the flight muscles of the moth Manduca sexta, synapses are formed de novo on maturing fibers. In the last week of pupation, increasingly large junction potentials can be recorded. Intracellular recordings were made from fibers of the dorsal longitudinal muscles, each bundle of which is innervated by a single separate motor neuron, and measurements made of endplate potential amplitude and time course, and response to multiple stimulation. Samples were then removed and prepared for thin section, freeze-fracture, and scanning microscopy. Early in the last week, we record small, long duration synaptic potentials that fatigue readily. Junctions are characterized by having postsynaptic structures seen either as small patches of particles on the external leaflet in freeze-fracture or small areas of electron dense membrane opposite the nerve in thin section. The membrane of the nerve terminal is specialized into plaque-like areas similar to those in adult junctions but smaller; there are irregular clusters of large particles in the center of the plaques in the cytoplasmic leaflet. In thin section the plaque area is seen as a region where the nerve membrane parallels the postsynaptic membrane, and the cluster of particles is in the same location as the presynaptic dense body or active zone. There are a few scattered or clumped, large round particles that lie both within the postsynaptic patches and adjacent to them. They appear to correspond to desmosome-like structures in thin section. Like similar structures found in other developing synapses, they may function in attachment of nerve to muscle and glial cell to muscle during the initial stages of apposition. Glial cells have a large volume of cytoplasm above the nerve terminal; they are beginning to form the processes that penetrate the muscle and curve around the nerve extensively in the adult. There is a difference in the complexity of structure from one end of a junction to another, with the greatest development of postsynaptic and glial processes at the end first contacted by the nerve. Junctions formed by a particular motor neuron are relatively uniform in length and complexity. Differences are seen between the populations of muscle fibers innervated by different neurons. On the basis of anatomical and physiological evidence, the developing muscle fibers are electrically coupled. Further development consists of increase in size and quantity of the pre- and postsynaptic structures and glial specializations.

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1656 ULTRASTRUCTURAL EVIDENCE OF MOTOR AXON RETRACTION DURING MATURATION OF RAT SOLEUS MUSCLES. Dan A. Riley. Dept. of Anatomy, U.C.S.F., San Francisco, California 94143.

Soleus muscle fibers of immature rats are polyneuronally innervated. Multiple motor axons converge upon the single endplate of each fiber. All but one of the multiple inputs per endplate are completely eliminated during the second postnatal week, generating the mature pattern of unineuronal innervation. My previous light microscopic studies of silver stained material indicated that terminal axonal branches are eliminated either by retracting from the endplate and absorption into the parent axon or by sloughing in situ and Schwann cell phagocytosis (Brain Res. 134: 279, 1977). In the present study, these mechanisms were examined further by electron microscopy of serial thin sections of soleus muscles from 7- to 15-day-old rats. The majority of the axonal profiles examined exhibited morphological features consistent with a scheme of retraction without marked degeneration. Axons interpreted as retracting were found between the neuromuscular junctions and the nearest intramuscular nerve bundles occupied by the parent axons of the transient terminal branches (a total distance of 20-40 μ). The retreating axon was characterized by a slender proximal shaft containing normally organized neurotubules and neurofilaments and terminating in a distal bulbous enlargement. The terminal swelling contained mostly closely packed 50 nm clear vesicles, a few dense core 100 nm vesicles (these are ubiquitous in developing axons), some larger irregular membranous sacs and mitochondria. The axoplasm of the bulb was generally electron lucent, except for flocculent material which probably represented depolymerized neurotubules and neurofilaments. Other than an occasional dense mitochondrion, there was a remarkable paucity of degenerating debris. Evidence of sloughed processes was sparse. Axons with fragmented plasma membranes and very densely clumped axoplasm and mitochondria were rarely (<1% of the nerve fibers) encountered in the nerve bundles. The identity of these profiles was not known, but some could have been skeletomotor because a vesicle-laden terminal swelling with dense axoplasm was found in a small nerve bundle. Despite extensive searching, dense degenerating boutons were never seen at the synapses. Sloughing apparently accounts for very little of the axonal loss. However, the real contribution of this mechanism may have been masked by a rapid rate of degeneration and removal of the not yet myelinated processes. The present results indicate that the majority of the transient terminal axonal branches retract rather than degenerate in situ.

1657 FURTHER ELECTROPHYSIOLOGICAL STUDIES OF NEUROMUSCULAR TRANSMISSION IN THE ANIMAL MODEL OF MYASTHENIA GRAVIS. Donald B. Sanders and Yong I. Kim* (SPON: R.N. Johnson). Dept. of Neurology, Univ. of Virginia Sch. of Med., Charlottesville, VA 22908

Neuromuscular transmission defects were examined in forelimb flexor digitorum longus (FDL) muscles from rats with experimental autoimmune myasthenia gravis (EAMG), produced by immunization with an initial subcutaneous injection of 50μg Torpedo acetylcholine receptor protein (AChRP) and a booster injection of 10 to 15 μg AChRP given two weeks or more before the experimentation.

Results show: (1) Miniature end-plate potential (MEPP) amplitude was reduced to 25 to 50% of control values; (2) The resting membrane potentials and MEPP frequency were unchanged; (3) End-plate potential (EPP) and MEPP amplitudes were reduced the same amount; (4) The waveform of indirectly elicited muscle action potentials (APs) was normal; (5) Curare sensitivity of EAMG muscles was markedly augmented as measured by twitch tension reduction; application of 4-aminopyridine restored the reduced twitch tension of curarized muscles; (6) The carbamylcholine-induced depolarization of EAMG end-plates was found to be significantly less than that seen at normal end-plates; (7) The mean number of ACh quanta released per nerve impulse in Mg^{++}-blocked preparations was not significantly different from control values.

These studies confirm that the major defect of neuromuscular transmission in EAMG results from postsynaptic abnormalities at the end-plates, presumably secondary to reduction in the number of functional ACh receptors, and support the concept that EAMG is primarily a postsynaptic disorder. EAMG in rats serves as a reasonable model of human myasthenia gravis in which a similar defect of neuromuscular transmission is present.

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1658 AN ACETYLCHOLINE RECEPTOR AGGREGATION FACTOR IS RELEASED BY SYMPATHETIC AND SPINAL CORD NEURONS. A.E. Schaffner,* R.L. Schnaar,* Z. Vogel,* and M.P. Daniels. NHLBI, NIH, Bethesda, MD 20205

Neuromuscular synapse formation is characterized by a redistribution of acetylcholine receptors (AChR) which results in receptor localization at the postsynaptic region of the muscle plasma membrane. Recently it was reported that neuroblastoma x glioma hybrid cells which form synapses with cultured muscle cells release a factor which increases the number of AChR aggregates on cultured striated myotubes, independent of new receptor synthesis (1). We now have evidence for release of such a factor by neurons in primary cell cultures.

Coculture of rat superior cervical ganglion (SCG) cells for 4 days with rat myotubes resulted in a 5.8 fold increase in the number of AChR aggregates per myotube (A/M) over control cultures which contained myotubes alone. The A/M was determined by staining cultures with tetramethylrhodamine labeled α-bungarotoxin (TMR α-BT) and counting fluorescent patches and myotubes. Incubation of myotube cultures for 24 hrs with conditioned medium (CM) from SCG cultures grown in L-15 medium without bicarbonate and containing only neurons ("adrenergic condition") caused a 1.8-2.6 fold increase in A/M over cultures which received control medium. Four day incubation with this medium yielded a 2.9 fold increase. CM from SCG cultures grown in bicarbonate buffered medium, allowing growth of glial cells ("cholinergic conditions"), caused a 3.4 fold increase in A/M. However, CM from SCG glial cells cultured without neurons did not increase the A/M in myotube cultures. AChR on myotubes were stained with TMR α-BT before application of CM from "adrenergic" cultures. This resulted in a 2.1 fold increase in the A/M, indicating that the factor caused incorporation into aggregates of AChR already on the muscle cell surface. Receptor aggregation activity was not blocked when myotubes were exposed to 100 μg/ml cycloheximide; thus the factor was effective in the absence of protein synthesis. Six day chick embryo spinal cord cells were fractionated on a Metrizamide density gradient and 3 fractions maintained in culture. CM from a fraction with a high ratio of choline acetyltransferase to lactate dehydrogenase activity (CAT/LDH) caused a 2.4 fold increase in A/M while fractions with low CAT/LDH activity caused only a 1.3-1.5 fold increase.

Studies in progress may indicate whether release of an aggregation factor is limited to cell types which can form cholinergic synapses.

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1659 A STUDY OF THE MOTOR NERVE ENDING AS A DRUG RECEPTOR FOR DIVALENT CATION AGONISTS-A RE-EVALUATION OF THE TRADITIONAL MASS ACTION APPROACH. E.M. Silinsky. Dept. Pharmacol.,Northwestern Univ. Med. Sch., Chicago, IL 60611.

Studies on the activation of depolarization-secretion coupling by divalent cations have generally relied upon the mathematical framework of Henri-Michaelis-Menten (HMM) enzyme kinetics.Such an interpretation assumes that the responses mediated by the activating cations (e.g. Ca^{++},Sr^{++}) are in accordance with the principle of mass action,whereby receptor occupation by the agonist provides a direct measure of the response. Recent results suggest however, that the binding site for Ca^{++} at the motor nerve ending behaves more in the fashion of a drug receptor (e.g. Silinsky, Brit. J. Pharm.63:485, 1978). Implicit in this pharmacological interpretation is that the full agonist, Ca^{++} may produce a maximal response with only a small percentage of the binding sites occupied and in turn leave 'spare receptors'. This study was undertaken to determine if spare Ca^{++} binding sites are present at motor nerve terminals.

Experiments were made on isolated frog cutaneous pectoris nerve-muscle preparations using conventional electrophysiological methods. Lanthanum (La^{+++}) in micromolar concentrations has been shown to antagonize irreversibly evoked transmitter release by obstructing Ca^{++} entry into nerve endings. Such an effect has been confirmed in this study. In addition, however, a small elevation in the extracellular Ca^{++} concentration in the presence of La^{+++} caused an immediate, forceful increase in evoked transmitter release. It can be demonstrated that this effect of Ca^{++} is not due to displacement of La^{+++} by Ca^{++} nor to the effect of Ca^{++} on events that follow entry of the ion into the nerve terminal. The simplest explanation appears to be that increasing the external Ca^{++} concentration surmounts the La^{+++} antagonism by 'mopping-up' spare external receptor sites for Ca^{++}. If this interpretation is correct then the following should hold: a) The partial agonist, Sr^{++} (which must occupy a large fraction of binding sites to support transmitter release), would be irreversibly antagonized by La^{+++} and an increase in the external Sr^{++} concentration should not restore transmitter release;b) Comparison of equal levels of transmitter release evoked in the presence of Ca^{++} and Sr^{++} should provide an affinity constant for Sr^{++} that is similar to the value determined for Sr^{++} as an antagonist of Ca^{++}-mediated release. The observed behaviour is in accord with these predictions.

These results suggest that spare receptors for Ca^{++} exist at frog motor nerve terminals and demonstrate that the mathematical approach of modern drug receptor theory is more appropriate than HMM kinetics when studying the agonist effects of divalent cations on transmitter release.

1660 DEVELOPMENT OF SKELETAL MUSCLE AND ACETYLCHOLINE SENSITIVITY IN THE ABSENCE OF INNERVATION. G. S. Sohal, R. K. Holt and Tony L. Creazzo*. Dept. Anat., Sch. Med., Medical College of Georgia, Augusta GA 30912.

An attempt to understand the role of nerve fibers on the development of skeletal muscle and on its acetylcholine sensitivity is being made by examining the superior oblique muscle of the duck embryo in which contact by the trochlear nerve is experimentally prevented. Trochlear nerve fibers normally reach the muscle on day 10 of incubation and the muscle at this time is primarily composed of myoblasts and a few myotubes. In order to destroy the trochlear nucleus dorsal midbrain lesions were made on day 7 of incubation by using electrocautery. The absence of the trochlear neurons and fibers was verified both from the histological serial sections of the midbrain and from silver-esterase preparations of the muscle. The control muscle on day 18 is composed of myotubes and myofibers and the motor endplates are morphologically identifiable with cholinesterase stain. Our limited observations based on a few samples of the aneurogenic muscle processed so far indicate that the overall muscle mass is greatly reduced as compared to the control on day 18. Muscle appears to be comprised of mainly myoblasts with a few myotubes. No cholinesterase deposits could be observed at this time. On day 24 the striated appearance of the muscle fibers at the light microscope level is apparent. Large deposits of cholinesterase mainly located in the belly of the muscle were also seen on this day. Ultrastructural localization of such deposits is being currently sought. The appearance, distribution and content of acetylcholine receptors in the anuerogenic muscle is currently being examined.
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1661 A STUDY OF THE PRE- AND POSTSYNAPTIC REACTIONS OF A POTENT AGONIST OF THE NICOTINIC RECEPTOR. C. E. Spivak*[1], E. X. Albuquerque[1], R. S. Aronstam*[1], M. E. Eldefrawi*[1], and B. Witkop[2]. Dept. Pharmacol. & Exptl. Therap.,[1]Univ. MD, Sch. Med., Baltimore, MD 21201 & Lab. Chem.,[2] NIAMDD, NIH, Bethesda, MD 20205 .

(+)-Anatoxin (ATX), a stable toxin in the presence of junctional acetylcholinesterase (AChE), is a bicyclic tertiary amine having potent agonist activity as revealed by our initial electrophysiological studies. ATX was shown by biological assay on frog rectus abdominis muscle to be 10-fold more potent than carbamylcholine. Frog sartorius endplates rapidly depolarized to about −45 mV in 5 μM ATX. This was accompanied by the disappearance of miniature endplate potentials (mepp) and was followed by spontaneous repolarization that continued for 50 min at a rate of about 0.6 mV/min. Upon washing, mepps returned in about 20 minutes. ATX caused no alteration of the time course of the endplate current (epc), and at high concentrations, i.e., 1 μM, it depressed the peak amplitude of the epc without, however, changing the epc's voltage and time-dependent properties. At these concentrations, when 90% reduction in epc peak amplitude was recorded, there was no change in the equilibrium potential, which is similar to that of the natural agonist acetylcholine (ACh). Iontophoretically applied to the endplate region, ATX induced an inward current whose similarity to that of ACh disclosed that the single channel conductance and lifetime were unaltered. The two agents, ACh and ATX, applied to the same endplate region using a double-barreled micropipet disclosed, however, a slower onset and offset for ATX as compared to ACh, this effect being mostly related to the inability of AChE to hydrolyze ATX. Because of its depolarizing action, the toxin markedly decreased the amplitude of the miniature epcs while producing a marked presynaptic effect, increasing the rate of release by several fold during iontophoretic application of ATX. At similar concentations, ATX inhibited 90% of the binding of [^{3}H] ACh to the membrane bound ACh receptor isolated from the electric organ of the Torpedo ocellata. ATX reduced the rate as well as equilibrium binding of [^{125}I] α-bungarotoxin. ATX also blocked the binding of [^{3}H] 3-quinuclidinyl benzilate (50 pM) to muscarinic acetylcholine receptors from rat brain with an IC_{50} of 20 μM. In conclusion the reaction of ATX with the nicotinic receptor appears remarkably similar to that of ACh. Since ATX does not change the properties of the single channel, it is suggested that neither diffusion nor hydrolysis of transmitter plays a major role in the ultimate transmitter action. Thus, ATX promises to serve as a most important tool for the study of the nicotinic receptor. (Supported in part by USPHS Grant NS-12063.)

1662 ANTICHOLINERGIC EFFECT OF AMANTADINE IN MOTOR NERVE TERMINALS P.C.Su, M.D., W.D. Niemi, PhD* and A.D. Rosen, M.D. Division of Neurology, SUNY at Stony Brook, N.Y., Dept of Phisology, Columbia University, N.Y.

Amantadine is an effective antiparkinsonism agent especially for the symptom of tremor and rigidity. The mode of action has been suggested that Amantadine causes release of donamine (Grelak, et al, Science 169:203, 1970). Due to loss of dopaminergic neuron in parkinsonism such a mechanism probably plays little role. We have, hence studied the effect of Amantadine on cholinergic nerve terminals and our results indicate that Amantadine possess significant anticholinergic effects in motor nerve terminals.

We used in vitro mouse phrenic nerve-diaphragm preparations. Intracellular recording of membrane potentials (MP); endplate potentials (EPP) and minature endplate potentials (MEPP) were made by glass microelectrode filled with 3M Kcl. The EPP's were obtained by nerve stimulation in high megnesium Kreb's soloution or in glycerol treated muscles. At least 100 to 150 EPP's were recorded on FM tape along with 60 to 100 MEPP's in each muscle fiber. Off-line analysis using an IBM 360/44 was used. The analysis includes the quantal content, probability of release (p) and available store (n). The quantal content were determined by direct methods using the ratio of mean amplitude of EPP's and MEPP's after correlation of non-linear response of EPP. The p and n were determined assuming binominal distribution of quantal release and according to formula given by Johnson and Wernig (J. Physiol. 218:757, 1971).

Amantadine at 50 uM and 150uM concentration significantly reduce the amplitude of EPP and MEPP to 60% and 90% of control respectively. The muscle membrane potentials were depolarized from -78 mV to -58 mV at 150 uM concentration and the depolarization was not noticeable at 50 uM concentration. Quantal content evokes at 1 Hz showed a 50% to 60% reduction at 150uM and a 15% to 20% reduction at 50 uM. No change in the p and n were noted. The effect on quantal content has slow onset which usually takes 60 minutes, whereas the effect on amplitude of EPP and MEPP is rapid and complete within 10 minutes. The time course of reduction in quantal content is quite similar to muscle membrane depolarization. We susnect that reduction in quantal content might be caused by the depolarization of presynaptic nerve terminals.

Based on our findings and others (Nastuk, et al, Nature 264: 76, 1976), we propose that anticholinergic effect of Amantadine both presynaptically and postsynaptically accounts for its action in antiparkinsonism.

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1663 BINDING OF ANTI-SYNAPTIC VESICLE ANTISERUM TO NERVE TERMINALS IN VERTEBRATE SKELETAL MUSCLE. Randall J. von Wedel, Joshua R. Sanes, Steven S. Carlson & Regis B. Kelly. Dept Biochem. Biophys. Univ. Calif. San Francisco, CA 94143

Antibodies directed to specific components of motor nerve terminals would be valuable molecular probes of presynaptic function, such as transmitter release, at the neuromuscular junction. Recent success in obtaining purified cholinergic synaptic vesicles from the electric organs of Narcine brasiliensis (Carlson et al., 1978) has enabled us to raise anti-synaptic vesicle antibodies in rabbits, and to demonstrate by indirect immunocytochemical techniques that antibodies in Rabbit Anti-Synaptic Vesicle Antiserum (RASVA) bind to nerve terminals in rat, chick and frog skeletal muscle. Cryostat sections were incubated with a mixture of RASVA and rhodamine-conjugated α-bungarotoxin (α-BTx) and then with a fluorescein-conjugated second antibody (goat anti-rabbit IgG). Appropriate filter combinations allowed us to excite and view fluorescein or rhodamine selectively and thus to compare the distribution of the immunohistochemical stain with that of α-BTx, which binds tightly and specifically to the acetylcholine receptors that are clustered in the postsynaptic membrane of the neuromuscular junction. Antiserum stained a variety of membranes, but most intensely stained were small patches on the muscle fiber surfaces, which could be identified as neuromuscular junctions because they also stained with rhodamine α-BTx. The RASVA-stained structures were identified as nerve terminals on the basis of their size, shape and position, and because they were absent from denervated muscle. After adsorption of RASVA with vesicle-free membranes from Narcine electric organ, nerve terminals stained intensely, but axons on the nerve or in intramuscular nerve branches did not stain well, nor did the cytoplasm of motor neuron somata. Thus, antiserum distinguishes terminal from preterminal portions of motor axons. It is particularly fortunate that antibodies in RASVA bind to nerve terminals in frog skeletal muscle, for it is in frog muscle that the best evidence has been obtained for the exocytosis hypothesis of transmitter release. This hypothesis predicts that antigens on vesicles should be hidden from antibodies applied to whole muscle, but exposed when vesicles fuse with the plasma membrane during stimulation.

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1664 PRESYNAPTIC EFFECTS OF CHRONIC ADMINISTRATION OF NEOSTIGMINE ON NEUROMUSCULAR TRANSMISSION. David F. Wilson and Ted. W. Klein*. Dept. Zoology, Miami University, Oxford, OH 45056.

Previous investigations on rats have demonstrated presynaptic effects of neostigmine treatment using high dosages (1.0 mg/Kg/day) but the dosage dependency of this effect is uncertain. In the present study the presynaptic effects of daily treatment of rats with neostigmine at 0.88 mg/Kg/day for 7-10 days was compared with rats treated with 0.44 mg/Kg/day for 7-10 days, and control rats. Intracellular recording techniques were used to examine end-plate potentials (EPPs) and miniature end-plate potentials (MEPPs) in the rat diaphragm cut muscle preparation. Quantal release was determined by the direct method. The binomial parameters store and probability of release were examined. Neostigmine treatment with 0.88 mg/Kg/day reduced quantal release of the first EPP by 60%, the statistical store by 59% and the mobilization rate by 65%. The probability of release and facilitation properties of the nerve were not significantly affected. Neostigmine treatment with 0.44 mg/Kg/day did not significantly affect any of the presynaptic parameters. These results suggest that chronic treatment of Myasthenic patients with anticholinesterases normally does not lead to alterations in transmitter release unless high dosages are administered.

1665 IMMUNOHISTOCHEMICAL LOCALIZATION OF ACETYLCHOLINE RECEPTORS ON RAT MYOTUBE SURFACE WITH MONOCLONAL ANTIBODIES. Riley C. Yu, D.P. Richman* and C.M. Gomez*, Department of Neurology and Brain Research Institute, University of Chicago, Chicago, IL 60637.

Progress towards understanding neuromuscular conduction at the molecular level has been greatly aided by studies of binding properties of cholinergic ligands to membrane receptors using biochemical and morphological techniques. We have used anti-acetylcholine receptor (AChR) antibodies secreted by cloned hybridomas produced by fusion of a myeloma cell line with spleen cells from animals immunized with purified Torpedo AChR (Gomez, et al., Biochem. Biophys. Res. Com., 1979, in press). Dissociated cells from leg muscle of new-born Wistar rats were first preplated for myoblast enrichment before being seeded on collagen-coated coverslips and nourished with regular muscle culture medium. AChR appeared on the membrane surface as the myoblasts fused to multinucleated myotubes which manifested spontaneous contraction and cross-striations. Application of concentrated supernatant from one clonal hybridoma culture, followed by labeling with goat anti-rat IgG conjugated with fluorescein or with peroxidase resulted in stained patches only on the myotube surface. The background mononucleated cells showed no staining. These patches were thought to be indicative of specific AChR sites, because no staining was found in similar experiments with cultures preincubated with alpha bungarotoxin (α-Btx). When mature and fully developed, each myotube showed 2-3 ring-shaped patches of AChR staining located in the central portion of the myotube. On young myotubes, however, staining revealed numerous small, round patches, some of which were distally located on the myotubes. A similar staining pattern has also been observed with antibody from another hybridoma, but was not blocked by α-Btx. Antibody from two other hybridomas stained the myotube surface with a totally different pattern of numerous, scattered small spots. We are investigating further the identity of these surface receptors by providing neuronal input combined with electrophysiologic analysis in these myotube cultures. Antibodies from clones which showed negative results in our immunohistological experiment may still possess affinity to mammalian AChR which is not located on the membrane surface. This is suggested from an early observation (Gomez, et al., 1979, Fed. Proc. 38:1421) that one of these monoclonal antibodies produced experimental myasthenia when injected into animals intravenously. The specificity provided by monoclonal antibodies combined with the great sensitivity of immunohistochemical analysis in a tissue culture system has proved a valuable tool in our effort to understand the development and fine structure of AChR at the molecular level.

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1666 A FURTHER EXAMINATION OF FACILITATION OF TRANSMITTER RELEASE AT THE FROG NEUROMUSCULAR JUNCTION. J. E. Zengel & K. L. Magleby. Dept. of Anat., Emory Univ., School of Med., Atlanta, GA 30322 and Dept. of Physiol. and Biophysics, Univ. of Miami, School of Med., Miami, FL 33101.

There are a number of differences in the literature concerning the kinetic properties of facilitation of transmitter release. Mallart & Martin (1967) and Magleby (1973) were able to approximate the increase in transmitter release during short trains of repetitive stimulation at the frog neuromuscular junction using a linear model of facilitation. Younkin (1974), on the other hand, developed a power facilitation model to describe transmitter release in the frog. More recently, Balnave & Gage (1977) were unable to describe data they obtained from the toad neuromuscular junction with a linear facilitation model, but used instead a two-step kinetic model. In all of these previous studies the data were analyzed without correcting for changes in transmitter release that might arise from augmentation and potentiation, two processes with time constants longer than facilitation that also act to increase transmitter release during and following repetitive stimulation. We have reexamined several models of facilitation, taking into account the effects of augmentation and potentiation.

End-plate potentials (EPP's) were recorded from the frog sartorius nerve-muscle preparation under conditions of low quantal content (0.4-0.6 mM Ca, 5 mM Mg). The nerve was conditioned with short trains of impulses (10-40 impulses at 10-100/sec), then tested with single impulses applied at varying intervals after the conditioning stimulation. The decay of the testing EPP amplitude was corrected for the contributions of augmentation and potentiation and estimates of facilitation were obtained assuming three models: a linear model, such that

$$F = F_1^* + F_2^*$$

where F is facilitation and F_1^* and F_2^* are the factors responsible for the first and second components of facilitation; a power model, such that

$$F = (F_1^* + F_2^* + 1)^q - 1.$$

where q is the power relating the factors responsible for facilitation to facilitation; and a multiplicative model, such that

$$F = (F_1^* + 1)(F_2^* + 1) - 1.$$

Both the power and multiplicative models of facilitation could describe the effect of repetitive stimulation on facilitation of transmitter release; the additive model consistently gave poorer fits to the data. Supported by NIH grant NS 10277.

NEURONAL CIRCUITS AND PATTERN GENERATION

1667 BIOGENIC AMINE ACTIONS ON LIMULUS CARDIAC GANGLION NEURONS. George Augustine, Raymond Fetterer and Winsor Watson III*. Depts. of Zoology, Univ. of Maryland, College Park, MD 20742, Michigan State Univ., E. Lansing, MI 48824, and Univ. of New Hampshire, Durham, NH 03824.

The Limulus cardiac ganglion is a small neural network which generates the rhythmic heartbeat of this animal. It is known that a variety of biogenic amines alter the activity of this ganglion, but the cellular basis of their effects is unknown. We have therefore examined the effects of several amines on the physiological properties of cardiac ganglion neurons. The Limulus cardiac ganglion is composed of 100-400 neurons of two functionally different types. Pacemaker cells are spontaneously active neurons which generate the heartbeat rhythm, and follower cells are motorneurons which are postsynaptic to the pacemakers and innervate the cardiac muscle. Standard intracellular electrophysiological techniques were used to monitor the membrane potential of these two types of neurons. Most experiments were performed on the follower cells, which are larger and consequently more readily examined with intracellular electrodes. Bath application of octopamine (OCT), dopamine (DA), epinepherine (EPI) or norepinepherine (NE) produced increases in the frequency of bursting activity of follower cells. OCT was the most effective, increasing burst frequency by 120% (± 14% S.E.M.) at a concentration of 10^{-7} M. The excitatory effects of all four amines was reduced by the α-adrenergic antagonist phentolamine (10^{-5} M). In addition to their excitatory effects, DA and NE produced a transient inhibition of burst rate. This transient inhibition was blocked by the serotonin antagonist metergoline (10^{-5} M). Changes in follower cell burst frequency were a consequence of corresponding changes in pacemaker cell activity. Recordings from pacemaker neurons during amine treatment showed that the frequency of spontaneous action potentials increased, accompanied by a small (less than 5 mV) depolarization of membrane potential. A transient decrease in pacemaker action potential rate was observed with DA or NE perfusion. In addition to their effects on pacemaker cells, these amines also directly influence follower cell activity. DA and NE produced transient hyperpolarizations of follower cell membrane potential (up to 20 mV at 10^{-6} M), and OCT depolarized these cells (less than 10 mV at 10^{-6} M). These effects are not due to alterations in the synaptic input on to these cells, for they occurred in preparations where synaptic activity was blocked by addition of Co^{++} or Mg^{++}, or removal of Ca^{++}. Thus these biogenic amines are active on both types of neurons which comprise the Limulus cardiac ganglion.

1668 SUPPRESSION OF THE ACTIVITY OF SECONDARY IMPULSE INITIATION SITES BY THE ACTIVITY OF A PRIMARY INITIATION SITE IN RHYTHMICALLY ACTIVE NEURONS. Ronald L. Calabrese. Dept. of Biology, Harvard University, Cambridge, MA 02138.

The heart interneurons of the leech ventral nerve cord are normally active in rhythmic impulse bursts. Recent experiments indicate that this impulse burst rhythm is endogenous in origin (Calabrese, 1979, J. Exp. Biol., in press). The majority of the heart interneurons possesses impulse initiation sites in each segmental ganglion through which their respective axons pass and each of these initiation sites is capable of producing rhythmic impulse bursts endogenously. At any given time however, only one of an interneuron's initiation sites is rhythmically active, the others are apparently suppressed. The active initiation site is usually the one in the ganglion where the interneuron's cell body is located (Primary Initiation Site) and the activity of the other sites (Secondary Initiation Sites) is expressed only if the axon of the interneuron is severed or the primary site's activity is suppressed by injected hyperpolarizing current.

Experiments were performed to determine how the Primary Site suppresses the activity of the others. Two consistent observations emerged:

a) The free run period of the Primary Initiation Site's rhythmic activity is smaller than that of the Secondary Initiation Sites.

b) Intercalated impulse bursts from the Primary Initiation Site resets the rhythm of the Secondary Initiation Sites by phase delaying their next impulse burst.

From these observations the hypothesis was developed that suppression is accomplished through cycle by cycle reseting of the Secondary Initiation Sites impulse burst rhythm by the faster rhythm of the Primary Initiation Site. This hypothesis was tested directly by intercalating regular trains of impulse bursts from the Primary Initiation Site into the Secondary Initiation Sites' impulse burst rhythm. If the period of the intercalated bursts was greater than the free run period of the Secondary Initiation Sites suppression was not accomplished but if it were smaller then suppression was complete.

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1669 NONLINEAR ANALYSIS REVEALS CRITERIA FOR INTRINSIC OSCILLATIONS IN "BURSTING"NEURONS. N. T. Carnevale, Dep't. Neurol., Scho. Med., SUNY, Stony Brook, N.Y. 11794.

Certain features are common among neurons which display an intrinsic slow oscillation of membrane potential: a steady-state inward current associated with a negative slope conductance (NRC), and a depolarization-induced slow outward current (Sci 186:932 (74)). However, the exact circumstances under which oscillations will arise have remained unclear; for example, not all cells with an NRC oscillate, and some question has arisen whether an NRC is even a prerequisite for oscillations. (Br.Res. 94:161 (75), Neuro-Sci.Abstr. #565 (78)). Detailed models of oscillating neurons, while elegant, are cumbersome and have tended to obscure critical features of the interaction between these membrane current components. To delineate the conditions which must be met in order for oscillations to occur, and to determine the factors which underlie distinguishing characteristics of the oscillations, a model which incorporates the slow outward and NRC-associated inward current was studied.

This model led to a set of non-linear differential equations which describe a phase space in which changes of the state of the model are analogous to movement along paths (trajectories) of "rapid" or "slow" motion (Andronow et al., Theory of Oscillators, p. 660 (Pergamon, 1966)). Piecewise linear analysis of these equations, confirmed by analog computer simulation, demonstrated several points clearly, including the following: 1, the oscillation requires an NRC, which must persist throughout the accelerating portion of the depolarizing phase of the oscillation; 2, increased leak conductance can abolish the oscillation; 3, the inward and outward currents remain partially activated throughout the oscillatory cycle; 4, a too rapidly activating outward current can prevent oscillations; 5, the reversal potential of the slow outward current must be hyperpolarized relative to the NRC region, or else inactivation of the inward current must play a dominant role in the repolarizing phase of the oscillation. This model also nicely illustrated the mechanism by which polarizing currents influence the frequency and amplitude of the oscillation. The conclusions drawn from the piecewise linear analysis serve as the basis for quantitative criteria for the prediction of oscillations in the more general non-linear model and in the real cell.

1670 A SIMPLE VERTEBRATE LOCOMOTOR MODEL: "FICTIVE SWIMMING" IN THE IN VITRO LAMPREY SPINAL CORD. A.H. Cohen and P. Wallén.* Dept. of Physiol. III, Karolinska Institute, 114 33 Stockholm, Sweden.

Little is known of the cellular organization of vertebrate central pattern generators (CPG's). Swimming in fish is a complex motor behavior which is produced by CPG's in the spinal cord (Grillner et al., Brain Res., 109:255-269, 1976). The undulatory body movements of fish result from a specific activation pattern consisting of a strict alternation between the two sides of a single segment and a constant phase coupling between successive segments (Grillner, Exp. Brain Res., 20:459-470, 1974).

To investigate the spinal pattern generator for locomotion in detail, we have used an in vitro preparation consisting of the isolated spinal cord of Icthyomyzon unicuspis. 50-60 segments were dissected out along with the notochord and surrounding membranes. The tissue was maintained in cooled aerated lamprey Ringers (pH = 7.4). Curare was added to prevent the small amplitude movements sometimes produced by the residual muscle tissue. Low concentrations of D-glutamate or L-DOPA were used to induce swimming movements, following the technique of Poon in the lamprey "myotome" preparation (submitted and cf. Teräväinen and Rovainen, J. Neurophysiol., 34:990-998, 1971). The ventral root (VR) discharges were monitored with suction electrodes in the conventional manner.

In the presence of D-glutamate and curare the isolated cords were capable of generating efferent activity having all the characteristics of normal swimming. 1) There was strict alternation between two roots of a single segment. 2) The duration of a burst of a VR increased linearly with the cycle duration, as is the case in intact fish. 3) The latency between bursts in successive roots increased linearly with cycle duration, i.e. there was constant phase coupling between the segments. To date, a piece of cord consisting of as few as 6 segments could produce this pattern, although such reduced pieces may be unstable.

The fibers ensuring the phase coupling were found to be highly distributed, as aspects of the coordination were preserved even after extensive transverse lesions including 80% of the cord width. The location of the lesion had effects on the coupling, but no region was found to be essential. Microstimulation of many different regions could affect the structure of the cycle. In addition, the alternation between the roots of a single segment was not disrupted by longitudinal lesion of 1-4 segments in the midline region.

This is the first preparation in which a complete locomotor pattern has been produced by a portion of an isolated vertebrate central nervous system maintained under in vitro conditions. Moreover, the efferent pattern can be highly stable and show all the essential features of the normal pattern.

1671 DRIVER POTENTIALS ISOLATED IN CRUSTACEAN CARDIAC GANGLION CELLS BY LIGATURING. I. M. Cooke* and K. Tazaki* (SPON: J. E. Bardach). Békésy Lab. of Neurobiology, U. of Hawaii, Honolulu, HI 96822.

Crab (Portunus sanguinolentus) cardiac ganglion neurons exhibit 20 mV, 200 msec, TTX-resistant, Ca-dependent, depolarizing responses to depolarizing pulses (J. Neurophysiol., in press). We now report these 'driver potentials' (DPs) in another crab, Thalamita crenata, and in the lobsters, Panulirus penicillatus and Homarus americanus. Ligaturing experiments provide evidence that DPs are intrinsic responses and arise at a site near the soma separate from the site for impulse initiation, which is a distance of 1 mm or more along the initial axon segment. In Homarus, a ligature was placed between the anterior bifurcation and one of the most anterior cells (1 or 2); extracellular electrodes were positioned on each side of the tie, and intracellular electrodes recorded from the anterior cell and the cell at the bifurcation (Cell 3). All electrodes remained in place while the ligature was tightened. All impulse activity ceased anterior to the ligature while uninterrupted bursting activity continued posterior to it. Current-passing confirmed interruption of the electrotonic connection between Cell 3 and the anterior cell. Depolarizing current passed into the anterior cell elicited a DP, but no impulses. TTX caused no change in amplitude and a slight increase in duration of the mechanically isolated DP. In Portunus, intra- and extracellular electrodes were maintained in place while tightening a ligature midway between the anterior and posterior large cell groups. Posterior to the tie, small cell bursting remained; corresponding synaptic responses and slow depolarization, recorded in a large cell, occasionally led to DPs, but there were no posterior large cell impulses. Electrotonic pathways between anterior and posterior cells were blocked, but not those among anterior cells. Anterior to the ligature, all small cell activity ceased, while large cells showed spontaneous pacemaker potentials leading to 'escape' firing or to DPs driving impulse bursts. Depolarizing pulses elicited DPs with bursts. These observations provide evidence that DPs: 1) probably are a property of most crustacean cardiac ganglia; they may correspond to 'plateau potentials' observed in decapod stomatogastric ganglia; 2) represent an intrinsic response of individual neurons and are not dependent on neuronal interaction; 3) are generated in or between the soma and the impulse initiation zone; 4) can occur independently of impulse initiation mechanisms; 5) are not greatly distorted by the presence of $3X10^{-7}$M TTX, thus supporting the extension of TTX observations to normal functioning.
Supported by NIH grant NS11808.

1672 DETECTION OF RECURRING PATTERNS IN SPIKE TRAINS. Judith Dayhoff and George Gerstein. Dept. Physiology, University of Pennsylvania, Philadelphia, Pa. 19104

Repeating patterns in nerve spike trains could be important for information transfer in the nervous system. Patterns could be generated in response to internal stimuli occurring at times unknown to the experimenter. Thus, it is appropriate to search for patterns of firing which occur frequently but at unspecified times in the spike train. Such patterns might not be seen with an autocorrelogram or with other traditional spike train methods. We have developed a new pattern detection method based on comparing a series of template patterns to the spike train. This method differs from our previously presented method (Dayhoff and Gerstein, Neurosci. Abst. 4: 380(1978)) because it is able to detect frequently occurring spike patterns which are approximately the same each time they occur but can have extra spikes superimposed on the pattern. These extra spikes could be due to background noise or a variation in the pattern.

Our new pattern detection method tests whether a temporal pattern occurs more frequently than expected in a random model. The random model used is a shuffled version of the data spike train. The patterns detected consist of an arbitrary number of spikes. The computer program does not require a priori specification of a template pattern; it chooses a series of several hundred templates from the spike train, and tests to see if each occurs more than expected at random. The test is achieved by comparing each template pattern to every place in the data spike train and to every place in a number of shuffled versions of the spike train. In each comparison, extra spikes in the template or in the spike train are ignored. Those patterns which match the data spike train considerably more often than they match the shuffled spike trains are identified as patterns which occur unusually often in the data.

Results of analysis of both simulated and experimental data will be presented. Simulated spike trains containing known patterns were analyzed in order to assess the pattern detection sensitivity of the method. The experimental data analyzed consists of crayfish and cat spike trains.
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1673 MODEL CIRCUIT FOR THE CONTROL OF MOTOR NEURON ACTIVITY PATTERNS IN DROSOPHILA FLIGHT. W. O. Friesen and R. J. Wyman. Dept. Biol., Univ. of Virginia, Charlottesville, VA 22901 and Dept. Biol., Yale Univ., New Haven, CT 06520.

Each fiber of the fibrillar flight muscles of Drosophila melanogaster is innervated by a single motor neuron. Recordings from individual muscle fibers have revealed that motor neurons innervating different fibers of a single muscle fire cyclically, with each motor neuron firing once per cycle. Of the six possible firing sequences for the four motor neurons innervating the ventral fibers of the DLM (units c, d, e and f) two, c-e-d-f and c-f-d-e, are preferred. Subsequent to antidromic stimulation of one motor neuron, firing in the others is inhibited for tens of milliseconds and the phase of the activity cycle is shifted. To account for these results Harcombe and Wyman (J. Neurophysiol. 40:1066-1077, 1977) proposed that the motor neurons innervating the DLM form an inhibitory circuit in which each neuron inhibits each of the others and that this mutual inhibition is greatest between c-d and between e-f.

To determine whether this circiut can account for motor neuron firing patterns observed during Drosophila flight, we have constructed an equivalent circuit with electronic neural analogs (neuromimes). These neuromimes were designed by Lewis (Proc. Inst. Elect. Engrs. 56:931-949, 1968) and have been employed in previous modeling studies of neural networks (Friesen and Stent, Biol. Cybernetics 28:27-40, 1977). The Lewis neuromimes mimic the Hodgkin-Huxley parameters describing large-scale voltage fluctuations at the neural impulse initiating zone and can model both excitatory and inhibitory synaptic interactions. We provided four neuromimes with common excitatory input and adjusted thresholds so that each had a 6 Hz free-running impulse frequency. We then connected each neuromime to each of the other three by way of their inhibitory inputs, setting synaptic time constants to 30ms and synaptic amplitudes such that inhibition between those neuromimes modeling motor neuron pairs c-d and e-f was twice that of the other connections, since quantitative physiological data for these parameters is lacking.

This neuromime network produced cyclic activity patterns closely resembling those observed in Drosophila flight muscles, confirming the prediction that a mutually inhibitory circuit can account for the physiological firing patterns. The preferred motor neuron fixing sequences observed in Drosophila were also the sequences most frequently observed in modeling experiments. Finally, by producing additional impulses in individual neuromimes, the phase of the neuromime activity pattern was shifted as during physiological experiments.
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1674 THE PRIMARY ACOUSTIC STARTLE CIRCUIT IN THE RAT. D. S. Gendelman* and M. Davis. Dept. of Psychiatry, Yale Univ. Sch. Med., New Haven, CT 06508

The latency of the acoustic startle reflex in the rat is 6-7 msec., measured from tone onset to the beginning of the electromyographic response in the forepaw (Ison et al., Physiol. Behav., 1973, 10, 1035-1039). This extremely short latency indicates that only a few synapses could be involved in the primary acoustic startle circuit. Acoustic startle is being used as a model system for studying habituation, sensitization, pre-pulse inhibition, classical conditioning, fear or anxiety, and drug effects on behavior. Hence, the delineation of the exact acoustic startle circuit would provide critical information for further study in all of these areas.

Because of this, we have investigated the possible neural connections between the cochlear n., which receives the primary afferent input, and the reticulo-spinal tract, which most probably represents the final motor pathway of startle. Unilateral, single pulse electrical stimulation of specific ventral points within the nucleus reticularis pontis caudalis, the cell bodies which form the reticulo-spinal tract, elicit short latency, startle-like responses in unanesthetized rats. Bilateral lesions of these points abolish acoustically elicited startle responses. Reaction product from horseradish peroxidase iontophoresed into these points is found in the nuclei of the lateral lemniscus, which are known to be innervated by the cochlear nuclei. Unilateral, single-pulse stimulation of the nuclei of the lateral lemniscus elicit short latency startle-like responses. Bilateral lesions of these nuclei attenuate or abolish acoustically elicited startle. Latencies of electrically elicited startle become shorter as electrodes are placed progressively further down the circuit.

Simultaneous presentation of electric and acoustic stimuli elicit startle amplitudes greater than the sum of each presented alone. Temporal interactions between acoustic and electrical stimuli occur when one follows the other in time.

The data suggest that the primary acoustic startle circuit in the rat is: auditory nerve, cochlear nucleus, nuclei of the lateral lemniscus, nucleus reticularis pontis caudalis, spinal interneuron, lower motor neuron, muscles. Hence five synapses, plus the neuromuscular junction, are probably involved.

1675 NEURONAL BASIS OF THE SWIMMING RHYTHM IN TRITONIA.
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The swimming rhythm of the marine mollusc, Tritonia diomedea, is a series of 2-20 cycles of alternating dorsal and ventral flexions. The central pattern generating network mediating swimming consists of at least three populations of cerebral premotor interneurons designated C2, dorsal swim interneurons (DSI), and ventral swim interneurons (VSI). The temporal firing pattern of these interneurons during a swim sequence was analyzed to elucidate neuronal mechanism involved in the initiation, maintenance, and termination of the swim pattern. Over the time course of a swim sequence, cycle period progressively increases. For a given preparation, oscillation terminates when the cycle period attains a maximal duration independent of the number of preceding cycles. Each swim cycle may be subdivided into three segments by the firing times of the three interneuron populations. Variation in the initial segment of each cycle accounts for 80 percent of the total increase in cycle period. The latter two segments remain relatively constant independent of cycle period. Cyclic bursting within the swim network is superimposed on a large average depolarization (10-15 millivolts) which slowly dissipates over the course of a swim sequence. Oscillation rate appears to be directly proportional to the level of tonic network excitation (e.g. cycle period decreases as tonic excitation increases).

Swim network oscillation is initiated by a tonic depolarization established by a transient sensory stimulus. Pattern generation is maintained as long as phasic positive feedback between C2 and the DSI is established during the initial and middle segments of each swim cycle. Positive feedback between C2 and DSI is required for maintenance of oscillation but is not required for the initiation of a swim sequence. During a swim sequence, dissipation of the tonic depolarization level decreases the likelihood of the positive feedback. Oscillation terminates when the tonic network excitation falls sufficiently to prevent C2/DSI positive feedback. A swim sequence ends on a weak dorsal swim interneuron burst corresponding to the last dorsal flexion of the behavior.

1676 RHYTHMICALLY OCCURRING SYNAPTIC POTENTIALS IN TRIGEMINAL MOTONEURONS EVOKED BY REPETITIVE CORTICAL STIMULATION
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Chewing, lapping and suckling are motor behaviors which have a strong rhythmic component. The guinea pig is used in this study as an animal model for the investigation of central nervous system pattern generators which may be responsible for the production of these rhythmically occurring jaw movements.

A single shock or short train of shocks delivered to the motor face area of the cortex of ketamine anesthetized guinea pigs evokes a brief jaw opening response. Intracellular recording in trigeminal motoneurons innervating the jaw opener and closer muscles reveals that the stimulus evokes membrane depolarization and spikes in jaw opener, and a reciprocal hyperpolarization in jaw closer motoneurons. The latency from the cortical stimulus to the onset of the response is ≈ 10 msec and the duration ≈ 30 msec. The hyperpolarization in jaw closer motoneurons changes to a depolarizing potential with the injection of Cl^- and can be reversed by hyperpolarization of the membrane with current passed through the recording microelectrode. These results indicate that the hyperpolarization is postsynaptic. When the stimulus intensity is reduced to well below threshold to evoke a response to a single shock, continuous stimulation at frequencies of between 20 and 40 hz produces the following: the initial stimuli of the train do not evoke a response in the motoneurons; after a variable period of time, each succeeding stimulus gradually becomes more effective until a maximum level of response is reached. This is accompanied by a sustained jaw opening since each stimulus is producing depolarization and spikes in opener motoneurons and a simultaneous hyperpolarizing potential in closer motoneurons. In most instances a rhythmic pattern is then initiated; each successive stimulus becomes gradually less effective, the opener motoneuron spiking ceases, the hyperpolarization in the closer motoneurons diminishes, and the jaw returns to the original rest position. Stimulus effectiveness then cyclically waxes and wanes and rhythmic (≈ 2 hz) opening and closing of the jaw is produced. Cortical stimulation with the animal paralyzed evokes a similar pattern of synaptic response. The results suggest that, 1) a facilitatory mechanism is activated by the high frequency cortical stimulation which greatly increases the effectiveness of the motor cortex-trigeminal motoneuron pathway and, 2) a centrally mediated oscillation can be activated which rhythmically gates this facilitation of the pathway. These central mechanisms result in rhythmic jaw movements similar in basic form to those observed in chewing or lapping behavior.
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1677 ORGANIZATION AND GENERATION OF MOTORNEURON BURSTS DURING TRITONIA SWIMMING.
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Escape swimming in Tritonia diomedea is characterized by alternating bursts of activity in two populations of antagonistic motorneurons, the Dorsal Flexion Neurons (DFN) and the Ventral Flexion Neurons (VFN). The basic swim pattern is generated by an identified network of premotor interneurons. Here we report on the temporal organization of the firing pattern in the flexion neuron pools, and on the synaptic interactions between the premotor interneurons and identified flexion neurons.

The VFN pool is relatively homogeneous. Swim bursts in VFNs are 1.5-3 seconds in duration and begin at virtually the same time in all VFNs. The DFN pool is made up of two major subpopulations. The DFN-A bursts are of long duration (3-5 seconds), have two frequency peaks per burst, and increase in duration as the swim cycle period increases. The DFN-A burst begins at a constant latency after the onset of firing in the VFNs. In contrast, DFN-B bursts are of shorter duration (2-3.5 seconds), have a single frequency peak, and do not increase in duration as cycle time increases. The latency from the beginning of a VFN burst to the onset of a DFN-B burst increases as cycle time increases, such that DFN-B bursts occur at nearly a constant phase in every cycle. Recordings from whole animal preparations indicate that cells in the DFN-B pool are responsible for generating most of the dorsal flexion movement.

Simultaneous intracellular recordings were made from identified pattern generator interneurons (C2, DSI, VSI) and identified flexion neurons in a search for monosynaptic connections. Monosynapticity was judged by 1) postsynaptic potential (PSP) at constant latency following a presynaptic spike 2) ability to affect postsynaptic potential by presynaptic polarization and 3) maintenance of postsynaptic potential in 2 1/2 times normal Ca, Mg sea water. By these criteria all three classes of interneurons make monosynaptic connections onto most flexion neurons. Some of the monosynaptic events are discrete EPSPs or IPSPs with a rise time of ≈ 50 msec, but many of the monosynaptic PSPs are very slow, with a rise time of greater than 500 msec. Many flexion neurons receive dual component PSPs from the DSI and/or the C2. The synaptic connections from the interneurons to the two classes of DFNs are distinctly different. As an example, C2 excites DFN-A cells but inhibits DFN-B cells. No monosynaptic connections were observed from flexion neurons to interneurons, nor among flexion neurons. The identified monosynaptic connections from C2, DSI, and VSI appear to be sufficient to account for much of the temporal organization of flexion neuron activity during a swim.

1678 

PARALLEL RECORDING OF SINGLE UNIT ACTIVITY IN VIVO.
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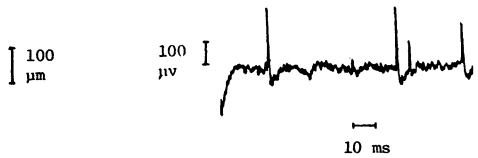
We have developed a high-density fixed-array multimicroelectrode for monitoring extracellular neural activity in vivo. The PRONG (Parallel Recording of Neural Groups) can be used to simultaneously record from neighboring neurons along any 2 mm extent of brain tissue that is accessible within 7 mm of the surface. It is fabricated using microelectronic technology and consists of a linear array of recording sites fixed on a hair thin substrate (20 μm thick, 2 mm long). In the prototype, an electrochemically etched molybdenum substrate supports gold leads insulated with photoresist. The leads start at contact pads at the top of the PRONG and end in a wedge-shaped area containing an array of 20 recording sites (100 μm spacing) at the bottom tip of the PRONG. The shunt capacitance is about 3 pf. The area of the exposed recording sites can be varied during fabrication from 40 to 120 μm^2, and each site is plated with platinum black. For implantation, the PRONG is supported by a connector fixed to a microdrive. The connector springloads each of the contact pads of the PRONG with thin beryllium copper wire.

The PRONG has been used to record single units in cat visual cortex with 250 μv amplitude spikes with a signal to noise ratio of 10:1.

New versions of the PRONG with more recording sites and a thinner active area are under construction.
(Research supported by NIH grants 5 R01 EY00676 and 1 T31 GM07484.)



1679 DIAMETRIC PATTERN ENSEMBLES IN NEURONAL INTERSPIKE INTERVALS DURING SEVERAL BEHAVIORAL STATES IN THE CAT. T.J. Marczynski and L.L. Burns* (SPON: L. Isaac). Dept. Med. Pharmacology, Univ. of Ill. Med. Center, Chicago, Ill. 60612.

The activity of single cells was recorded chronically in awake freely moving animals from the centromedian-parafascicular (CM-Pf) area of the cat thalamus using fine wire semimicroelectrode bundles. Neuronal spike trains were analyzed by a pattern detection technique which made inequality statements about consecutive interspike intervals; an interval was either longer (+) or shorter (-) than the previous interval. Thus a consecutive series of (+) and (-) signs was generated which corresponded to the interval lengths of the neuronal spike train. A movable window of variable size then moved through the spike train one sign at a time tabulating occurrences of patterns 3 through 6 signs in length. The number of occurrences of each pattern encountered was compared to the expected occurrence calculated from a theoretical model which assumes independence (Brudno and Marczynski Brain Res. 125: 65, 1977). Chi-squared tests of significance were used for all comparisons. Patterns which were emitted more often than predicted by the theoretical model and those which were suppressed, i.e. appeared significantly less often than predicted, were both seen in the data and often constituted an ensemble, or idiosyncratic profile of patterns for a particular behavioral state.

Many neurons were found which, in paired sets of data, showed diametric tendencies; patterns emitted in one behavioral state were significantly suppressed in another and vice versa. Several neurons showed this type of behavior during slow wave sleep (SWS) and quiet wakefulness; other neurons were diametric during SWS and paradoxical sleep. Still other neurons were diametric during various phases of motor behavior. Some of the patterns in an ensemble did not show diametric changes; in many cases only a few patterns would reverse. In many instances diametric shifts would occur without changes in the mean firing rate.

The results suggest that neurons may increase the signal-to-noise ratio in certain pathways by actively suppressing certain temporal patterns below their chance occurrences. Even a small diametric shift in these patterns would constitute a highly significant signal against an otherwise noisy backround. The observed neuronal behavior is comparable to an anacoluthon, a change in syntax to achieve rhetorical effect, hence we propose to term this neuronal behavior anacoluthic.

1680 DIRECT PROJECTION OF PAUSER NEURONS TO NYSTAGMUS-RELATED PONTOMEDULLARY RETICULAR BURST NEURONS. Charles H. Markham, Shozo Nakao* and Ian S. Curthoys*. Dept. Neurology, UCLA School of Medicine, Los Angeles, CA 90024.

Pauser neurons (PNs) which exhibit a tonic discharge ceasing prior to and during saccades, have been suggested to be important in the control of saccadic eye movements. The present study aims to demonstrate the role of PNs in vestibular nystagmus and to provide evidence that they project to and produce inhibitory effects in a class of reticular burst neurons which inhibit contralateral abducens motoneurons at the quick phase (BINs) (Hikosaka et al. Exp. Brain Res. 33:337-352, 1978).

Extracellular spike discharges of single PNs were recorded in the raphé nucleus near the rostral pole of the abducens nucleus during nystagmus in alert cats rendered pain-free by extensive local anesthesia. Abrupt cessation of PN tonic discharges during the slow phase was coincident with the onset of the negative field potential in the ipsilateral BIN area which preceded BIN burst activity at the quick phase. Microstimulation (less than 20μA) of the PN area using glass micropipettes suppressed burst activities of BINs.

Antidromic activation of PNs from the BIN area was examined. Of 54 PNs, 48 were antidromically activated from the ipsilateral BIN area. The spike latencies ranged from 0.3 to 1.2 msec (mean 0.75 msec). Fourteen neurons were also activated antidromically from the contralateral BIN area. Antidromic activation of PNs was further studied by systematic tracking with the stimulating microelectrode in saggital (L 0.5) and transverse (P 7.5) planes through the ipsilateral BIN area. There were two or three low threshold foci (less than 30μA) which were separated by nonresponsive sites. The effective sites were found mostly in the BIN area (around 0.5 mm lateral from the midline, and 1.0 to 3.0 mm deep from the floor of the fourth ventricle), indicating axonal branching in this area.

During intracellular recording of BINs, microstimulation of the PN area induced a hyperpolarization with latencies ranging from 0.7 to 1.5 msec (mean 1.0 msec) which were inverted into depolarizing potentials by passing hyperpolarizing currents. This indicates that the hyperpolarizations are monosynaptic IPSPs. The IPSPs induced on stimulating the PN area could explain IPSPs found in the same BINs during the slow phase of nystagmus.

It is concluded that burst activity of BINs may be caused by release of the direct inhibition from PNs (disinhibition).

1681 RAPID KILLING OF SINGLE NEURONS BY ILLUMINATION OF INTRACELLULARLY INJECTED DYE. John P. Miller and Allen I. Selverston. Biology Dept., UCSD, La Jolla ,Ca., 92093.

We have approached questions of neuronal function and connectivity with a new technique for rapidly killing all or part of single identified neurons. To examine the effect of removing one cell from a circuit, the lobster stomatogastric ganglion was used. This ganglion generates two motor patterns, and the connectivity of the 30 neurons it contains has been well worked out.The cell to be killed was first filled with the dye Lucifer Yellow CH via intracellular iontophoresis. The dye itself had no effect on the resting-, action-, or post synaptic potentials, or on the input resistance in any of the cells from which we recorded. To kill the dye-filled cell, the ganglion was illuminated with high intensity blue light. (This dye absorbs at 426 nm and fluoresces brilliantly at 540 nm, allowing visualization of the cell during the illumination.) Within 5 min. of illumination, the membrane potential of the cell depolarized to zero, all psp's onto follower cells disappeared, and action potentials were no longer observable on the cell's peripheral axon. Electron microscopy showed cytoplasmic vacuolization, mitochondrial disruption, and general membrane fragmentation in such dye-filled and irradiated cells. No structural or physiological damage was detectable in unfilled cells.

This method has been used to test our understanding of the circuitry underlying pattern generation by the ganglion. Elimination of a neuron predicted to be crucial to pattern coordination (such as the AB interneuron) causes disruption of the output pattern. Elimination of cells with roles predicted to be less crucial causes little disruption of ongoing activity.

In order to demonstrate the extent to which a dye-filled cell is damaged when only a portion of it is illuminated, one segment of a crayfish lateral giant axon was filled iontophoretically with Lucifer Yellow. After irradiation of a small portion (6%) near the center of the axon for 10 min., conduction of action potentials was blocked at this central point. However, the un-irradiated portions could still conduct action potentials. Thus, elimination of a fraction of a single neuron's dendritic tree may now be possible through a combination of this method and microbeam irradiation.

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1682 TWO FIRING PATTERNS IN AN IDENTIFIED MOLLUSCAN NEURON. Mark W. Miller* and Matej Stepita-Klauco. (SPON: W.A. Wilson,Jr.) Department of Biobehavioral Sciences, U.Conn., Storrs, CT. 06268

Experiments have been conducted on an identified cell in the buccal ganglion of the land snail, Helix aspersa. The activity of this cell was recorded extracellularly with a suction electrode on the ipsilateral salivary nerve and intracellularly with single- or double-barrelled microelectrodes in the desheathed soma. This cell exhibited two distinct patterns of firing (I & II) which appear to be generated by two separate mechanisms.

Firing pattern I consists of trains of three to ten evenly spaced action potentials. Interspike intervals within a train range from 200 to 400 msec. and trains are separated by 2 to 10 seconds. Hyperpolarization of the cell reveals a pattern of incoming synaptic activity which appears to produce this firing.

Firing pattern II is an intense burst of action potentials which is always preceded by a prolonged period of hyperpolarization. These bursts typically consist of 12 to 20 action potentials and are separated by intervals ranging from 20 seconds to over 4 minutes. Patterns I and II are both eliminated in high Mg^{++}-low Ca^{++} bathing medium.

A pressure injection pulse of 5-HT applied to the hillock region of this cell elicited the entire sequence of events associated with firing pattern II. The presence of this response was time dependent, requiring a sufficient interval (approximately 3 min.) to elapse following the previous instance of firing pattern II. If an insufficient period was allowed to elapse, 5-HT produced only a biphasic response consisting of an early hyperpolarizing phase followed by a depolarization without generating pattern II. A similar situation was observed when hypolarizing current pulses were passed through the recording electrode into the soma. Such a hyperpolarization was always followed by a post-inhibitory burst of action potentials. On trials having a sufficient delay from the previous pattern II the rebound burst was immediately followed by production of another pattern II. As was the case with 5-HT, the interval since the previous occurrence of firing pattern II appears to determine whether pattern II will be generated or not.

Single pulses of ACh to the hillock region produced depolarizing responses which were excitatory at resting membrane potential. ACh responses did not trigger either of the firing patterns. We suggest that this Helix cell may provide an opportunity to study the generation of two rhythms and their interactions at the level of a single neuron.

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1683 AUTORADIOGRAPHIC PROJECTIONS OF THE MIDBRAIN CENTRAL GRAY.
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The mesencephalic central gray area (CG) is not a functionally homogenous structure. For example, stimulation of the dorsal portion of the central gray produces aversive behavioral effects while the ventral portion, which contains the serotonergic dorsal raphe nucleus, has been implicated in stimulation-produced analgesia. Electrical stimulation of the lateral central gray has been shown to induce the lordosis reflex in the female rat, and luteinizing - hormone releasing hormone terminals have been located in this area. The present study examines autoradiographically the projections of these functionally different areas of the central gray. Iontophoretic injections of ^{35}S-methionine were made into the (1) dorsal CG (2) ventral CG and (3) lateral CG. Rats survived for 3 days. Following a 6 day exposure period, the tissue was developed, stained for Nissl and examined microscopically. From the dorsal CG injection site, extensive bilateral labelling was observed. The commissure of the superior colliculus was labelled both rostrally and caudally. Labelled fibres radiated laterally and ventrally in Weisschedel's radiatio grisea tegmenti. Intra-central gray projections went to the pretectal area and periventricular nucleus of the thalamus. From the ventral CG injection site caudal projections were seen bilaterally in discrete fascicles on the lateral edge of the central gray. Rostrally labelled fascicles coursed into the ventral tegmental area and the medial forebrain bundle. Light labelling was also seen in the periventricular nucleus of the hypothalamus. From the lateral CG, three rostrally projecting pathways could be identified. Heavy labelling was obtained in the commissure of the superior colliculus from the injection site to the pretectal area. Labelled fascicles were also seen to course ventromedially to ascend in the central tegmental tract to the region of the red nucleus and further rostral in the medial forebrain bundle. Labelling was seen as far rostral as the preoptic area. Finally, intracentral gray projections, mainly ipsilateral, ascended as far rostral as the N. parafasicularis and the ventral nucleus of the thalamus. These diverse projections from different parts of the CG may underlie the functional heterogeneity associated with this structure. (Research supported by BRS grants 5-S07-RR07175-03 and 5-S07-RR05426-16).

1684 IS THE NERVOUS SYSTEM A "BIT" OR A "BYTE" PROCESSOR?: AN EVALUATION OF THE MARKOV CHAIN LENGTH OF INTERSPIKE INTERVALS.
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The times between adjacent action potentials (ISI) are usually treated from both a theoretical and practical standpoint (Biophy. J. 7: 391, 1967) as if they form a memory-less Markov chain (i.e. knowing the value of an ISI does not allow prediction of succeeding ISIs). Based on empirical observations of statistically significant changes in the occurrence of patterns involving as many as 13 sequential ISIs (Life Sci. 11: 441, 1972), we decided to test the hypothesis that ISIs form higher-order Markov chains. The first step was to have the computer determine if the first interval in a sequential pair was >, =, or < the next sequential interval and code this relationship as a +, 0, or - in the computer memory. The occurrences of these patterns were tallied into a series of conditional probability matricies where the sequential relationships were specified as probabilities in a 'digram' matrix for 2 symbols, 'trigram' matrix for 3 symbols, and 'quadgram' matrix for 4. We tested the hypothesis that the data were generated by a process utilizing a Markov chain of either 1st, 2nd, or 3rd order (a 3rd order chain, for example, implies that the probability of occurrence of an ISI depends on the probability of occurrence of two immediately preceding ISIs. For each of the matricies, we calculated the expected and empirically observed values in each cell to determine the Chi-square statistic (Theoret. Biol. 29:427, 1970). For 12 neurons in the rat cerebellar cortex, we found that 7 neurons required at least a 3rd order Markov chain to describe the spike train adequately. The remaining 5 neurons required at least a 2nd order Markov chain.

These data suggest that at least part of the information processing in the nervous system may be analagous to computerized 'byte' processing.

1685 MODELLING OF GANGLION CELLS IN A REALISTIC VERTEBRATE CONE RETINA.
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Prototypes of ganglion cells (GC) of vertebrate cone retinas require 4 specifications:

1. an informational flow diagram relating the flow of signals from cones to GCs. Two types of GCs are present- - tonic (t-) GCs formed by direct convergence of bipolar cells (BC) and phasic (p-) GCs formed by convergence of 2 types of BCs- - hyperpolarizing- and depolarizing-center BCs;

2. spatial organization of cones into unit hexagons (UH) which form repetitive units across the entire retina. A UH is a geometrical pattern consisting of a central cone and spectrally like-cones located at each of the 6 corners of the UH. Receptive fields (RF) of GCs result from converging UHs and RF sizes can be increased by adding rows of cones to the UH. At the GC level, RF organizations develop from convergence of all or parts of 7 overlapping central UHs with their accompanying surround UHs. Six variations are possible- - within the central and surround regions, spectrally like-UHs are present and, in one case, are the same for the 2 regions and, in the other case, are different and finally, within each region different overlapping UHs are present in each region. Besides p- and t-GCs, there are color-coded (C-) and non-color-coded (L-) types of GCs. The L-types are formed with all cones, regardless of spectral type, within a UH connecting to appropriate retinal elements and C-types are formed with the appropriate spectral type of cone within the UH;

3. weighted inputs (a_x) for each cone depend on electrical coupling of like-cones and/or stray light given by, $a_x = at_x + bu_x + cv_x + dw_x + - - - - -$, where t_x, u_x, etc are the number of cones located in the 1st, 2nd, etc rows from the central cone and a, b, etc are their respective weighting factors;

4. a polarization factor (X) relating the amount of polarization change produced in a given GC by hyperpolarization of a given cone, $X = a_x E(t)(Zd_i)f(g_i)$, where $E(t)$ is hyperpolarization of the cone as a function of time, Zd_i is the sum of synaptic delays in the path from the cone to the GC and $f(g_i)$ is a complex linear function of synaptic gains assuming linearity and modifications due to negative feedback from L-HCs to cones and electrical coupling between like-HCs.

The model can generate all known RF organizations of vertebrate retinal GCs. Proper manipulations of the various parameters generate GC types for any given species of vertebrate.

1686 CENTRAL GENERATION OF THE SCRATCH REFLEX IN THE TURTLE.
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Scratching movements of a hindlimb can be elicited by tactile stimulation of specific regions of the shell of a turtle, Pseudemys scripta elegans, whose spinal cord is cut posterior to the forelimb enlargement at D2 (Valk-Fai and Crowe, J. Comp. Physiol. 125: 351, 1978 and in press). The present study has demonstrated that this scratch reflex is centrally programmed within the spinal cord of the turtle. These results are similar to those obtained with the scratch reflex of the cat (Berkinblit et al., J. Neurophysiol. 41: 1040, 1978).

In low spinal turtles with intact sensory input, the knee extensor muscle (KE), triceps femoris, pars femorotibialis, vastus medialis, was active during the protraction phase of the scratch and the hip retractor and knee flexor muscle (HR-KF), flexor cruris, pars flexor tibialis internus, was active after the termination of KE activity and during the retraction phase of the scratch. A scratch in response to a single tactile stimulus was either (i) a half cycle, i.e. just one KE burst, (ii) a full cycle, i.e. a KE burst followed by a HR-KF burst, (iii) several full cycles, or (iv) several full cycles and a half cycle. A small burst of activity in HR-KF was sometimes also seen during KE activity. In addition, during a few scratch episodes a burst of HR-KF activity was seen prior to the first onset of KE activity.

Sensory activity entering the spinal cord from the hindlimbs via dorsal roots was eliminated by bilateral dorsal rhizotomies of segments D7-S2. In addition, all inputs from the caudal segments were isolated from the hindlimb enlargement by a complete cut of the cord posterior to the S2 roots. The patterns of muscular activation during the scratch in these preparations were similar to those observed in turtles with intact sensory input. Moreover, when further total section of the cord was made posterior to either D8 or D9 and the cord posterior to the section removed, then a normal burst pattern of KE was observed in response to tactile stimulation.

Muscular paralysis with gallamine triethiodide (5 mg/kg, i.m.) was utilized as an alternate way of demonstrating the central program for the scratch. In these preparations, KE motor neuron activity was recorded from the femoral nerve and HR-KF motor neuron activity was recorded from the flexor tibialis nerve. Tactile stimulation in these immobile preparations elicited neural activities, "fictive scratching", whose patterns were similar to the muscular activities observed in preparations with moving limbs.

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1687 BIOGENIC AMINES AND REGULATION OF THE CARDIAC GANGLION OF LIMULUS. W. Watson III*, G. Augustine, E. O'Connor*, R. Fetterer and G. Wyse* (SPON: R. Steinman). Marine Biological Laboratory, Woods Hole, Mass. 02543.

Cardiac ganglia of arthropods are favorable preparations for determining how neurotransmitters or neurohormones modulate neuronal activity. We have addressed this question by using the cardiac ganglion and heart of the horseshoe crab, Limulus polyphemus. Little is known about the regulation of cardiac ganglion activity in vivo; we therefore monitored the heartbeat and cardioregulatory nerve activity of intact Limulus using chronically implanted electrodes. The heartbeat provides a direct indication of cardiac ganglion activity, since it is generated by this ganglion. The Limulus heartbeat is altered in a variety of situations. Decreases in rate are observed in response to stimuli such as touch, changes in light intensity, or the presence of food objects. Acceleration of the heartbeat occurs during swimming. The heartbeat appears to be coordinated with respiratory activity, and thus also changes under conditions which affect respiration. All decreases in heart rate are associated with clear increases in the electrical activity of efferent units recorded extracellularly from the cardioregulatory nerves connecting the CNS and cardiac ganglion. There are other efferent units whose activity is correlated with increases in heart rate. This suggests that the CNS contains both excitatory and inhibitory cardioregulatory neurons. We have attempted to determine the neurotransmitters or neurohormones involved in cardioregulation. As amines are thought to serve a cardioregulatory role in other systems, we examined the catecholamine levels of the cardiac ganglion, cardioregulatory nerves, and CNS. Dopamine, norepinepherine, and epinepherine were found in all three of these structures using high pressure liquid chromatography with electrochemical detection. Octopamine has also been found in the cardiac ganglion by others. The presence of biogenic amines in these structures has been verified by localization with a glyoxylic acid histofluorescence technique. All four amines excite the isolated ganglion, increasing the rate of bursts of electrical activity recorded extracellularly. In summary, the activity of the cardiac ganglion of Limulus is modulated in vivo by neurons within the CNS. Biochemical, histochemical, and physiological data suggest that one or more amines may be involved in this cardioregulation.

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1688 A 'QUANTAL' ELECTRIC CONNECTION BETWEEN NEURONS IN THE LEECH CNS. Birgit Zipser, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724, U.S.A.

A synaptic connection exists between leech neurons that is quantal in nature but is not blocked under conditions which interfere with chemical synaptic transmission. The connection is important for the neural control of mating since it occurs between two pairs of penile evertor motor neurons, the lateral and rostral cells. These neurons have previously been found to be directly coupled via an electrotonic synapse. In addition to this electrotonic transmission, I have found another excitatory lateral-to-rostral interaction with unusual properties. Transmission across this synaptic connection is unidirectional with a latency of about 4 msec. Intermittent failures in transmission are seen. These failures are not due to conduction block in the lateral cell since lateral cell spikes invariably give rise to small electrotonic coupling potentials in the rostral cell. The amplitude of the postsynaptic potentials (PSPs) fall into two discrete classes, with the larger PSPs being about twice the size of the smaller ones. In some preparations, spontaneous PSPs occur; these have amplitude distributions similar to evoked PSPs. These findings would ordinarily suggest that the interconnection is chemical; however, ion substitution experiments rule out a conventional chemical synapse. The PSPs persist in Ca-free solutions, including those to which the Ca-chelator EGTA (0.5 to 1mM) has been added. The single and double modes of the PSP amplitudes are not only maintained in Ca-free Ringer, but in fact, the number of failures decreases. Increasing the external Ca concentration inhibits transmission by increasing the number of failures. Ca channel blockers such as Ni (2.5mM) do not block transmission either. The mechanism by which this Ca-independent quantal transmission occurs is not clear. One way that the transmission could proceed is via the presence of 2 interposed interneurons with parallel electric connections to both rostral and lateral cells. The single and double PSPs could be generated by action potentials in one or both of these neurons. The facilitatory effects of Ca-deletion could then be attributed to removal of chemical inhibition from these hypothetical interneurons. Consistent with this hypothesis is the finding that the PSPs are blocked by tetrodotoxin at concentrations that do not eliminate action potentials in lateral and rostral neurons. Supported by NSF grant 78-13064.

NEURONAL SHAPE AND FUNCTION

1689 THE MORPHOLOGY OF MECHANOSENSORY NEURONS IN THE SKIN OF THE LEECH. Susanna Blackshaw* and John Nicholls. Dept. Neurobiol., Stanford Med. Sch., Stanford, CA 94305.

In the leech, the cell bodies of mechanosensory neurons lie within the central nervous system. They are easily identified and much is known about their electrical properties, their arborisation within the ganglia, their synaptic connections, and the way in which they regenerate to reform connections after injury. The receptive fields of individual neurons have been mapped physiologically and each cell innervates a clearly defined area of skin, its receptive field. By contrast, there is little information on their morphology in the skin. By injecting horseradish peroxidase into the cell body of a neuron in the ganglion we have now been able to follow the way in which the axon branches and terminates within its receptive field.

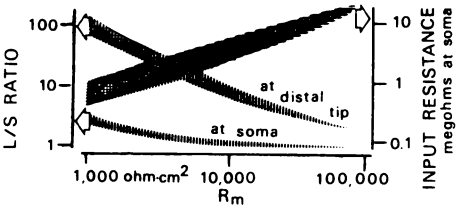
Neurons that respond to light touch branch repeatedly in deeper layers of the skin. The terminal branches of the axon then turn towards the surface between the skin epidermal cells. If the skin surface is viewed with Nomarski optics the touch cell endings can be seen as well-defined structures 2 - 3 μm in diameter lying between the epidermal cell profiles. An individual touch cell makes over a 1000 of these endings at the skin surface.

In contrast to the touch cells neurons that respond to pressure branch less profusely within their receptive fields, and branches of the axon end in deeper layers of the skin.

The use of peroxidase makes it possible to study the terminals of identified neurons by electronmicroscopy. Such information about fine structure provides a basis for further experiments on the specificity of skin innervation and reinnervation by identified mechanosensory neurons.

1690 SOMATIC *VS.* DENDRITIC SYNAPSES: MEMBRANE RESISTIVITY IS OFTEN UNIMPORTANT IN DETERMINING RELATIVE STRENGTHS. William H. Calvin and Katherine Graubard, Univ. of Washington, Seattle, WA 98195.

Not all synapses are equal; indeed, length constants have been interpreted to suggest that synapses on distal dendrites are "virtually ineffective" compared to somatic synapses. Dye injected cells have permitted estimates of membrane resistivity, R_m, using cell reconstructions and measured input resistance; unfortunately, R_m estimates vary many-fold, depending on how adequately the fine distal processes are visualized. Here we report that, for the same synaptic conductance change and driving potential, distal synapses deliver nearly as much charge (area beneath PSP, hereafter simply called the "PSP") to the soma as do somatic synapses. Furthermore, wide variations in R_m do not change this basic result. PSPs are calculated from the steady-state cable equation; our figure is based on the range of data (shading) from two cells of quite different appearance (a lobster stomatogastric cell and a model spinal motoneuron with d3/2 conserved) and is, we believe, representative of many dendritic trees. We plot the PSP size ratio (largest/smallest, i.e., local and most distant synapses). *For most R_m, the somatic PSP from a local synapse is less than twice as large as the PSP arriving from the most distant synapse* (L/S < 2, "at soma"). While this indicates relative synaptic strengths for impulse initiation, one must also consider presynaptic regions in the dendritic tree which could release transmitter in response to summed PSPs. The local PSPs near a distal tip are quite large due to the high input resistance, *often being 10-40X larger than PSPs spreading from somatic synapses or those on other dendrites* (L/S > 10, "at distal tip"). This potential for "regional computation," while always considerable (c.f., The Neurosciences Fourth Study Program 1979), is enhanced by lower R_m. Reasoning based upon analogies with axon length constants is often misleading when applied to dendritic trees, primarily because a dendrite is terminated centrally by a heavy load, that of the soma and other dendrites. *Voltage attenuation is also misleading: even if there is ten-fold attenuation between a distal tip and the soma, moving to a synapse from soma to tip will usually cause the local PSP to increase by a similar factor, so that the PSP seen in the soma changes less than two-fold.* (NIH grants NS04053, NS 09677, TW 00181.)



1691 ENDOGENOUS FLUOROCHROMES IN THE CAUDATE NUCLEUS. Joseph T. Cummins and Roger S. Rahn*. Dept. Psychiat. Sch. Med. UCLA, Los Angeles, CA, and VA Medical Center, Sepulveda, CA; Dept. Biol. Chapman College, Orange, CA.

Sections from the caudate nucleus were illuminated with a 325 nm He-Cd laser and studied with a triocular microscope modified with a dichroic mirror and quartz objective. Tissue fluorescence could either be photographed or measured by a monochrometer and photon counting system. 325 nm laser illumination of caudate sections revealed a number of endogenous fluorescent structures of potential neuroanatomical importance. These included: long fibers with bright blue fluorescence, triangular "nerve cell-like" structures and unresolved areas of fluorescence. The long fibers contain a fluorochrome with an emission maximum at 455 nm. Most caudate structures have a blue fluorescence; but green and red fluorescing structures are also seen. Counterstaining with thionin demonstrated that the blue fluorescing fibers contact nerve cells and that certain nerve cells have a blue fluorescence. Blue fluorescing structures were seen in fresh caudate slices perfused with Krebs-Ringer and following several methods of fixation of frozen caudate sections such as air drying, treatment with dimethylsulfoxide or dimethylsulfoxide plus dithiothreitol. The latter treatment suggests that the fluorescence does not arise from oxidation within the tissue section. Similar endogenous fluorescent structures were seen in caudate sections from mouse, rat or beef. These observations suggest that the fluorochromes have some importance in brain function and that the U.V. laser microscope may be a new approach to studying certain aspects of the microchemistry of the nervous system.

1692 CONDUCTANCE CHANGES DURING RECURRENT IPSPs IN HIPPOCAMPAL PYRAMIDS IN VITRO. Raymond Dingledine and Iver A. Langmoen*. The Institute of Neurophysiology, Univ. of Oslo, Norway.

Recurrent inhibitory synapses are located predominantly on the soma of CA1 pyramids, while excitatory synapses appear to be made exclusively on pyramidal cell dendrites (see review by Andersen in The Hippocampus, Isaacson & Pribram (ed), Plenum, 1975). The question is raised as to how these spatially separate inhibitory and excitatory synapses interact in this cortical neuron.

Intracellular recordings were obtained from antidromically identified CA1 pyramidal neurons in an in vitro slice preparation of the guinea pig hippocampus. Tungsten microcathodes were placed both in the alveus and among the afferent fibers in the apical dendritic layer of the pyramids. The time course of the impedance change underlying recurrent ipsp's was estimated by injecting current through the recording electrode in an a.c. bridge configuration (method of Hagiwara & Tasaki, J. Physiol., 143:114, 1958).

The dependence of the recurrent ipsp on the membrane potential was examined. A conductance increase was implicated, which at the resting potential was sufficient to drive the ipsp 30-60% of the distance to its reversal potential. By eliciting ipsp's in the presence of a 10 Hz sine wave current injected across the cell membrane, it was found that the timecourse of the membrane impedance change matched that of the ipsp itself, except that the maximum impedance decrease usually occurred slightly before the peak of the ipsp. Both the ipsp and its associated impedance change decayed nonmonotonically over a period of about 150 ms.

The inhibition afforded by the 2-10 mV peak hyperpolarization of the ipsp was considerable since the normal firing threshold, when measured in the same cells, was 5-12 mV above the resting potential. Furthermore, the probability of spontaneous discharge was depressed throughout the duration of the ipsp. The membrane potential at which spikes were initiated did not appear to be altered during the recurrent ipsp.

The ipsp conductance, if great enough, was able to shunt epsp's generated in the apical dendrites. This was demonstrated by adjusting the membrane potential to the ipsp reversal potential and showing non-linear summation of epsp's with recurrent ipsp's. However, such inhibitory conductance shunting was usually weak and could only be detected during the period of most intense impedance decrease (up to 15-25 ms).

Thus, the inhibitory actions of hippocampal recurrent ipsp's are mediated by a hyperpolarization and, if the ipsp is properly timed with excitatory potentials, by a conductance shunting effect. In these respects recurrent inhibitory mechanisms in the hippocampus appear similar to those for motoneuron ipsp's.

1693 "RECTIFICATION" UNDERLYING DIFFERENTIAL CHARACTERISTICS OF ANTI- AND ORTHODROMIC ACTIVATION OF A VERTEBRATE CENTRAL NEURON. Donald S. Faber and Paul G. Funch. Div. Neurobiology, Dept. Physiol., SUNY at Buffalo, Buffalo, NY 14214 and NYS Research Institute on Alcoholism, Buffalo, NY 14203.

The goldfish Mauthner cell (M-cell) is characterized by its large soma and a correspondingly large myelinated axon (M-axon), with the fiber diameter being 40-60 μm. These two regions are electrically coupled by a tapering axon hillock and a thin unmyelinated axon no more than 10-12 μm in diameter and 75 μm long. The functional role of such a constriction, which is typical of many vertebrate neurons, is not well established, although it is often assumed to electrotonically separate the soma and axon. We report here that the electrical properties of these different regions and their associated extracellular resistances provide for a "rectification". Specifically, there is minimal potential decrement from the soma or axon hillock to the M-axon, while there is significant attenuation in the opposite direction. This results in a low safety factor for antidromic invasion of the axon hillock but not for orthodromic activation of the M-axon.

Simultaneous intracellular recordings and current injections were used to measure input resistances of the M-cell soma and axon and the transfer resistance (R_T) between the two. R_T is approximately 140 kΩ, while the lumped resistance of the axon hillock and somatic membranes is about 200 kΩ. In contrast, the input resistance of the caudal M-axon is at least one order of magnitude higher. Furthermore, there is minimal decrement in the amplitude of eighth nerve-evoked EPSPs from the soma to the axon (typically < 20%), while the axon spike recorded somatically is reduced in amplitude by 80-90%. A simple electrical model based on our resistance measurements and the geometrical relations described above predicts such a "rectification". Furthermore, it provides a basis for analyzing previous observations: 1) There is a low safety factor and consequent delay for antidromic invasion of the axon hillock; 2) Antidromic invasion may spontaneously fail; and 3) Orthodromic propagation into and along the M-axon is nevertheless secured with no observable delay between activation of the axon hillock and the M-axon. Thus, antidromic stimulation of the M-cell may be used as a sensitive test for studying pharmacological and environmental alterations in membrane excitability, and may serve as a model for other regions of low safety factor, such as axonal branch points. (Supported in part by PHS Grant No. NS 12132.)

1694 ELECTROTONIC STRUCTURE OF HIPPOCAMPAL NEURONS. Russell A. Fricke, Thomas H. Brown and David A. Prince. Neurology Department, Stanford University Medical School, Stanford, CA 94305.

The hippocampus contains several spatially segregated classes of morphologically distinct neurons. Using the guinea pig in vitro slice preparation, we have examined the passive electrical membrane properties of neurons which we penetrated in the pyramidal layer of regions CA3 or CA1, or in the granular layer of the dentate gyrus. These layers principally contain the cell bodies and proximal dendrites of pyramidal and granule cells, respectively. Rallian "neuron parameters" were estimated in current clamp experiments. The method of analysis assumes, as a first approximation, that the cells can be represented by an isopotential "soma" compartment (consisting of a capacitance C_s in parallel with a conductance G_s) which is attached to a single "equivalent" dendritic cylinder having an input conductance G_d and electrotonic length L. Hyperpolarizing current steps were delivered to the cell and from the charging time constants and their weighting factors we estimated (See Norris et al, Neurosci. Abstr. 5, 1979) C_s, G_s and L, along with ρ, the "dendritic dominance." Average values for these electrical parameters are given below. The interpretation of these parameters, and their possible contribution to the characteristic firing patterns of granule and pyramidal cells will be discussed. (Supported by NS 06161 and NS 12151).

Electrical Constant	Presumed Neuronal Cell Type: Pyramidal Cell (N = 7)	Granule Cell (N = 7)
R_N (MΩ)[1]	42.4 ± 2.9	37.8 ± 3.8
τ_m (msec)[2]	17.1 ± 2.6	12.3 ± 1.9
G_s (nS)	6.9 ± 2.1	14.1 ± 2.3
C_s (pF)	139 ± 55	153 ± 9
L (X/λ)	1.0 ± 0.2	0.9 ± 0.1
ρ (G_d/G_s)	5.3 ± 2.5	1.4 ± 0.4

[1]Input resistance [2]Membrane time constant

1695 "CONTINUOUS" IMPULSE PROPAGATION ALONG A MYELINATED VERTEBRATE AXON. Paul G. Funch and Donald S. Faber. Dept. Physiol., SUNY at Buffalo, Buffalo, NY 14214, and NYS Research Institute on Alcoholism, Buffalo, NY 14203.

The goldfish Mauthner axon (M-axon) is a large myelinated fiber, 40-60 μm in diameter, which lacks nodes of Ranvier. Its impulse conduction velocity is well below what would be predicted for saltatory conduction along a myelinated vertebrate fiber of similar diameter with a typical ratio (100-150) of internodal distance (L) to fiber diameter. The unusual character of impulse propagation suggested by these early observations has been studied by recording intracellularly in the soma while making successive penetrations of the M-axon with a second microelectrode. Hyperpolarizing current injections into the axon and soma during orthodromic and antidromic activation, respectively, separated the axon hillock and axon spikes from the composite action potentials observed in the M-axon. The magnitudes of these spike components as well as those of eighth nerve-evoked EPSPs and the maximum rate of rise of the axon spike have been plotted as a function of distance from the axon hillock (up to 7.6 mm). The resulting spatial profiles of the axon hillock spike and eighth nerve-evoked EPSPs indicate the space constant (λ) of the rostral M-axon is approximately 4.5 mm. In contrast, the caudal to rostral decrement in the axon spike amplitude is much less than predicted on the basis of these estimates of λ, and this profile is paralleled by that of the magnitude of its maximal rate of rise. Furthermore, the latency of the maximal rate of rise of the axon spike component is linearly related to distance and yields a conduction velocity of 47 m/sec. Morphological studies by Celio et al. (J. Neurocytol., 8:19-29, 1979) have shown that collaterals project about every 300 μm from the M-axon in the spinal cord of 20 cm long goldfish. These collaterals appear to be the only possible active membrane sites, suggesting that for the 13.5 cm fish used in this study there would be a very short electrotonic distance of about λ/20 for L. While saltatory conduction and very rapid conduction velocity normally accompany myelination, these findings lead us to suggest that impulse propagation in the M-axon is an essentially continuous process which, while slower, provides a high safety factor for conduction along the M-axon in the face of significant current loss through the many collaterals. (Supported in part by PHS Grant No. NS 12132.)

1696 PERIPHERAL NERVE MYELIN MORPHOLOGY IN ANIMALS OF DIFFERENT PLOIDY. S. A. George and M. J. Lemanski.* Biology Department and Neuroscience Program, Amherst College, Amherst, MA 01002.

As part of a continuing study of the morphology and function of neurons in animals of different chromosome number, we studied the relations between internodal length (L), axon diameter (d), and total fiber diameter (D) in individual sciatic nerve fibers from normal (diploid) and triploid Xenopus. Triploids (3 sets of chromosomes) were prepared by subjecting fertilized eggs to a hydrostatic pressure shock. Previous work in this laboratory and by others has shown that the nervous system and other organs of triploid amphibians contain larger, but fewer, cells than diploids of the same species. We confirmed this relation in Xenopus sciatic nerve, through fiber counts and measurements of fiber cross-sectional area.

Lengths of single fibers containing from 6 to 17 nodes were isolated after treating sciatic nerves with glycerol, and myelin dimensions were measured in 1351 internodal segments from these fibers. The relation between axon and fiber diameter was linear, but the relation was different for diploids and triploids: the average value of the ratio g=d/D was .625±.008 in diploids, compared to a value of .693±.006 in triploids, a highly significant difference. Thus the myelin sheath (D-d) was thinner around triploid fibers compared with diploid fibers of the same diameter. The internodal length L was also strongly correlated with axon diameter, and again the relation was a function of ploidy: L in triploid fibers was, on the average, longer than in diploid fibers of the same diameter. The volume of myelin in each internode was calculated from the values of L, d, and D, and in this case the triploid-diploid differences were more complex: triploids had a smaller myelin volume than diploids in fibers less than 10 μm in diameter, but triploids had more myelin per internode in fibers above 10 μm. Thus myelin morphology in animals of different ploidy is not simply scaled according to cell size differences in these classes of animals.

We also recorded sciatic nerve compound action potentials. The amplitude of these potentials in triploid nerve was significantly smaller than in diploid nerve, indicating a smaller total nodal area in triploid nerve. The average conduction velocity in triploid nerve was nearly identical to the diploid average, though the variability between animals was large. The conduction velocity might be expected to be greater in triploids because of their larger average fiber diameter, but we suggest that the altered myelin structure in triploids may reduce the conduction velocity in fibers of a given diameter below the value achieved in normal diploid fibers.

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1697 AN INTERACTIVE CAMERA LUCIDA COMPUTER-MICROSCOPE. E.M. Glaser, M. Gissler* and H. Van der Loos*. Institute of Anatomy, University of Lausanne, CH1011 Lausanne, Switzerland.

Quantitative light microscopy has experienced a significant evolution in recent years due to its wedding with the laboratory minicomputer, a wedding facilitated by such ancillary devices as motor driven stages, video scanners, graphics displays, and graphics tablets. The resulting computer-microscopes have enabled the microscopist to acquire data rapidly and accurately and to analyze these data in a multiplicity of ways. Nonetheless, the microscopist has had to pay a substantial price for these assets: he has been divorced to a certain extent from intimate contact with the original image of the preparation. We have designed a computer-microscope system which reunites the investigator and preparation and which provides a highly flexible means for him to interact with the data acquisition and analysis procedures. The system is based upon image superimposition as provided by a camera lucida. The microscopist sees at one and the same time in a single visual field (a) the original image of the preparation, (b) a graphics display of the data acquired from this image, (c) a command 'menu' permitting him to control the system without taking his eyes from the oculars. The superimposition of the original image and the graphics display is maintained regardless of how the stepping motor driven stage is moved during the course of data acquisition. In practice, data are acquired either by stepping the stage, by 'drawing' with a graphics tablet stylus, or both. The combination of the two techniques greatly enhances the speed and accuracy of tissue examination. Because it is the original high resolution, fully chromatic image that the microscopist examines, the only optical limitations to the system are those inherent in light microscopy itself.

The computer-microscope is particularly well suited to the study of neurons stained in their entirety, as by Golgi methods. Since 3-dimensional stepping motor stage control is provided, sections of any thickness can be dealt with. The maintenance of image superimposition regardless of stage position permits viewing an unlimited expanse of the preparation with high magnification optics. This maximizes the amount of information that can be acquired. Chromatic aspects of the image can also be studied.

Other important tasks can be performed with the instrument. For example, serial sections can be aligned by letting the graphics display present the relevant contours from a neighboring section while the section on the stage is maneuvered to obtain the best registration. Another type of operation facilitates stereological analysis by having the graphics display present stereological grids selected from files stored in the computer.

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1698 A COMPARISON OF RAPID GOLGI STAIN QUALITY IN FRESHLY PERFUSED HIPPOCAMPAL SLICES AND HIPPOCAMPAL SLICES MAINTAINED AND STIMULATED IN VITRO. Kristen M. Harris & Timothy J. Teyler, Program in Neurobiology, Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272.

A rapid Golgi staining technique for brain tissue less than 0.5 mm thick (Harris, K. M. Soc. Neurosci. Abs. 1978, 4, A1053) was applied to hippocampal slices (0.3-0.4 mm thick). One Group of slices (Group A) was immediately perfused with 4% buffered paraformaldehyde and processed by this rapid Golgi technique. Other slices were placed in an incubation chamber (Alger & Teyler, Brain Res 1976, 110, 463-480) for up to 6 hours. Hippocampal slices from this second group were either stimulated (Group B) or allowed to remain undisturbed in the incubation chamber (Group C). All slices from Groups B and C were tested for spontaneous activity at the end of an incubation period. Those slices showing spontaneous activity were transferred to 4% buffered paraformaldehyde and rapid Golgi stained. Slices showing no spontaneous activity were discarded.

Qualitative light microscopic analysis of the rapid Golgi stained slices showed fully impregnated cell bodies, dendrites, dendritic spines, axons, and axonal varicosities in groups A, B, and C. Good stain quality was obtained whether the tissue was fixed in paraformaldehyde for 24 hours or for several days, from slices in all three groups.

It is suggested that this technique can be successfully applied to hippocampal slices that are experimentally manipulated in vitro. (NSF Grant BNS-78-23947)

1699 CABLE PROPERTIES, NEURONAL GEOMETRY, AND TRANSIENT POTENTIALS IN DENDRITIC SYSTEMS. Barry Horwitz, Physics Department, Texas Woman's University, Denton, Texas 76204.

A theoretical model has been developed which explores the way in which the geometrical structure of a neuron's dendritic tree affects the time course and amplitude of transient potentials generated at different locations on dendritic branches. The model, based on the work of Butz and Cowan[1], allows one to investigate dendritic systems which are not of the equivalent cylinder class of Rall[2]. We have examined a number of such systems and have developed an analytical way to evaluate the strength of the time-varying potentials that would be observed at the neuron's soma. Examples will be presented which will demonstrate how, for an input on a given branch, the potential observed at the soma depends upon such geometrical parameters as (1) the number of sister branches, (2) the branch lengths, (3) whether or not the dendrite bifurcates at a point distal to the input, and (4) given such a bifurcation, the lengths of the daughter branches.

(Supported by TWU Institutional Grant 0997)

1. Butz, E. and Cowan, J., Biophysics J. 14, 661-689, 1974.
2. Rall, W., Biophysics J. 2, 145-167, 1962.

1700 EARLY EXPERIENCE EFFECT ON DENDRITIC BUNDLES. Frances E. Jensen* and D. N. Spinelli. Departments of COINS and Psychology, University of Massachusetts, Amherst, MA 01003. SPON. A.Treehub

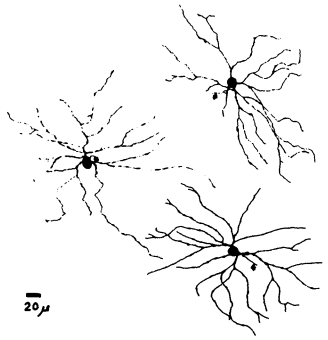
Dendritic bundles have been suggested as a possible anatomical substrate for movement sequencing (Scheibel and Scheibel, 1973). In a previous study, kittens received daily training sessions requiring the lifting of 1 forearm to avoid a small shock. Electrophysiological recordings from single cells in the Somato-Sensory cortex revealed a 200-300% enlargement of the somatotopic representation of the trained forearm relative to the untrained arm (Neurscience abstracts Vo 4. No. 2075, Science, 1979). We have now examined neuronal architecture of the brains of the same cats using Golgi-Cox staining. We compared the 2 hemispheres for cell count, dendritic density, dendritic length, dendritic bundling, shape of bundles, and the angle of branching of the dendrites incorporated in bundles. Dendritic trees in the cortex corresponding to the enlarged area associated with the trained forearm appeared more extensive (much larger) than those seen in the contralateral representation of the untrained forearm. The number of cell bodies stained in the 2 hemispheres was not significantly different, indicating that the staining had been uniform. In this study we specifically concentrated on an analysis of dendritic bundles to investigate the possibility that their formation may be altered, or guided, by early experience. Two notable findings relating to this were: 1) statistical analysis showed that the number of bundles in the trained area is significantly greater than that of the untrained one for all cats studied 2) in some cases the length of the bundles is also significantly greater in this cortical area. This data seems to indicate that early experience has a measurable impact on dendritic growth and bundling. As bundles are structurally bound and embedded into a matrix of nerve cells and axons, clearly once formed, they must be permanent. It seems unlikely that new bundles will form once dendrites have reached the end of their growth. If bundles are indeed implicated in motor sequences, a concept supported by these results, then it would become clear why behaviors involving precise motor sequences, such as in speech, if not learned during childhood - are never learned as well later. Further implications for the education of children will be discussed.

1701 DISPLACED GANGLION CELLS IN RAT RETINA. Peter Land and Neil Segil*. Dept. Biol. Str. Univ. Wash., Seattle, WA 98195.

The morphology of disolaced ganglion cells in rat retina was examined after diffusely filling these neurons with horseradish peroxidase by optic nerve injection. The somata of displaced ganglion cells measure between 10 and 18 μ in diameter (ave = 14 μ). One to four stout primary dendrites issue from the vitreal surface of the somata. The primary dendrites most often branch within 20 μ of the soma, and the axons generally arise from one of the primary dendrites. Most secondary and all higher order dendritic branches are extremely fine, and except for occasional bead-like swellings, their surface is smooth and spine free. The elliptical dendritic trees of these neurons as seen in flat mounted retinae averaged 240 μ in the long axis. Three examples of displaced ganglion cells drawn from three retinae are shown in the accompanying figure (arrows = axon).

In retinae embedded in celloidin and sectioned perpendicular to the optic fiber layer, the dendrites of disolaced ganglion cells are seen to ramify in the outer 1/3 to 1/2 of the inner plexiform layer. As described previously (Bunt et al., Brain Res. 73 [1974]), displaced ganglion cells were most common in the central portion of the retina.

Our results demonstrate a remarkable morphological homogeneity for displaced ganglion cells in rat retina. This result is perhaps less surprising in view of the information which currently is emerging with regard to the central connection of these neurons in other species. Determination of the central connections of displaced ganglion cells in rat and elucidation of their detailed morphology in other species should be useful in unraveling the significance of these neurons in the organization of the vertebrate visual system. (Supported by USPHS Grant EY 05185 from the National Institutes of Health.)



1702 FUNCTIONAL CHARACTERIZATION OF MORPHOLOGICALLY DISTINCT GIANT MOTOR SYNAPSES IN THE CRAYFISH CNS. Joseph F. Margiotta* and B. Walcott. Dept. Anat. Sci., HSC, SUNY at Stony Brook, Long Island, N. Y. 11794.

In a classic study, Furshpan and Potter (1959) showed that synaptic transmission at segmental giant motor synapses (GMS) in the crayfish CNS is electrical and that the impedance of these synapses between lateral giant axon (L.G.) and axons of giant flexor motoneurons (F_1) is much lower for applied orthdromic (L.G. to F_1) than antidromic currents. Our anatomical experiments involving cobalt backfilling and injection of fluorescent dyes reveal consistent differences in the gross morphology of F_1 at thoracic versus abdominal synapses. At the level of the last thoracic ganglion (T_8), the axon of F_1 is closely apposed to the L. G. for over 800 μm. At the second abdominal ganglion (A_2) this "contact length" extends for only 200 μm.The cell-to-cell contact in both cases is characterized by the presence of small dendrites which protrude from F_1 and presumably provide the basis for electrical coupling. We have exploited the difference in contact length to assess whether size in any way determines the functional characteristics of these morphologically distinct yet homologous rectifying synapses. This expectation assumes that F_1 acts as a distributed current source/sink along the entire contact length of the GMS. The individual length constants for L.G. and F_1 are 4.23 ± 0.70 mm and 1.90 ± 0.28 mm respectively. However, the trans-synaptic length constant of F_1 when measured by applying current to L.G. and recording the membrane potential in F_1 at different interelectrode distances is 3.37 ± 0.03 mm. This data strongly supports the assumption that F_1 acts as an effective current source or sink along the entire contact length of the GMS.

Differences in current-voltage (IV) properties are being used as an assay for functional comparison of these morphologically distinct synapses. Using a multiple microelectrode technique adapted from Watanabe and Grundfest (1961) we have obtained a family of IV curves for the GMS at T_8 and A_2. Although synapses at both locations display rectification, there are obvious quantitative differences. Consistent with its morphology, the T_8 GMS, having a larger contact length, typically has a lower impedance to antidromic currents (R_s = 1.43 ± 0.53 x 10^6 Ω) than does the A_2 GMS (R_s=6.72 ± 3.78 x 10^6 Ω). Synapses at both T_8 and A_2 show a nearly identical decreased impedance to orthodromic currents beyond a positive value of synaptic potential (V_s). The change to lower orthodromic impedance (R_s = 0.10 ± 0.01 x 10^6 Ω), however, always occurs at a lower value of V_s for thoracic (V_s = 19.9 ± 7.6 mV) than abdominal (V_s = 40.0 ± 8.3 mV) synapses. Both these features reflect an improved safety factor for transmission at thoracic giant motor synapses. Supported by NIH AM 18750.

1703 DIFFERENTIAL SOLUBILITIES OF CYTOSKELETAL PROTEINS IN SQUID AXOPLASM. James R. Morris* and Raymond J. Lasek. Dept. Anat. and Neurobiology Ctr., CWRU, Cleveland, OH, & Marine Biological Laboratory, Woods Hole, Mass.

Our goal is to study the forms which cytoskeletal proteins take under physiologic conditions. We have chosen to study squid giant axon axoplasm because it consists principally of cytoskeletal proteins (tubulin, neurofilament proteins (NFP), and actin) and can be rapidly separated from its axolemma by extrusion without detergents. Additionally, the solution conditions of squid axoplasm have been defined with respect to the dialyzable low molecular weight components. Thus, it has been possible for us to design a physiologic buffer (buffer P) which effectively replicates the solution conditions in the axon. A cylinder of fresh axoplasm was extruded from the giant axon directly into buffer P. Using SDS-PAGE, proteins diffusing into buffer P were compared to those remaining in axoplasm.

We found that axoplasmic cytoskeletal proteins have different solubilities. While essentially all of the NFP (>95%) remained in the axoplasm, most of the tubulin and actin diffused into buffer P (≈5/6 and ≈3/4 of the total respectively). The axoplasm swelled but maintained its cylindrical morphology in buffer P for 24 hrs. at 20°C. The mitochondria were also retained in the axoplasm. Electron microscopy showed the presence of intact neurofilaments (NF) and mitochondria and the absence of microtubules. The axoplasmic structure which retains the insoluble axoplasmic proteins is subsequently referred to as the axoplasmic ghost.

Because all the NFP remained in the ghost and electron microscopy showed the ghost to be principally NF, we conclude that essentially all axonal NFP are normally polymerized in NF and that little or no NFP are normally dissociated from the NF. A fraction of the tubulin and actin also remained attached to the ghost. This fraction must also exist as stable polymer. However, most of the tubulin and actin diffused into buffer P. This diffusible component must exist in the axon as monomers or as a polymer which is dissociable under physiologic conditions. It is possible to distinguish between monomeric and polymeric forms by analyzing the kinetics of their diffusion from the ghost into the buffer. Such an analysis demonstrates that the diffusion of tubulin and actin is slowed when compared to the kinetics predicted by the physical equations describing diffusion for monomeric proteins. Thus, three forms of cytoskeletal proteins exist in the axon: diffusible monomer, soluble polymer, and stable polymer. NFP differ from both tubulin and actin in that NFP exist solely as stable polymer while tubulin and actin may exist in all three forms in the axon.

1704 BRANCHING CHARACTERISTICS OF HUMAN CORTICAL NEURONS FROM TWO PRETERM INFANTS OF IDENTICAL CONCEPTUAL BUT DIFFERENT GESTATIONAL AGES. Albert M. Paldino and Dominick P. Purpura, Dept. Neuroscience, Rose F. Kennedy Center for Research in Mental Retardation and Human Development, Bronx, New York 10461.

The computer microscope was utilized to obtain quantitative information concerning the branching characteristics of human cortical neurons. Hippocampal pyramids from two normal human brains were analysed in regards to their angles of branching and spatial distribution of terminals. The infants who succumbed had similar conceptual ages (33 weeks c.a.) but differed in their gestational ages (g.a.). One pre-term infant was born at 33 weeks (g.a.) while the other was born at 29 weeks (g.a.) and survived for 4 weeks in the Neonatal Intensive Care Unit. Branch and fission angles were calculated for the neuronal processes and the results indicated that (1) the average dendritic branch and fission angles of neurons from the older g.a. specimen were significantly greater than those of neurons from the younger g.a. specimen; (2) the average axonal branch angle was slightly greater in the older g.a. specimen; and (3) the average axonal fission angle was slightly lower in the older g.a. specimen. Information regarding the bifurcation frequency vs soma distance will also be presented.

Spatial analyses of axonal and dendritic terminals reflect, in part, the maturational status of neurons. In regards to these pre-term infants, the data suggest that (1) the average dendritic radial separation of terminals was approximately 40% greater in the older g.a. specimen and (2) the average dendritic cylindrical radius was approximately 50% greater in the older g.a. specimen. These results suggest that dendritic development was more advanced in the older g.a. specimen. Data concerning axonal development will also be presented. Dendritic terminals were then classified as either apical or basilar. Spatial and angular distributions were obtained for each class which enabled quantitative assessments to be made regarding dendritic differentiation. For example, each class of terminals dominated, as expected, a different portion of the polar angle distribution; that is, most apical dendritic terminals were found to possess low (less than 60°) polar angle values, and most basilar dendritic terminals were found to possess high (greater than 100°) polar angle values. Information regarding the length of neuronal processes will also be presented.

The present study is intended, in part, to augment information concerning the developmental and structural patterns of neuronal processes. Attention to such patterns as they appear in normally and aberrantly developed human brain may elucidate the morphological substrate regarding certain forms of mental retardation.

1705 CABLE PROPERTIES OF MOUSE HIPPOCAMPAL NEURONS IN CELL CULTURE. John H. Peacock and Charles R. Walker*. Dept. of Neurology, Stanford University Medical School, Stanford, CA 94305.

Flow of electric current from a cell body into its branches is governed by a combination of geometric and electric factors. Estimates of distribution of current flow between soma and processes can be made from the cable equations derived by Rall (1) who has suggested that nerve cell cultures provide an opportunity to compare cable properties which have been separately determined from geometric and electrophysiologic measurements. We have studied hippocampal neurons during their first 21 days in culture. Young neurons have less extensive branching than in older cultures and are well visualized because the background nonneuronal cell layer is thin. These neurons have been studied in growth medium plus 5-10 mM added $CaCl_2$. The mean resting potential was -55 ± 1 mV, 98 cells; action potentials were recorded from each cell; and postsynaptic potentials occurred over the entire time span of study.

Detailed measurements of cell geometry were made from photographs (1250X) with a 10X ocular. Geometric measurements were used to calculate electrotonic lengths (L_D) of processes and summed conductances (ΣG_D) of processes in 8 neurons under sealed end conditions for a membrane resistivity (R_m) of 2000 Ωcm^2 and an internal resistivity of 70 Ωcm. Electrophysiologic data give input resistance (R_N), membrane time constants (τ_m and τ_1) and estimates of electrotonic length for the entire neuron (L_N).

L_D (from geometry) ranged from 0.4-1.8 whereas L_N (from electrophysiology) was 1.0-1.9 space constants. In these cells, the ratio of diameters of 55 daughter to parent branches raised to the 3/2 power was 0.61 ± 0.04 $(10^6)cm^{3/2}$. From branching analysis the weighting factor for current flow into primary trunks (Bo) was 0.29 ± 0.05. Thus mean current flow into the 18 primary trunks was about 29% of the current flow into an equivalent infinite cylinder. ΣG_D ranged from 0.6-26.6 nS. Corresponding soma conductances (G_S) were 2.3-4.4 nS. The sum of ΣG_D plus G_S equals G_N, the input conductance, which ranged from 2.9-29 nS. The reciprocal of G_N is R_N which varied from 35-345 MΩ.

In 3 cells, measured R_N was compared to predicted values from branching analysis. Here, R_m's of 1725, 1610, and 610 Ωcm^2 plus detailed geometry yield the recorded R_N's of 100, 100, and 106 MΩ's respectively. Furthermore, predictions of R_m from electrophysiology (see Rall, 1) are respectively 1330, 1620, and 640 Ωcm^2; hence geometric and electrophysiologic estimates of R_m and R_N fit reasonably well in these cases. The issue of whether cable properties change with development in culture will be addressed in subsequent work. (Supported by NIH grants NS 12151 and NS 07012).

[1]Rall, W., Handbook of Physiology, Section 1: The Nervous System, Vol. 1, Part 2, 1977, pp. 39-97

1706 SYNAPTIC ULTRASTRUCTURE OF SINGLE PHYSIOLOGICALLY IDENTIFIED NEURONS USING COBALT. Christine E. Phillips, Department of Zoology, University of Cambridge, Cambridge CB2 3EJ,England.

Techniques for intracellular recording and iontophoresis of cobalt dye into identified neurons in the locust Schistocerca americana gregaria have been in routine use for a number of years. Many motorneurons to flight and leg muscles and some of the presynaptic interneurons have been physiologically characterised and their morphology examined at the level of the light microscope after silver intensified cobalt staining.

A technique has been developed to preserve the synaptic structures of dye marked motoneurons and local non-spiking interneurons. Electrodes containing a mixture of cobalt chloride and potassium chloride were used for recording, physiological identification and staining of the neurons. Ganglia were prefixed prior to sulfide precipitation of the cobalt, then fixed for electron microscopy and embedded in Epon 812.

Ganglia were serially sectioned at 2.5um. Sections were silver intensified on glass slides, photographed and re-embedded. Thin sections were stained with uranyl acetate and lead citrate and photographed in a Philips EM300.

The cobalt-silver complex appears as descrete dense particles ranging from 15nm to 40nm in diameter. The highest concentration was found in the cell body. The perineurium and glia lacunae exhibited non-specific silver reaction. Frozen sections of unfixed, control ganglia which were then silver intensified, showed that was due to an endogenous reaction. Silver precipitate in the cell body layer and in the neuropil was restricted to the injected neuron and could be found in terminal arborizations.

Using this technique, synaptic structures of identified motorneurons and presynaptic, non-spiking interneurons in the mesothoracic and metathoracic ganglia of the locust have been clearly seen.

This work was supported by N.I.H. postdoctoral fellowship No. 1 F 32 NS 06028 01.

1707 A NON-RANDOM PROCESS DETERMINES THE DIRECTION OF MYELIN WRAPPING. Whitman Richards, Ronald Kalil[1] and Claire L. Moore*.[2] Department of Psychology, M.I.T., Cambridge, Massachusetts 02139.

Over ten years ago, Peters (1964) observed that the internal and external mesaxons of developing myelin sheaths were often found in the same quadrant during the early stages of myelination. This observation, together with more recent work (Moore et al, 1976), suggests that the initial glial wrapping proceeds step-wise with complete 360° revolutions about a fiber. Little attention has been paid to the direction of wrapping, however. To explore this question, approximately 1100 fibers were examined at 10,000X in an EM montage covering 0.04 mm^2 of the optic tract of a 4-week-old kitten. The analysis shows that the direction of wrapping of neighboring fibers does not proceed independently of one another. For fibers in contact with the same glial cell, the probability of adjacent fibers being wrapped in the same direction is 75% rather than the 50% chance value. Furthermore, as one proceeds from one fiber to the next in a progression around a glial cell, then rarely do more than four reversals of the direction of myelin wrapping occur. A relaxation oscillator model for myelination is proposed, whereby glial "beats" of elongation and contraction about two axes cause its membrane to wrap fibers on opposite sides of the glial core in the same direction.

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Peters, A. (1964) Further observations on the structure of myelin sheaths in the central nervous system. J. Cell. Biol. 20, 281-296.

Moore, C.L., Kalil, R. and Richards, W. (1976) Development of myelination in optic tract of the cat. J. Comp. Neur. 165 (1), 125-136.

1708 MEMBRANE RESISTANCE AND ELECTROTONIC DECAY IN GUINEA PIG CA1 HIPPOCAMPAL PYRAMIDAL CELLS. Dennis A. Turner* and Philip A. Schwartzkroin. Dept. Neurol. Surg., Univ. Wash., Sch. Med., Seattle, WA 98105 U.S.A.

Input resistance (R_{in}) and other intracellular parameters were measured in CA1 cells in the in vitro hippocampal slice preparation. Subsequently, horseradish peroxidase was injected intracellularly and then reacted with diaminobenzidine and H_2O_2. Twelve pyramidal cells and 2 interneurons were successfully stained, with average R_{in} 27.8 ± 7.4 (mean ± S.D., megohm) for the pyramidal cells and 27.0 ± 1.0 for the interneurons. The specific quantitative anatomy of each cell was then reconstructed using computerized finite cable analysis (Rall, W. Exp. Neurol. 1:491, 1959). Specific membrane resistance (R_m, ohm-cm^2) averaged 2450 and 4860 for the pyramidal cells, comparing sealed-end and infinite cable terminations, respectively. R_m ranged between 915 (sealed-end) and 1050 (infinite cable termination) for the 2 interneurons, one of which appeared to be a basket cell histologically. Evaluation of the three-halves power of the diameters at branch points revealed an average of 1.001, for 2680 branch points.

Apical dendrites of pyramidal cells terminated at 1.2-2.5 length constants, while the basilar dendrites ended at 0.7-2.0. For voltage injected at the most distal apical and basilar dendritic terminations, only 0.4% would appear at the soma. For terminals within the stratum radiatum, 3.3% of the injected voltage would reach the soma. However, for current injected at the apical terminations, an average of 34% would arrive at the soma, as compared to 51% for the most distant basilar dendritic regions and 72% for the stratum radiatum terminations. Input resistances of these apical and basilar dendritic segments averaged 2.9×10^9 ohms. Thus, current in these cells achieves a higher transfer efficiency than voltage in electrotonic conduction from distal parts of the cell to the soma. The dendrite to soma conductance ratio in pyramidal cells averaged 3.5 ± 1.3 for the sealed end termination and 7.7 ± 3.1 for the infinite cable termination.

These measurements compare well with those on the motoneuron, and indicate that distal synaptic events may significantly influence ongoing activity at the soma, without active conduction.

NEUROPATHOLOGY AND NEUROIMMUNOLOGY

1709 MICROSCOPIC STUDY OF A TRAUMATIC/HYPOXIC BRAIN INJURY MODEL. E.L. Auen, K.D. Barron, L.R. Nelson and R.S. Bourke, Div. of Neurosurgery and Depts. of Anatomy and Neurology, Albany Medical College, Albany, NY 12208.

Light and electronmicroscopic techniques were used to examine various neuroanatomical sites in a brain trauma model presented elsewhere (see Nelson et al. this volume). The intent was to establish the neuropathological changes resulting from traumatic insult to brain in conjunction with and separate from hypoxic insult. The cats were divided into 4 groups. Group I cats received the traumatic insult and were sacrificed at various times beginning immediately after the insult. Group II cats received hypoxic insult (respired with 6% O_2 in N_2 for 30-60 min) and also were sacrificed at various times after the insult. Group III cats received the combination traumatic/hypoxic (60 min) insult and likewise were sacrificed at various times up to 24 hours post trauma. Group IV was the control receiving all surgical manipulations except trauma and hypoxia. Animals were killed under anesthesia with a paraformaldehyde-glutaraldehyde perfusion sequence and tissues from one hemisphere were embedded in epon while the other half of the brain was embedded in paraffin. The neuroanatomical sites studied included motor cortex, caudate,thalamus,cerebellum, brain stem, periaqueductal gray, ectosylvian gyrus, hippocampus, and spinal cord.

Group I animals showed a conspicuous degree of perivascular astroglial cell and process swelling. In 1µm epon sections stained with toluidine blue the perivascular astroglial cell swelling was easily observed. These expanded electron-lucent profiles in the perivascular position were scattered throughout the brain but particularly evident in motor cortex and cerebellum. The perivascular astroglial swelling was not observed immediately after trauma but was seen at 40 min post trauma. The Group II animals demonstrated a scattered and subtle perineuronal astroglial swelling mainly confined to motor cortex. Group III animals receiving the combined traumatic/hypoxic insult had a 50% mortality rate. Animals in Group III apparently destined to survive demonstrated variable degrees of astrocytic swelling depending on the neuroanatomical location and time of sacrifice. Group III animals destined to expire based on electroencephalogram criteria showed not only perivascular and perineuronal astroglial cell swelling but also chromatolytic changes in neurons and electron-dense nerve cells. Clumping of nuclear chromatin was observed with extrusion into the cytoplasm of both neuronal and astroglial cells. These pathological conditions occurred within 20 minutes from the traumatic insult. Group IV animals were normal in appearance. Supported by NIH Grant NS 13042.

1710 MULTIPLE FORMS OF THE COPPER(II)-CARNOSINE COMPLEX AND THEIR POSSIBLE INVOLVEMENT IN WILSON'S DISEASE. Charles Eric Brown and William E. Antholine*, Departments of Biochemistry and Radiology, The Medical College of Wisconsin, Milwaukee, Wisconsin 53226.

The copper(II)-carnosine complex has been well characterized in the crystal as a magnetically coupled cupric dimer that is chelated by two molecules of carnosine (β-alanyl-L-histidine). The complex in solution was assumed to consist only of a single copper(II) ion complexed to one molecule of carnosine with crystallization occurring when two of these complexes joined to form the dimer. Since the monomeric complex in solution was shown to be weak, no copper-carnosine was expected to form in vivo. We have found that the cupric monomer is formed in solution only when carnosine is in excess. There are four molecules of carnosine bound per copper(II) ion instead of only one as indicated by the nitrogen hyperfine splitting of the $g_{\parallel}$ low field $I=+\frac{1}{2}$ transition in the S-band ESR spectrum at 77°K (Fig. 1). In addition, the

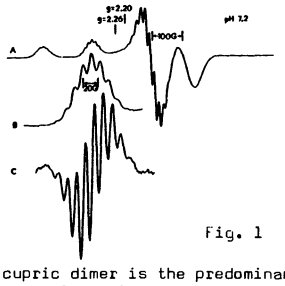

Fig. 1

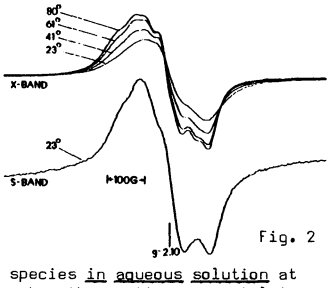

Fig. 2

cupric dimer is the predominant species in aqueous solution at physiological pH and temperature when the peptide and metal ion are at equimolar concentration. This stability in aqueous solution is demonstrated by the increase in ESR spectral intensity as the temperature is raised (Fig. 2). We have detected the dimeric species at concentrations as low as 10µM at which the sensitivity of the spectrometer is limiting. Similar results were obtained with anserine (β-alanyl-1-methylhistidine) but not with homocarnosine (γ-aminobutyryl-L-histidine). These observations may explain the finding that patients with carnosinemia, carnosinuria, anserinuria and deficient carnosinase activity in the serum (i.e. with elevated carnosine or anserine in the plasma and/or urine) exhibit neurological damage that mimics that of Wilson's disease (i.e. hepatolenticular degeneration, impaired copper metabolism by the liver resulting in deposition of copper in brain and kidney). (Supported by a Biomedical Research Support Grant from The Medical College of Wisconsin and NIH Grant RR-01009)

1711 IMPAIRED PROLIFERATIVE RESPONSE OF LYMPHOCYTES TO MEASLES ANTIGENS IN MULTIPLE SCLEROSIS. Vinh Cam*, Hon-Sum Ko*, Michael Lyons*, Charles Plank* and John Zabriskie* (SPON: N. E. Miller) Rockefeller Univ., New York, NY 10021.

The blastogenic index to measles antigens was studied in 23 patients with multiple sclerosis (MS) and compared to that in 23 normal controls and in patients with other neurological diseases. MS patients exhibited a consistently low response to measles antigens when compared to controls (mean response 45% lower than controls). However, responses to mumps, parainfluenza, bacterial antigens and PHA were normal.

Two factors appeared to be most important for successful cell proliferation to measles antigens: 1) the optimal cell concentration (1×10^6 cells/ml) and 2) the selection of a correct batch of human AB+ plasma. The HI titers to measles antigens in AB+ plasma and in the sera of all MS and control subjects, and their level of immune complexes were measured, and their HLA typed.

Further investigations demonstrated that approximately 70% of MS patients had increased levels of $Fc\gamma$ receptor bearing T cells ($T\gamma$) among their peripheral blood lymphocytes. The elevated $T\gamma$ level appeared to correlate with the depressed blastogenic response. Adherent, B, T, $T\gamma$, and $T\gamma$-depleted T cells were separated and their reactivity to measles antigen studied. The blastogenic response of MS patients was not restored by depletion of $T\gamma$ cells.

1712 UTILIZATION OF COMPUTERIZED TOMOGRAPHY IN THE DETECTION AND MANAGEMENT OF PEDIATRIC BRAIN TUMOR PATIENTS. D. DE MICHELE*, M. CERRONI*, L. SINKS*, D. MC CULLOUGH*, B. HAMILTON , W. NORMAN*, P. WEISS*, D. ROBERTSON*, D. SCHELLINGER*, H. MANZ*, AND J. MAZZIOTTA*. (SPON: R. ADAIR) GEORGETOWN UNIVERSITY MEDICAL CENTER WASHINGTON D.C. 20007, AND U.C.L.A. MEDICAL CENTER, LOS ANGELES, CA 90024.

Advances in therapy for pediatric cancer patients has been disproportionate among the different types of cancers. Clinical success rates with cerebral neoplasms has not been particularly consistent. This is due in part to a lack of precision methods whereby patient response to surgical, radio, and adjuvant chemotherapy can be assessed. In an attempt to resolve this situation we have developed a system for monitoring glioma development and response to treatment through employment of Computerized Tomography (CT). We believe the information gained from this system will enable us to treat our patients more effectively. Three parameters and how they interact are followed over time. These are, lesion volume, ventricular volume and total normal brain volume. This information is critically reviewed taking into account the drugs that are in use at the time. Therapuetic regimens are developed based on this volumetric data and continually updated. Secondly, neurological signs and symptoms can be correlated to neuroanatomical scan data. Ventricular, lesion and total brain volumes change unpredictably over time within the same patient and among different patients with the same histologically verified lesion. Changes in lesion volumes can be examined in conjunction with fluxes of ventricular and total brain mass determinations thus providing a detailed view of complete intra cranial dynamics in disease and during the entire course of multimodal therapy. This system will be described, advantages and disadvantages weighed, and its worth assessed by illustrative longitudinal case studies.

1713 TRANSPLANTATION OF RAT SCHWANN CELLS CULTURED IN VITRO INTO DEMYELINATED AREAS OF THE MOUSE SPINAL CORD. I.D. Duncan*, A.J. Aguayo, Dept. Neurol., McGill Univ., Montreal, Que. H3G 1A4, R.P. Bunge and P. Wood, Dept. Neurobiol. Washington Univ., St. Louis, Mo. 63110.

It has been shown in the rat by Blakemore (Nature 266:68,1977) that segments of the autologous sciatic nerve can be transplanted into regions of the spinal cord. The aim of the present study was to determine the feasibility of grafting cultured xenogenic Schwann cells to the mouse demyelinated spinal cord.

Laminectomies were performed on C57BL/6J mice and quaking (qk) mice in mid-lower thoracic area and the dura exposed and then incised. A hand held 20 µm tip glass micropipette connected to a microsyringe containing lysophosphatidyl choline (LPC) (10 µg/ml) was inserted into the dorsal columns and 1 µl injected at three sites. A small breach in the pia was made in the center of this area. Holtzman rat Schwann cells, cultured and separated from neurites (Brain Res. 115:36, 1976) were inserted into this breach and teased gently under the pia. The wound was closed and the animals allowed to survive from periods of between 2 and 15 weeks during which immune suppression was carried out by the twice weekly injection of host mice with antilymphocytic serum (ALS). From 2 weeks it was possible to detect spinal cord axons which were myelinated by Schwann cells. Myelination by Schwann cells was observed to extend along the dorsal columns of the spinal cord for a distance of approximately 200 µm, rostral and caudal to the site of the graft. These were especially obvious in the qk mice where axons well myelinated by Schwann cells were adjacent to and mixed with the host's hypomyelinated axons.

Early evidence that these Schwann cells were foreign was deduced by graft rejection techniques. These findings suggest that Schwann cells grown in vitro can ensheath and myelinate both demyelinated axons in the CNS as well as regenerating axons in the PNS (Aguayo et al, Neurology 29:589, 1979).

1714 DOPAMINE DEPLETION AND THE VISUAL EVOKED POTENTIAL. Robert S. Dyer, William E. Howell* and Robert C. MacPhail*. Neurotox. Div. Health Effects Res. Lab., U.S.E.P.A., Research Triangle Park, NC 27711.

It has recently been reported (Brain, 101:661, 1978) that patients with Parkinson's Disease have significantly longer visual evoked potential (VER) latencies than nonparkinsonian patients. This altered response to photic stimuli may result from the brain dopamine depletion known to correlate with Parkinson's disease, or from other nonspecific factors. We report here that brain dopamine depletion significantly increases the latency of the flash evoked potential from the visual cortex of rats. Chronically prepared rats were injected with the following drugs, presented in counterbalanced order once every three days: Alpha-methyl-para-tyrosine (AMT) (75 mg/kg 6 hrs + 75 mg/kg 2 hrs before testing), FLA-63 (25 mg/kg 4 hrs before testing), scopolamine (2 mg/kg 15 min before testing), naloxone (1 mg/kg 15 min before testing), and saline (2 ml/kg 6 hrs + 2 ml/kg 15 min before testing). In a separate experiment, ten animals were injected with (150 mg/kg) para-chlorophenylalanine (PCPA) and ten others were injected with saline. In this experiment all injections were given 3 days before testing. Peak-to-peak amplitudes and peak latencies of the potential evoked by 64 flashes were recorded. AMT significantly increased latency to the first negative peak (N1) (34.5±0.8 msec) compared to the saline control (30.1±0.3 msec), while FLA-63 did not alter latency (30.6±0.4 msec). AMT also increased latency of peaks P1 and P2. Dopamine depletion was apparently responsible for the altered latency, since depletion of norepinephrine by FLA-63, a dopamine-β-hydroxylase inhibitor, did not produce these alterations. AMT did not alter latencies of later peaks (N2, P3 or N3), thus demonstrating independence of these peaks from earlier ones (P1, N1, P2). Since the effect of AMT occurs in the first peak of the evoked potential, since the retina contains dopamine, and since thioridazine has been shown to increase the latency of the ERG A wave in humans (Int. Pharmacopsychiat. 13:151, 1978), it seems likely that the effect of AMT on the visual evoked potential occurs primarily at the retinal level. This interpretation is supported by the finding that AMT does not alter latencies of potentials recorded in the cortex following optic tract stimulation.

1715 LONG-TERM LEARNING DEFICIT IN THE RAT PRODUCED AFTER METHYL MERCURY EXPOSURE IN LATE GESTATION. C.U. Eccles and Z. Annau. The Johns Hopkins University, School of Hygiene and Public Health Baltimore, MD 21205.

Prenatal exposure to methyl mercury (MeHg) is known to cause central nervous system damage in a variety of species. The nature of the resulting deficits and the parameters involved in the manifestation of them is currently under investigation. A previous report from our laboratory demonstrated that the offspring of rats treated with 8 mg/kg MeHg on day 7 of gestation sustained long lasting learning deficits when tested in a two-way shuttlebox avoidance task as adults (Eccles and Annau, Neurosci. Abst., 4, p. 396, 1978). We have employed a similar design to examine the effects of the same dose of MeHg administered on day 14 of gestation.

Pregnant Long-Evans rats bred in our laboratory received 8 mg/kg of MeHg on day 14 of gestation. The control groups received sodium carbonate alone. On the day of birth, litters were culled to a standard size of 8, if possible. The pups were weighed on days 1, 7, 14 and 21.

Adult males were again weighed at 9 weeks of age. The spontaneous locomotor activity of each of these animals was measured for 1 hour in a Stoelting electronic activity meter. The rats were then tested in a two-way shuttlebox avoidance task. Training was carried out in daily 50-trial sessions until the criterion level of 10 consecutives avoidances was met. Avoidance behavior of animals that met the criterion was extinguished the following day. This was followed by a reacquisition period in which the animals were again required to make ten consecutive avoidances.

Neonatal weight data revealed a significantly reduced weight gain in the MeHg treated pups. The mean weight of the MeHg treated males at 9 weeks of age (235 g) was also reduced compared to that of controls (276 g).

There were no significant difference in spontaneous locomotor activity level between the two groups, although there was greater variability in the MeHg treated group for this measure. In the avoidance task, 80% of mercury treated rats did not reach criterion level of responding during the acquisition period. The rats that did meet the criterion required significantly more trials than controls. When treated rats that previously met criterion were tested during reacquisition, they again required a greater number of trials to reach criterion compared to controls.

The results indicate that MeHg administered on day 14 of gestation can result in a severe and long-lasting learning deficit in the absence of general motor debilitation. The effects seen after administration of MeHg on gestional day 14 were more severe than those observed after administration on day 7.

1716 FOCAL LACERATION AND CONTUSION OF RAT CEREBRAL CORTEX. D.M. Feeney, H.M. Murray, W.G. Dail Jr., M.G. Boyeson & R.T. Linn Depts. of Psych. & Anat., Univ. of New Mexico, Alb. NM 87131.

Changes in behavior, lesion histochemistry and morphology were studied at various times after unilateral laceration or contusion of rat cerebral cortex. As a model of laceration, four mm diameter undercuts were made in the area from which hindpaw movements can be evoked by electrical stimulation. In the first hours after injury, EEG was reduced to half prelesion amplitude at loci several mms distant from the undercut. At 24 hours, the lesion was not organized and only minor cell loss was seen in overlying cortex, however, an oxidative enzyme stain (alpha glycerophosphate dehydrogenase) showed extensive paling far beyond undercut cortex. By 4 days, cells adjacent to the undercut stained intensely for dehydrogenase. These apparently are macrophages and are filled with vacuoles containing hemosiderin-like material. Hypertrophied astrocytes now appear at the lesion as well as mitotic figures presumed to be hematogenous in origin. Between 9 and 15 days the undercut in the white matter enlarges to maximum size and alkaline phosphatase stain indicates a transition to a lined cavity. Hypertrophied astrocytes stain intensely for alpha glycerophosphate dehydrogenase by 15 days and superficial cortex above the cut is invaded by tissue from the meninges which appears vascular. The widespread cortical paling seen earlier with dehydrogenous stain is now restricted to cortex over the undercut. The EEG has returned to normal amplitude but high voltage spikes are seen in the vicinity of undercut cortex. Behavioral changes include: (1) At 4 days a 700% decrease in speed of locomotion on a narrow plank and a 10 fold increase in footslips. This recovers by 30 days. (2) Recovery is not dependent on practice since animals with identical injuries not tested during the 30 days post injury are also recovered by that time. (3) Spontaneous activity begins to increase at 24 hours, peaks at 6 days and returns to baseline by 40 days.

For cortical contusion, an apparatus delivered graded impacts to the "hindpaw" area of rat cortex. A tube guides a falling weight onto a footplate resting on exposed dura. Force is expressed as weight x distance dropped. Moderate and severe focal contusions, without subcortical involvement are obtained with a 4.5 mm footplate, penetration limited to 2.5 mm by use of a sleeve, and forces of 200 or 600g/cm. This model shows a contralateral hemiparesis with some recovery by 15 days. Hemorrhaging appears to begin in the white matter and is followed by the development over 15-30 days of a necrotic cavity lined with fibroblasts.

Supported by NIH Grant NS 13684-02.

1717 MORPHOLOGICAL AND BIOCHEMICAL ALTERATIONS IN A NEW RAT MODEL OF SPONTANEOUS ALCOHOL INTAKE. Barry Goldstein*, Ronald P. Hammer, Jr., Gaylord Ellison, and David S. Maxwell. Dept. Anat., Sch. Med., UCLA, Los Angeles, CA 90024.

This is a report on a method for producing rats which spontaneously and voluntarily ingest ethanol. The morphology of the brains and livers from a population of these animals has been examined. In addition, their liver alcohol dehydrogenase (AdH) activity has been studied and their brain AdH activity is currently under investigation.

Thirty-two rats were housed in a semi-naturalistic rat colony environment with ad lib access to 10% ethanol and water. After seven months of colony housing, all animals were captured, placed in individual cages and alcohol and water consumption were monitored. There was a wide range of alcohol appetites, five animals showed a marked alcohol preference (4.5-8.1 g/kg/day) while another eight animals consumed virtually no alcohol (0.3-0.7 g/kg/day.) The remaining nineteen animals consumed intermediate amounts of alcohol (0.9-4.3 g/kg/day.) These extremes of alcohol preference were apparently due to the colony housing since they did not occur in twenty-seven rats kept in isolation for the same length of time and also offered ad lib access to ethanol and water. Blood ethanol levels, spontaneous withdrawal signs, food consumption, and behavioral studies are currently being examined in a similar colony.

After the seven months in the colony, the animals were sacrificed and the brains and the livers were removed. The morphology of the nervous system was examined on an ultrastructural level and correlated with a Golgi study and a conventional light microscopic study. Fine structural alterations have been observed in the hippocampus, the mammillary bodies, the cerebral cortex, and the cerebellar cortex of the alcohol consuming rats. These alterations include numerous dendritic varicosities which contain smooth membranous vesicles. Some of these vesicles are continuous with the dendritic membrane while others are contiguous but not connected to the membrane. In the Golgi preparations, these dendritic varicosities often appear as vacuolated nodularities along the length of the dendrite.

The morphology of the livers from the alcohol consuming rats has also revealed structural alterations. Light microscopic examinations have indicated normal hepatic architecture in the animals that did not consume alcohol and a fatty metamorphosis in the alcohol consuming rats. The fine structure of these livers is currently being examined. In addition to the morphological studies of the liver, liver alcohol dehydrogenase activity was examined. The alcohol consuming rats had significantly higher liver AdH levels. (Supported by Grant #AA03513 from the National Institute on Alcohol Abuse and Alcoholism.)

1718 CONTENT AND FUNCTIONAL CAPACITY OF CEREBELLAR RNA FROM RATS WITH GRAFT VERSUS HOST DISEASE (GVHD). W. S. T. Griffin, M. Morrison*, and J. R. Head*. University of Texas Health Science Center at Dallas, Texas 75235.

The possibility of adverse effects on developing fetuses due to the passage of mature lymphocytes from the maternal to fetal circulation has long been recognized. Such passage can result in GVHD occurring during the period of brain development. However, our series of experiments, utilizing the rat cerebellum as a model, is the first to investigate the effects of such an immunological assault on the developing nervous system. We simulate maternal-fetal cell passage by intravenous injections of 40×10^6 parental strain lymph node cells (PSLNC) from Fischer rats (FI) into (FI x DA)F_1 hybrid rat pups on the day of birth. The F_1 hybrids do not recognize the PSLNC as nonself, but the PSLNC recognize the DA antigens present on the F_1 cells and attack host lymphoid tissue, the first of a cascade of events called GVHD. We have previously reported that GVHD decreased cerebellar DNA synthetic capacity, cell number and cellular components, perhaps due to a blood-borne factor released during lymphocyte interaction which inhibits proliferation. Here, we report the effects of GVHD on total RNA content (amount of rough endoplasmic reticulum, rER), RNA synthesis (^{3}H-uridine uptake into the RNA fraction), and RNA message capacity (separation of the various RNA species and the ability of mRNA to direct protein synthesis in a cell-free wheat germ culture). During acute GVHD (postnatal day 14) there was a decrease in 1) the amount of rER as shown by less staining by cresyl violet of cerebellar sections from animals with GVHD when compared to litter mate controls, 2) total RNA content (65% of control) as measured both by colorimetry and optical density, 3) ^{3}H-uridine uptake into the RNA fraction (65% of control), and 4) the overall translational capacity of mRNA (85% the biological activity of the control per unit of RNA). Two dimensional gel electrophoresis of the proteins synthesized by control and GVHD RNAs in in vitro cell-free protein synthesizing systems showed that about 200 to 250 separable proteins are synthesized by both fractions including α and β tubulins and β actin. The vast majority of these proteins appear qualitatively and quantitatively unchanged, but there were several spots in the experimental gels which were absent in the controls and vice versa. The overall conclusion is that there was a generalized reduction in the synthesis of the 200 to 250 proteins with isoelectric points between 4.5 and 7, suggesting a reduction in the amount or biological activity of the corresponding experimental mRNAs.

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1719 MEASUREMENT OF ACETYLCHOLINE RECEPTOR AND ANTI-ACETYLCHOLINE RECEPTOR IMMUNOGLOBULINS USING THE ENZYME-LINKED IMMUNOSORBENT ASSAY. I. J. Griffith, J. A. Lettieri*, and N. L. Norcross*. Dept. of Microbiology, New York State College of Veterinary Medicine, Cornell University, Ithaca, NY 14853.

Recent evidence indicates that the pathogenesis of myasthenia gravis (MG) involves antibodies directed against nicotinic acetylcholine receptors (AChR). Antibodies to the AChR in both MG and an animal model of the disease, designated experimental autoimmune myasthenia gravis (EAMG), are routinely measured using a radioimmunoassay (RIA) utilizing 125-I-labelled α-bungarotoxin (α-BGT) as a specific label for the AChR. We have developed an alternative method for the quantitation of both AChR and anti-AChR immunoglobulins employing the enzyme-linked immunosorbent assay (ELISA).

In its simplest form, purified AChR is adsorbed to the walls of a polystyrene microtitre plate by incubation in carbonate buffer. This is followed by sequential incubation with excess anti-AChR serum, peroxidase-conjugated anti-immunoglobulins, and a suitable substrate. The degree of substrate catalysis is then determined by optical density measurements. This procedure can be adapted to measure AChR or anti-AChR immunoglobulins. With the use of an additional reagent, it is also possible to quantitate specific classes of anti-AChR immunoglobulins.

If α-BGT is adsorbed to the polystyrene, followed by AChR in phosphate buffer, and sequential incubations with reagents as described above, the assay becomes more broadly applicable. With this modification, it is possible to measure AChR in extracts of both electric organ and mammalian muscle tissue. Ligands which inhibit the binding of α-BGT and AChR demonstrate the specificity of the assay.

It has been demonstrated that there is a significant correlation between the presence of anti-AChR immunoglobulins and MG. Due to the relative simplicity of the ELISA in comparison to RIA methods, it is possible that this test may prove valuable as a clinical diagnostic tool for MG.

1720 EFFECTS OF LOCUS COERULEUS AND ANTERIOR HYPOTHALAMIC BRAIN LESIONS ON ANTIBODY FORMATION IN MICE. Nicholas R. Hall, John K. Lewis*, Richard T. Smith* and Steven F. Zornetzer. Dept. Pathol. University of Florida College of Medicine, Gainesville, Florida

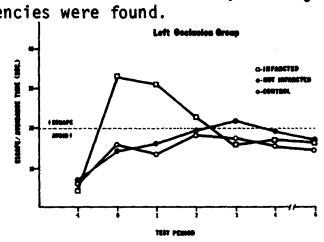
Previous data have shown that electrolytic lesions of the nucleus locus coeruleus are capable of inhibiting the subsequent formation of both granulocyte-macrophage colonies in tissue culture and hemopoietic colony forming units on the spleen induced by radiation exposure. Hemopoietic stem cells arising in the bone-marrow give rise to lymphocytes under the influence of the appropriate microenvironmental factors. The possibility that lesions of the nucleus locus coeruleus might exert an inhibitory influence over the immune system as measured by antibody formation was subsequently investigated.

Adult C57B/6J female mice received bilateral lesions induced electrolytically (300 uA for 10 seconds) in either the nucleus locus coeruleus or the anterior hypothalamus. Control animals were given nembutal anesthesia but were not manipulated surgically All subjects were ovariectomized to eliminate differences that might have been due to cyclic fluctuations of ovarian hormones. Animals were allowed 6 weeks recovery time before being injected with antigen. Sheep red blood cells were washed in hemaglutination buffer and injected ip in 0.25 cc of RPMI 1640 media. Five days later, the subjects were sacrificed by decapitation and trunk blood was collected as a source of serum for measuring hemaglutinating antibody titers. Brains were removed and sectioned for histologic verification of the lesion site.

Animals in both lesion groups were found to be good antibody responders. There was no statistical difference between those animals that had received lesions of the locus coeruleus and those with anterior hypothalamic lesions. Neither of the values obtained from the lesioned animals differed from the unlesioned control titer. These data suggest that the inhibitory influence of locus coeruleus lesions upon bone marrow stem cells is not manifested by a functional impairment of the immune system as assessed by using the above paradigm.

1721 THE RAPID AVOIDANCE TEST OF GERBILS AFTER UNILATERAL CEREBRAL ISCHEMIA. Charles J. Hannan, Jr., Andree J. Lloyd* and John J. McCloskey*. Clinical Investigation and Neurosurgery Service, and Pathology Department, Eisenhower Army Medical Center, Ft. Gordon, GA 30905.

Ninety male Mongolian gerbils completed 6 daily evaluations on the rapid avoidance test (RAT) in an attempt to quantitate the effects of cerebral ischemia produced by occluding a common carotid artery. Animals were trained in a shuttle box on a RAT schedule of 4 escape trials (tone and shock presented simultaneously for 40 sec) followed by 2 avoidance trials (20 sec of tone then 20 sec of tone and shock) with a 5 minute intertrial interval. Criterion for entry into the study was at least one successful response in the avoidance trials. One hundred and ninety six gerbils were trained with 26.5% rejected for not meeting the criterion. Animals were randomly assigned to groups for 1) right, or 2) left carotid artery occlusion, and 3) surgical controls. Animals were given the RAT just prior to surgery (animals failing to avoid were also rejected); at 3,5, or 7 hours after surgery; and then on 1,2,3,4 and 6 days post surgery. If animals died or were judged to be physically unable to perform the RAT they were eliminated from the study and additional animals were trained so that each group contained at least 30 animals at the end of the study. Of 104 animals entered into the occlusion groups, 40 died (right occlusion 17/49, left 23/55), therefore 64 occluded animals completed the study. Infarctions were histologically demonstrated in 14 of the 64 (21.9%) surviving animals (9 left, 5 right hemisphere). Mean values of performance on the RAT exhibited differences between groups, with animals which were found to be infarcted doing poorer than non infarcted or control animals, and left side infarcted animals doing worse than those with right side infarctions. No measure of escape/avoidance time or patterns of time was uniquely predictive of infarction, although significant population tendencies were found.



1722 IMMUNOCYTOCHEMICAL OBSERVATIONS ON DEMYELINATING LESIONS IN EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS (EAE). Yasuto Itoyama*, Nancy H. Sternberger*, Richard H. Quarles*, Steven R. Cohen* and Henry deF. Webster. NINCDS, NIH, Bethesda, MD 20205.

Focal changes in the distribution of myelin sheath constituents that occur during myelin breakdown are not well understood. To investigate these changes, we induced experimental allergic encephalomyelitis (EAE) by injecting an emulsion of spinal cord and complete Freund's adjuvant into strain 13 guinea pigs and Lewis rats. In guinea pigs, the first symptoms occurred 10-11 days after injection; all of them had a rapidly progressive illness and died on the 15-16th day. The rats became ill after 9-10 days; their illness was more severe 3-4 days later and all of them were recovering when sacrificed 17-19 days after injection. Animals were perfused with fixative before symptoms appeared, within 48 hrs of the first symptoms, and during severe illness. Rats also were perfused during recovery. Vibratome and paraffin sections were immunostained with antisera to myelin basic protein (BP), a major constituent of compact myelin, myelin-associated glycoprotein (MAG), located periaxonally in myelinated fibers, and immunoglobulin G (IgG) according to our modification of the peroxidase-antiperoxidase (PAP) method. The major findings were: 1) Early focal fragmentation of myelin sheaths was easier to identify with BP immunostaining than with LFB or other histological methods. 2) In more advanced lesions, fragmenting sheaths and demyelination were found beyond margins of perivascular infiltrations especially in the gray matter. 3) Demyelinating lesions in the gray matter also contained substantially fewer mononuclear cells than the white matter lesions of the same size. 4) In periventricular lesions, BP-stained myelin fragments were present extracellularly and in subependymal macrophages. Some macrophages were found between ependymal cells and a few were located intraventricularly. Their presence in the CSF may explain the elevated BP levels described by others in acute attacks of EAE and multiple sclerosis. 5) In zones of myelin fragmentation and breakdown, periaxonal staining by MAG antiserum was abnormal also. 6) In early EAE lesions, IgG was present perivascularly. Its penetration into the surrounding parenchyma increased as the lesions progressed. The IgG staining pattern was similar to that reported by others for EAE blood brain barrier experiments using tracers.

1723 TRIMETHADIONE INCREASES THE REDUCTION IN BRAIN MICROVASCULAR VOLUME AFTER PROLONGED CEREBRAL ISCHEMIA IN GERBILS. D.M. Jarrott*, and F.R. Domer. Depts. of Neurosurgery & Pharmacology, Tulane Univ. School of Medicine, New Orleans, La. 70112.

Qualitative methods of outlining the cerebral microcirculation with intravascular India ink have been used to describe a "no-reflow phenomenon" following ischemia. In this process reperfusion of the brain is hampered after ischemia by microvascular factors which obstruct blood flow to some capillary beds. The cause of the microvascular obstruction, its relevance to the various models of cerebral ischemia, and its quantitative determination have not as yet been accomplished. We have used a plasma label, ^{131}I-RISA, to investigate the microvascular volume of the gerbil and the changes that occur after one hour of bilateral carotid arterial occlusion. The normal, pentobarbital-anesthetized, gerbil has a cerebral blood volume of 14.4±0.7 µl/gm. After carotid occlusion this volume is decreased to 11.6±0.6 µl/kg ($P < .05$). When the gerbils are treated with trimethadione 300 µg/kg, the cerebral blood volume after ischemia is reduced even further to 10.0±0.5 µl/gm, which is significantly lower than that of the saline-treated gerbils ($P < .05$). Mongolian gerbils exhibit spontaneous seizures. The mortality rate after bilateral carotid occlusion is related to frequent and severe postischemic convulsions. Both spontaneous and stress-provoked convulsions are blocked by trimethadione and phenobarbital but not by phenytoin or pentobarbital. Administration of trimethadione 300 mg/kg to gerbils after one hour of bilateral carotid occlusion reduced the resulting mortality rate from 100% to 50% whereas phenytoin 10 mg/kg had no protective effect. Thus, the reduction of postischemic neuronal hyperexcitability produced by trimethadione might result in less postischemic hyperemia.This would explain the further reduction in microvascular volume observed in the postischemic period in trimethadione-treated gerbils.
(Supp. in part by an Institutional Biomedical Sciences Research Grant).

1724 MORPHOLOGICAL CORRELATION OF THE NEURONAL AND GLIAL RESPONSE TO COMPLETE CEREBRAL ISCHEMIA. Larry W. Jenkins*, John T. Povlishock, and Donald P. Becker (SPON: D.P. Becker). Depts. of Anatomy and Neurological Surg., Med. Col. of Va., Virginia Commonwealth Univ., Richmond, VA. 23298.

Adult cats were subjected to 1.5 to 15 minutes of reversible complete cerebral ischemia both with and without subsequent post-ischemic reperfusion. In all animals rigorous physiological controls were employed both prior to and following the ischemic insult. Following the desired duration of complete ischemia and if permitted post-ischemic recirculation, all animals were perfused with aldehydes. Tissue samples from selected brain loci were then processed for light and electron microscopy. Through such a regimen the termporal development of both neuronal and glial morphological alterations were ascertained.

In those animals perfused immediately following 1.5 to 15 minutes of complete cerebral ischemia both the neurons and astrocytes manifested homogeneous cellular alterations. Such alterations consisted of progressive chromatin clumping, nucleolar condensation, and moderate dilation of the endoplasmic reticulum, Golgi complex and mitochondrial intracristal spaces. In contrast, in those animals subjected to comparable insults followed by post-insult recirculation, heterogenous neuronal and glial alterations were now observed. These were more pronounced with increased insult durations and reperfusion periods. Four characteristic types of neuronal responses were observed, while three characteristic types of glial responses were noted and as such were confined to the astroglia. At the ultrastructural level these characteristic neuronal responses ranged from subtle neuronal involvement as manifested by chromatin clumping and minimal cytoplasmic changes to markedly altered neurons displaying shrunken and electron dense perikarya. The astroglia demonstrated either discrete cellular changes reflected in minimal chromatin clumping or more pronounced perturbations as evidenced in dramatic cellular swelling. A reciprocal relationship between neuronal shrinkage and astroglial swelling was observed. At the ultrastructural level shrunken and electron dense neurons were always seen in relation to swollen astrocytic cell processes. Such an observation suggests that neuronal and astroglia cell volume shifts following complete cerebral ischemia may be related.

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1725 CATALASE ACTIVITY IN NORMAL AND DENERVATED HEREDITARY DYSTROPHIC HAMSTER MUSCLE. Robert R. Jenkins and Diane Newsham*. Biology Dept., Ithaca College, Ithaca, NY 14850.

Catalase (H_2O_2 oxidoreductase, E.C. 1.2.1.6) has recently been shown to be present in skeletal muscle (Stauber and Schottelius, Exp. Neurol. 48: 524-33, 1975). The exact biological role of catalase has eluded investigators despite the voluminous amount of research which has been conducted on this enzyme. Work from the lab of Stauber et al. (Exp. Neurol. 55: 381-89, 1977) and our own lab has shown that catalase activity increases during various forms of muscle wasting such as tenotomy, casting and denervation. Our work has suggested that catalase may serve as a suitable marker for denervation since significant increases may be observed within two days of transection as compared to one week for other forms of atrophy. The present study was designed to determine whether a similar post denervation rise in catalase could be observed in genetically dystrophic hamsters. The study involved a comparison between Syrian normal control hamsters and dystropic (UM-X7.1) control and denervated animals. Catalase was analyzed on tissue homogenates of extensor digitorum longus, soleus, adductor magnus and myocardial muscles by the oxygen cathode method. Protein was determined by a modification of the biuret method. We have found that catalase activity is significantly greater in muscles which metabolize greater amounts of lipids. We also observed significantly greater catalase activity in the muscles most involved by the muscular dystrophy. Furthermore, denervated dystrophic muscles were found to have catalase activities which were significantly elevated above that of dystrophic controls. These results support the notion that catalase may serve as a possible marker in denervation.

1726 CAPRINE NEUROVISCERAL STORAGE DISORDER RESEMBLING MANNOSIDOSIS. Margaret Z. Jones, James G. Cunningham, Albert W. Dade and David M. Alessi*. Depts. Path. and Physiol., Michigan State Univ., East Lansing, MI 48824.

Of the genetically determined metabolic disorders which are expressed in animals by markedly abnormal neurological function at birth, the most common is bovine mannosidosis. Storage of mannose and N-acetyl glucosamine (glcNAC) in neurons and other cellular elements is evident in this disorder as cytoplasmic vacuoles bound by a unit membrane. Deficiency of alpha-D-mannosidase can be demonstrated in various tissues of affected animals. This report describes a similar disorder which has affected both male and female offspring of clinically normal Nubian goats. Affected neonates were unable to rise or bear weight on any limb. A coarse tremor in the head and all four limbs was exaggerated by movement. Miosis, ptosis, enophthalmos and intermittent tremor of the extraocular muscles were noted. Joints were hypermobile and proximal muscle atrophy was present. General physical examination was otherwise unremarkable. Radiographic studies revealed increased density of the distal femoral and proximal tibial metaphyses. Necropsy studies revealed no abnormalities of the joints. Marked diminution of myelination and ventricular dilatation were observed in the smaller brains of affected animals.

Light and electron microscopic studies of formalin and glutaraldehyde fixed tissues demonstrated numerous vacuoles bound by a unit membrane in the neurons of the cerebral and cerebellar cortex, in Schwann cells, endothelial cells, hepatocytes, Kupffer cells, renal tubular epithelium and reticuloendothelial cells of bone marrow, spleen, liver and lymph nodes. Vacuoles contained finely dispersed floccular material or sparse membranous fragments. Golgi type 2 neurons of the cerebellum were most affected of all neurons. Fewer oligodendroglial nuclei were seen in the myelin-poor white matter of affected animals. Axonal spheroids throughout the white matter contained aggregates of dense bodies and mitochondria. Biochemical studies (Dr. Glyn Dawson) revealed an eight-fold increase in urinary mannose and glcNAC. The activity of several hepatic lysosomal hydrolases, including alpha mannosidase, was elevated. Brain gangliosides, neutral glycolipids and phospholipids were not increased. Hepatic mucopolysaccharide levels were normal. The biochemical defect responsible for this caprine neurovisceral storage disorder remains to be determined.

This research was supported by the NIH Biomedical Research Support Grant of the College of Veterinary Med., Mich. State Univ.

1727 IN VIVO TRACER STUDIES OF SKELETAL MUSCLES IN GENETICALLY DYSTROPHIC HAMSTERS BEFORE AND AFTER CALCIUM IONOPHORE ADMINISTRATION. George Karpati and Stirling Carpenter*. Montreal Neurological Institute - McGill University, Montreal, Quebec, Canada H3A 2B4.

In genetically dystrophic hamsters (UM-X7.1), in the florid necrotic phase (60-120 days of age), 1% sodium fluorescein or horseradish peroxidase (HPO) was injected into the abdominal aorta. After 15 minutes the quadriceps, plantaris and soleus were removed. Cryostat sections were examined for peroxidase activity or fluorescence. Necrotic fibers with phagocytosis were usually brightly fluorescent and sometimes showed modest peroxidase activity. Occasional clusters of tracer-positive fibers were not obviously necrotic at the level of section. Only rare hypercontracted fibers were present. They were strongly fluorescent but did not react for HPO. In other dystrophic animals, one of two calcium ionophores (CaI), (A 23187-Lilly Res. Lab, or RO 2-2985/13) in dimethyl sulfoxide (DMSO) was injected into the abdominal aorta 15 minutes before the tracer. Numerous clustered and isolated hypercontracted fibers were then found in all muscles with or without in situ fixation. They were strongly fluorescent, but showed only faint peroxidase activity. Fluorescence was present diffusely in these fibers, but was especially prominent in a distribution corresponding to the surface membrane. In animals killed 24 hours after CaI administration, the prevalence of necrosis was not increased and hypercontracted fibers were rare. Controls (normal hamsters given CaI, and normal and dystrophic hamsters given DMSO) proved negative. Dystrophic hamster skeletal muscle cells respond to CaI with hypercontraction but, unlike their counterparts in Duchenne dystrophy, do not show tears of myofibrils and do not take up HPO. Their marked fluorescence suggests that only the smaller tracer was able to enter the muscle cell where it may bind to components of the surface membrane. The studies also suggest that this type of hypercontraction develops in vivo, but it does not necessarily lead to necrosis.

1728 RESOLUTION OF VASOGENIC BRAIN EDEMA. Igor Klatzo, Maria Spatz, Esther Chui* and Keigo Fujiwara*. Lab. Neuropathology & Neuroanatomical Sciences, NIH, Bethesda, MD 20205.

Development and resolution of the vasogenic brain edema (VBE) were studied in cats subjected to cryogenic cortical lesions. The basic parameters of VBE, i.e., an increment in water content of the tissue, an increased vascular permeability for various substances and especially for serum proteins, were assessed by specific gravity measurements, use of Evans Blue and application of immunohistochemical staining for cat serum proteins with peroxidase-antiperoxidase (PAP) reagents. Our observations indicate that resolution of the VBE proceeds from the periphery towards the original site of the lesion. This process coincided with a vigorous uptake of serum protein by the glia cells in the edematous area, as demonstrated by immunocytochemical staining according to the PAP procedure. Our studies suggest that the resolution of VBE is related to reduction of colloidal osmotic pressure in the extracellular spaces by the intracellular uptake of extravasated serum proteins in the edematous areas releasing the water to diffuse away and leading to resolution of edema.

1729 LYSOPHOSPHATIDYL CHOLINE INDUCED DEMYELINATION IN THE RABBIT CORPUS CALLOSUM. J.D. Kocsis*, S.G. Waxman*, R.E. Foster, and K.C. Nitta*. Dept. Neurol., Sch. Med., Stanford Univ., V.A. Hosp., Palo Alto, Ca. 94304.

Local injection of lysophosphatidyl choline (LPC) into the corpus callosum of the Dutch rabbit produces a focal demyelinating lesion. LPC (1% in normal saline) was injected in volumes of 1-4 µl through a glass micropipette (tip diam. 10 to 30µ) fixed to a microsyringe. The LPC was delivered over a period of 20 to 40 min. Survival times ranged from 1 hr. to 6 weeks. The lesions were assessed histologically using the Luxol fast blue stain for myelin and the Holmes silver nitrate method for axon cylinders. With these injection methods, the entire vertical extent (about 0.5 mm) of the corpus callosum could be demyelinated with minimal amounts of axonal degeneration. Ultrastructural analysis reveals pathological changes in the demyelinated zone ranging from sparse axonal degeneration to complete demyelination.

In some animals chronic stimulating electrodes were implanted in the corpus callosum near the midline and field potential recordings were obtained from the LPC pipette. The pipette was positioned at a point corresponding to the maximal negativity of the evoked callosal field potential, and LPC was injected at this level. After the injection the callosal field potentials were recorded with tungsten electrodes on consecutive days from the same animal. The fields evoked from single stimuli were reduced in amplitude minutes after LPC injection and this reduction persisted for several days. Another effect of the LPC injection was the reduction in the amplitude of the response evoked by the second of two paired stimuli at interstimulus intervals of up to several hundred msec. This is in contrast to the evoked response in control animals where the amplitude reduction occurs only with interstimulus intervals of up to about 3.0 msec. The present study indicates that it is possible to produce focal demyelinating lesions in fine caliber myelinated axons of the mammalian cerebrum. Therefore, it will be possible to study, over protracted periods of time, the physiological and morphological properties of focally demyelinated cerebral axons.

(Supported in part by the National Multiple Sclerosis Society and the U.S. Veterans Administration).

1730 BEHAVIORAL DEFICIT OF MICRENCEPHALIC RATS IN AVERSIVELY MOTIVATED LEARNING. Moon He Lee*, R. Haddad, Ausma Rabe and Ruth Dumas*. Neuroteratology Laboratory, New York State Institute for Basic Research in Mental Retardation, Staten Island, NY 10314.

Micrencephalic rats, the progeny of rats injected with 30mg/kg of methylazoxymethanol acetate (MAM Ac) on gestation day 15, are deficient in learning to reverse a previously learned position habit in a T maze. This deficit, originally observed in adult rats, has since been demonstrated in immature rats both before and after weaning. In all of these experiments, appetitive motivation was used. However, we have recently found that micrencephalic rats that had to learn to reverse a previously learned position habit to escape from a water filled T maze differ much more sharply from the normal rat. Similar results were obtained both before and after weaning. These findings, along with the previously reported differences in "behavioral arousal" of the micrencephalic rat (Rabe & Haddad, Federation Proceedings, 1972, 31: 1536-1539), suggest that, regardless of any deficit in cognitive function, micrencephalic rats (produced by prenatal treatment with MAM Ac) differ in motivational and/or emotional aspects. The behavioral differences are consistent with those that might be expected to result from the alterations in neurotransmitters in the cerebral cortex of the micrencephalic rat reported by Johnston et al. (Science, 1979, 203: 369-371). It would appear that estimates of the nature and extent of cognitive or other functional deficits in micrencephalic subjects may be grossly in error if the methods of appraisal do not take into account their altered emotional reactivity.

1731 UNILATERAL DFP NEUROPATHY: EVIDENCE FOR AXONAL SITE OF INITIATION. Herbert E. Lowndes, Richard D. Howland*, Thomas Baker and Rudy J. Richardson*. Dept. Pharmacol., CMDNJ, N.J. Med. Sch., Newark, N.J. 07103.

A method for producing a localized mono-neuropathy by intra-arterial injection of DFP has been described (Lowndes et al, Europ. J. Pharmacol. 29: 66, 1974) and morphologically characterized (Glazer et al, J. Neurocytol. 7: 741, 1978). The present studies were performed to confirm that the neurotoxin remained largely confined to the periphery of the injected limb, thus ruling out involvement of cell bodies in the etiology of the neuropathy. DFP (2 mg/kg body wt) labelled with ^{3}H-DFP (5 µCi/kg) was injected into the left femoral arteries of cats. One hr later, portions of the medial gastrocnemious (MG) and soleus (SOL) muscles, dorsal and ventral roots, triceps surae (TS) nerves, the sciatic nerve (SN) from roots to heel and spinal cord were removed bilaterally. DFP concentration was determined by liquid scintillation counting. The proximal-distal distribution of DFP in the sciatic was measured in 1 cm segments. Little DFP was found in the tissues of the non-injected leg. No significant differences were found in the content of left and right dorsal and ventral roots (range: 0.09-0.15 µg/g). Spinal cord segments were divided laterally and contained 0.38 and 0.37 µg/g of DFP on the right and left side, respectively. Left MG and SOL had 4.8 and 7.1 times as much DFP as the respective muscles on the right side. The SN from the injected side showed a proximal-distal gradient of concentrations of DFP: the most proximal segment had a mean conc. of 0.39±0.08 µg/g (SE) which increased over the next 12 cm to a peak of 2.49±0.33 µg/g. The most distal segment had a conc. of 1.11±0.13 µg/g. The left TS had a mean content of 2.56±0.48 µg/g. The contralateral SN showed no proximal-distal gradient and had a mean content for all segments of 0.24 µg/g. This neuropathy does not appear to involve perikarya but results from a direct action of DFP on axons and/or nerve terminals.

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1732 CELL PROLIFERATION AND REMYELINATION. Lidia M.N. Macedo* and Robert M. Herndon. Center for Brain Research, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642

The processes of cell proliferation and remyelination were studied in 1 month old rats using mitotic arrest with colchicine to identify proliferating cells. Demyelination was induced by the focal injection into the spinal cord of 2 µl of a 10 mg/ml solution of lysolecithin containing a small quantity of colloidal carbon to serve as a marker. 200 µg of colchicine was injected intraperitoneally 20 to 22 hrs before the animals were sacrificed. This procedure produced arrest in metaphase of any cells entering mitosis during this period. Animals were killed by perfusion at 1, 3, 5, 8, 12 and 28 days after lisolecithin. 24 hrs after lysolecithin injection there is a central area of necrosis, free of viable cells, surrounded by a large area in which there is virtually complete myelin breakdown and destruction of glial cells and invasion by a few macrophages with relatively good preservation of axons. By day 3, numerous lipid laden macrophages are present throughout the lesion. Many of the axons are denuded of myelin while others are surrounded by degenerating myelin remnants. There is already a suggestion of beginning remyelination at 5 days and easily recognizable remyelination is evident by day 28. Mitotic glial cells were scarce day 1. A few were seen at day 3, but they were more numerous at day 5 and day 8, apparently decreasing in numbers subsequently. The rapid appearance of dividing oligodendroglia which can be clearly identified by electron microscopy three days after lysolecithin injection, suggests that the cell proliferation may begin as a direct response to injury rather than requiring induction by denuded axons.

1733 MORPHOLOGICAL ALTERATIONS IN THE HIPPOCAMPUS OF CHRONIC ALCOHOLIC RATS. A Golgi study. Jesús P. Machado-Salas, Ernesto Espinosa* and José Trujillo*. Lab. de Neuromorfología Exp. y Apl. Depto. de Anatomía, Fac. de Med. UNAM. México, 20 D.F. MEXICO.

Review of the literature offers a good deal of physiological or biochemical studies regarding changes in the central nervous system, secondary to alcohol administration. On the other hand, recent anatomical studies are less common and, as far as we know, the tridimensional status shown by neurons, their prolongations and the neuropile as a whole, has not been described.

We have been studying, with Golgimethods and some other neurohistological techniques, the morphological changes that have developed in brains of alcoholic rats. For this purpose, we have raised three groups of rats under the following conditions: Control group: this group has been allowed to eat regular pellets and drink, ad libitum, common tap water. Experimental group A: this one also eats regular pellets and drink from a 10% alcohol solution. Experimental group B: these rats also receive the same type of pellets and drink from a 20% alcohol solution, as the only source of liquid. They have been kept, under constant conditions, for over a year. The liquid intake has been recorded every day, and the weight increment every other day.

We have been impressed by the qunatity and quality of changes occuring in the CNS of these rats. In this communication we describe the observations made in the hippocampal-dentate complex. 1. Increased glial population, including microglia. 2. Slender and spineless dendrites. 3. Swollen and fragmentated dendrites. 4. Enlarged mossy tufts (terminals), and 5. Evidence of dendritic proliferation with abnormal features. These changes are not age-related. The possible functional significance of these changes shall be discussed.

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1734 DIENCEPHALIC INJECTIONS OF KAINIC ACID PRODUCE MYOCARDIAL NECROSIS. Patrick L. McGeer, C. Kek Galabru*, Edith G. McGeer and William J. Boyko*. Kinsmen Laboratory of Neurological Research, University of British Columbia, Vancouver, B.C., Canada, V6T 1W5, and St. Paul's Hospital, Vancouver, B.C., Canada.

Bilateral injections of 3 nmoles of kainic acid (KA) into the thalamus produce the following triad of peripheral consequences: 1) periarteriolar myocardial necrosis, 2) elevated serum fibrinogen, 3) gross hematuria. Electrolytic lesions of the thalamus and intracerebral injections of KA at several other locations fail to produce this result. KA at much higher doses subcutaneously or intraperitoneally is also inactive. Some protection against myocardial damage is produced by reserpine or 6-hydroxydopamine, but atropine confers no protection. Urinary noradrenaline levels are markedly elevated following effective but not ineffective KA injections suggesting sympathetic "storm" may be one factor. Elevated serum fibrinogen is presumed to be related to tissue damage, while gross hematuria comes from bladder necrosis. The fact that myocardial damage may result from intracerebral lesions and/or pathological stimulation by KA may have clinical implications. Cardiac damage occasionally results in humans from strokes and intracerebral hemorrhage and no satisfactory explanation has ever been offered for the phenomenon of interstitial myocardial fibrosis. Such an end result might follow on repeated stress of cerebral origin. (Supported by the B. C. Health Sci. Res. Fund).

1735 VIRUS-INDUCED IMMUNORETINOPATHY IN NEWBORN RATS. Andrew A. Monjan and Manuel del Cerro, Department of Epidemiology, School of Public Health, Johns Hopkins University, Baltimore, MD and Center for Brain Research, University of Rochester School of Medicine, Rochester, NY 14642

A destructive developmental retinopathy can be induced by infecting rats, within the first 2 days of life, with lymphocytic choriomeningitis virus (LCMV). The lesion is immunologically mediated as attested to by the sparing effects of neonatal thymectomy or antilymphocyte serum. We now present a correlative optical and electron microscopic histopathological analysis of the changes occurring in the LCMV retinopathy. These data indicate that there are, within the cell population of the retina, various degrees of sensitivity to the immune reaction to LCMV. Thus, within the neural retina, there is a distinct gradient of degeneration from the outer to the inner layers. The rods are the least resistant with the ganglion cells being the most persistent of the neurons. Muller glial cells first react with a hypertrophic proliferation of the cytoplasmic lamellae which fill up some of the space vacated by the destroyed neurons and later they undergo pronounced atrophy. The least affected elements are the retinal pigment epithelial cells which, though infected, invariably survive, with comparative minor alterations, even after all the neuronal cells have degenerated. It is noteworthy that the progress of the lesion through the layers follows an exactly opposite direction than that of the spread of viral antigen as observed by immunofluorescence. Thus, while at 10 post-infection days, the antigen is still localized in the inner nuclear layer, morphological alterations are only seen in the outermost regions of the outer nuclear layer.

Finally, although the fluorescent antibody technique shows extensive distribution of the LCMV antigen, no unequivocal identification of viral particles has been made yet. This fact stands in sharp contrast with the profuse presence of viral particles observed in the LCMV infection of the immature cerebellum. Work is in progress to determine whether this difference originates from a sampling problem or represents a unique feature of the LCMV retinopathy.

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1736 EARLY SEGMENTAL DEMYELINATION IN EXPERIMENTAL DIABETIC NEUROPATHY S.A. Moore*, R.G. Peterson, D.L. Felten, and B.L. O'Connor*, Anatomy Department, Indiana University School of Medicine, Indianapolis, Indiana 46223

Studies in this lab revealed a small but significant number of nerve fibers undergoing segmental demyelination (SD) in nerves showing a reduced nerve conduction velocity (CV) in 2 month diabetic rats made diabetic by alloxan and streptozotocin (STZ). Although SD is a consistent pathological finding that may account for reduced CV in diabetic humans, its presence in animal models is disputed even though decreased CV in these models is well documented.

As part of a comprehensive, long term, longitudinal study, the sural and tibial nerves from the mid-calf region of 2 month STZ and alloxan diabetic rats were studied using a teased fiber preparation and compared to age matched controls. Approximately 50 nerve fibers were teased from one sural and one tibial nerve of 9 STZ, 9 alloxan, and 11 control rats. A total of 13 nerve fibers from 8 of the diabetic rats showed evidence of SD. Only one nerve fiber in one control rat had similar evidence. Other pathology observed in diabetic nerves included myelin globules indicative of Wallerian degeneration, swollen axons, and apparent loop formation in internodal myelin.

Although the number of SD nerve fibers may seem small, it is significant in that only a small percentage (about 5%) of the total number of fibers was sampled and a relatively short length (<10% of total) of nerve was examined. Many more nerve fibers than those examined (as well as other areas of those fibers that were studied) may be involved in a demyelinating process.

That a significant number of nerve fibers are involved is supported by CV studies done in this lab on the same nerves showing evidence of SD. While as yet tentative, these studies revealed a slowing of CV in the sural nerve but not in the tibial, suggesting a preferential sensory involvement.

These data, suggesting preferential slowing of sensory CV that is accompanied by a possible morphologic correlate (SD), correspond well with descriptions of human diabetic neuropathy in the literature. In light of this, experimental diabetes in the rat induced by STZ and alloxan appear to be appropriate models for the investigation of human diabetic neuropathy.

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1737 ENDONEURIAL FLUID PRESSURE DYNAMICS

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Recent technical advances have made it possible to record endoneurial fluid pressure (EFP) and have thus allowed investigation of nervous system transport phenomona in the peripheral nervous system, a system which is more amenable to control and experimental manipulation than the brain. We have employed the active, servo-null, micropressure system to record rat sciatic nerve pressures in four experimental groups of animals to explore the mechanisms of edema in peripheral neuropathy and to quantify the biophysical relationships between edema and EFP. Control EFP was 2.0 ± 1.0 cm H2O for adult, Sprague-Dawley rats. Significant differences were observed between controls and the following experimental groups:

Insult	Peak EFP
1. 6% $PbCO_3$ in water	9 cm H2O
2. 1000 ppm hexachlorophene in diet	17 cm H2O
3. 40% galactose diet	10 cm H2O
4. proximal crush injury	9 cm H2O

These studies reveal several mechanisms responsible for increased EFP. The blood-nerve-barrier (BNB) may be damaged by toxic injury to the endothelial cell as is seen in lead neuropathy. By contrast, hexachlorophene damages the myelin sheath without affecting the BNB. Edema is strictly confined to the myelin lamellae. In galactose neuropathy the synthesis of a poorly soluble macromolecule inside the BNB changes the local osmotic force causing fluid retention and elevated EFP. EFP changes following crush injury are the result of perturbations in all these mechanisms. Additional analysis of transverse sections in lead neuropathy suggest that the perineurial sheath has a compliance which is analagous to brain. As the edema fluid begins to accumulate, transfacicular area (TFA) increases but EFP does not change. Continued expansion of endoneurial volume is restricted by the perineurium which begins to stretch. This is associated with a rise in EFP. At approximately 10 cm H2O, EFP peaks but TFA continues to increase. This may be explained by proximo-distal transport of endoneurial fluid which is accentuated by high local EFP.

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1738 A NEW HEAD INJURY MODEL FOR EVALUATION OF TREATMENT MODALITIES.

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The evaluation of the effectiveness of various treatment modalities in head injury has been hampered by the lack of an appropriate animal model exhibiting extended coma and delayed death. We have developed an animal model that utilizes a repetitive translation plus rotation acceleration injury to the skull encased brain of the anesthetized cat (1400 positive and negative 80-85g impulses applied over 67 seconds) followed after 40 minutes by a one hour period of controlled respiration with 6% O_2 in N_2 which results in an arterial pO_2 of 24 ± 4 torr. This results in delayed death in approximately 50% of animals with an impaired rate of neurological recovery in many survivors. All surviving animals are given a numerically quantifiable neurologic examination at regular intervals up to 24 hours and all animals undergo autopsy examination. Approximately 10% of animals die early following the trauma alone and thus are not usable in evaluation of treatment modalities. Control studies evaluating anesthesia alone compared with anesthesia plus hypoxia show no mortality or morbidity from the hypoxia alone. Individual dose response characteristics for variation in duration of hypoxia, magnitude of repetitive acceleration and duration of repetitive acceleration have been worked out and demonstrate that an increase in any of the three parameters results in a corresponding increase in mortality. The data we have demonstrates that death can occur during or immediately following the repetitive acceleration injury if the magnitude or duration is great enough, however, extended coma or delayed death only occurs when the acceleration injury is followed by a period of hypoxia. This finding further underscores the importance of proper ventilatory support and oxygenation in the severely head injured patient. We have evaluated five treatment modalities to date on our model, three of which have resulted in a significant reduction in mortality rate and an improvement in the rate of neurologic recovery. The data from these studies will be presented. Supported by NIH grant NS13042.

1739 INCREASED AXONAL PROTEOLYSIS IN MYELIN-DEFICIENT MUTANT MICE.

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Neurons appear to play a critical role in mediating the formation of myelin. In this study, we found specific alterations of axonal proteolysis in three mutant strains of mice which exhibit CNS hypomyelination. The degradation of axonal proteins was measured within a single neuron type, the retinal ganglion cell. Axonally transported proteins in the ganglion cell were labeled in vivo with ^{3}H-L-proline. After 5 days, intact optic nerves containing labeled axonal proteins were incubated in vitro in HEPES buffer, pH 7.4 (Dunlop & Lajtha, 1975, Brain Res. 34:333). Protein synthesis inhibitors were added to prevent reutilization of amino acids released by degradation. The rate of protein degradation was calculated as the ratio of TCA-soluble dpm to total dpm after 1h at 37° minus background which is the ratio from the contralateral nerve similarly incubated at 0°. An enzyme system with different properties, presumably Cathepsin D, was measured at pH 3.8.

In 3-4 wk. old myelin synthesis deficiency (msd) mice, the rate of axonal protein degradation was elevated 300% at pH 7.4 ($p<0.01$) and 170% at pH 3.8 ($p<0.001$) compared to the rates in littermate controls. In preliminary experiments comparable elevations of axonal protein degradation were measured in 3-4 wk. old jimpy (jp) mice (an allele of msd). In 4 wk. and older quaking (qk) mice, axonal protein degradation was normal at pH 7.4 (112%, n=16) but was increased 260% at pH 3.8 ($p<0.001$). The different abnormalities in qk and jp/msd suggest that the axonal proteolytic defects are not solely a consequence of hypomyelination. In isolated neural retina, protein degradation at pH 7.4 and at pH 3.8 was normal in each mutant. Finally proteins in glial elements of the optic nerve were labeled by incubating freshly sectioned nerves in vitro with ^{3}H-L-leucine and protein degradation was measured as above. In each mutant, glial proteins were degraded at the same rate as those in littermate controls at pH 7.4 and pH 3.8.

These results indicate that 1) axonal proteolysis is increased in mutant mice with defective myelination; 2) the proteolytic defects differ in mutants affected at different genetic loci but are similar in alleles; and 3) the defects are specific for axons (neurons) and are absent in the unmyelinated, histologically normal retina. The hypothesis is advanced that the primary molecular lesion in these mutants may be in neurons and that enhanced axonal proteolysis may be a major pathological mechanism (Supported in part by the William F. Milton Fund).

1740 STUDIES OF THE SODIUM AND CALCIUM COMPONENTS OF EVOKED ACTION POTENTIAL (EVAPS) FROM CULTURED RAT DORSAL GANGLIA NEURONS INFECTED WITH HERPES SIMPLEX VIRUS.

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Dissociated neuron cultures from rat dorsal root ganglia were maintained for 4-6 weeks. Normal electrophysiological parameters were first established. Morphologically similar cells showed a plateau in the falling phase and consistent EVAP properties.

Cultures were infected with 10^4 p.f.u. of herpes simplex virus type 1. Recordings were made at 2, 4, 6, 12, and 24 hours (hrs) (± 30 minutes) post-infection (PI) in Eagle's Minimum Essential Media (EMEM). The signals were recorded and analyzed using a PDP-12 computer. Changes in spike amplitude (SA) and duration (D) were most significant. EVAPS at 2 hrs PI were essentially normal. At 4 hrs PI there was a decrease in SA and a small increase in D. 6 hrs PI recordings showed a recovery in the SA to normal values and a slight increase in D compared to the normal. Recordings from the 12 hrs PI sessions showed a large decrease in SA and a very marked increase in D. Only local responses could be evoked at 24 hrs. Light microscopic studies showed fragmentation of the nucleolus and granulation in the cytoplasm at this time. Mock-infected cultures exhibited normal EVAPS for at least 48 hrs.

Recordings were also obtained in Na^+ free (NF), 10^{-8}g/ml tetrodotoxin (TTX), Ca^{++} free (CF) and 10 mM $CoCl_2$ recording solutions at the same time intervals after virus infection. 2 hrs PI, recordings in NF, TTX, CF and $CoCl_2$ were the same as those for uninfected cells in these recording solutions. EVAPS from the 4 hrs PI cultures in TTX were much like those from 4-hr PI in EMEM. Readings in $CoCl_2$ and CF recording solutions showed a decrease in SA compared to EVAPS from uninfected cells in these solutions. Recordings 6 hrs PI, in $CoCl_2$ and CF solutions were not significantly different from EVAPS of uninfected cells in the same solutions. 12 hrs PI there was a marked similarity in the characteristics of the EVAPS in NF and those in EMEM. Recordings in $CoCl_2$ and CF showed a decrease in SA.

Initial results indicate there is a transient block in the active Na^+ channels due to the virus infection at 4 hrs PI. Progressively the passive Na^+ component also appears to be altered at 12 hrs PI. A slight increase in D of the EVAP of infected cells recorded in TTX from 4 hrs PI indicates a possible change in the Ca^{++} component. Supported in part by USPHS Grant 1-R01-NS-13326-01A1.

1741 "CYTOPLASMIC BODIES" IN HUMAN SKELETAL MUSCLE CO-CULTURED WITH FETAL MOUSE SPINAL CORD COMPLEX. Edith R. Peterson*, Edmund B. Masurovsky, Alfred Spiro* and Stanley M. Crain. Depts. of Neuroscience and Neurology, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

Human skeletal muscle innervated by co-cultured explants of fetal mouse spinal cord complex leads to greatly enhanced differentiation of many regenerated muscle fibers (Pet. & Cr., Exper. Neurol. '72). "Cytoplasmic bodies" (W.K. Engel, Neurol. '62) developed abundantly (100-500 per culture) in muscle explanted from a biopsy of Duchenne dystrophy (9-month child). Many of these "bodies" were observed in mature muscle fibers that still showed characteristic contractions in response to spinal cord stimuli (3-4 months in vitro). Considerably fewer bodies (3-25 per culture) have been observed, at times, in cultures of normal or pathologic human muscle. These inclusion bodies are clearly discernible by high-power light microscopy in the living cultures as a central dense core, round or ovoid, surrounded by a clear halo -- not membrane-bounded. The bodies are most numerous in distended regions towards the ends of both undifferentiated or well-differentiated muscle fibers. They are less numerous in areas of cross striations. In the living cultures, birefringence of the halo can be demonstrated with polarized light microscopy, and Nomarski optics supports the view that this halo is not membrane-bounded. In fixed preparations, the central core is deeply stained with phosphotungstic hematoxylin. Occasional "cytoplasmic bodies" of a similar light microscopic appearance were reported in cultures of uninnervated chick (W.K. Engel,'62) and human muscle (Askanas et al. Acta Neuropath. '78). In our co-cultures, cytoplasmic bodies appear to be extruded near the ends of some muscle fibers, and to a lesser degree along intermediate zones of cross striated as well as less mature fibers.

Electron microscopy of these bodies in 7-16 week cultures reveal fine-structural features consonant with our light microscopy and with EM studies of certain myopathies in situ (McDonald and A. Engel, Acta Neuropath. '69). The central core consists essentially of a dense tangle of ~3-6 nm filaments, which merges with the surrounding halo composed primarily of radially arrayed ~5-7 nm filaments that may dovetail with nearby myofilament lattices. Thicker (~10-30 nm) filaments are seen at times in this zone which otherwise excludes such organelles as mitochondria, endoplasmic reticulum and triads.

McDonald and Engel ('69) considered these bodies to be anomalies of the Z-disc, but neither the complete organization of the structures nor the significance of their occurrence in myopathies is clear. This culture model may facilitate further analyses.

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1742 THE OCCURRENCE OF ENDOTHELIAL LESIONS WITHIN THE CEREBRAL VASCULATURE AND THEIR RELATION TO THE PASSAGE OF HORSERADISH PEROXIDASE. John T. Povlishock, William I. Rosenblum, Hermes A. Kontos*, and Donald P. Becker. Dept. of Anat., Path. (Neuropath.), Med. and Surg. (Neurological), Med. Col. of Va., Virginia Commonwealth University, Richmond, Va. 23298.

The present investigation was undertaken to determine, if within the cerebral vasculature altered endothelial morphology always correlates with dysfunction of the blood-brain barrier. To this end 40 cats having received an IV injection of horseradish peroxidase (HRP) were subjected to either mechanical brain injury or an acute episode of systemic hypertension induced by angiotension. Following a brief survival the animals were perfused with aldehydes and their brains were sectioned on a vibratome. Serial sections were then collected in compartmentalized trays and the alternate serials were processed for either light microscopy or transmission (TEM) or scanning (SEM) electron microscopy. Light microscopy demonstrated that various loci throughout the neuraxis displayed peroxidase exudation. TEM examination of such sites revealed that the vasculature possessed endothelia displaying numerous HRP laden vesicles, vacuoles and tubules. Serial sections suggested that these HRP containing elements participate in the sequestration and transendothelial passage of the protein to the underlying perivascular basal lamina and brain parenchyma at the sites of HRP effusion. SEM study of such sites revealed that the vascular luminal surfaces possessed endothelia manifesting numerous vesicles. In contrast vasculature at sites with no peroxidase effusion lacked numerous endothelial vesicles; however, SEM revealed conspicuous endothelial balloon-like protrusions and crater-like concavities. These endothelial lesions ranged from 0.5 to 7.0 μm in diameter and were distributed randomly on the endothelial surface and along the marginal lines. TEM confirmed the SEM findings and demonstrated that the endothelial balloons were formed by large cytoplasmic vacuoles within the endothelia. Frequently, these large vacuoles were open to the luminal surface. These ruptured vacuoles were often collapsed and thereby reminiscent of the craters seen with SEM. It is apparent that conspicuous endothelial lesions may occur without any concomitant increase in the passage of proteins such as horseradish peroxidase and without the appearance of vesicles filled with HRP. A parallel study of pial vessels also showed severe endothelial damage without vesicle formation or HRP transport.

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1743 ALTERED PERMISSIVENESS OF AUTONOMIC NEURONS FOR HERPES SIMPLEX VIRUS REPLICATION: AN EPIPHENOMENON OF THE AXON REACTION? Richard W. Price. Dept. Neurology, Cornell University Medical College, New York, NY 10021.

A model of herpes simplex virus (HSV) infection of the superior cervical ganglion (SCG) was used to investigate the variability in outcome of neural infection with this virus. BALB/c female mice were injected intraocularly with 8 x 10^4 plaque-forming units (PFU) of HSV Type 1, and the course of SCG infection monitored by assessing viral titers in ganglion homogenates. Delayed passive (antibody) immunization administered one day after viral challenge was employed to reduce intraganglionic viral replication and to preserve the integrity of the ganglion; such immunization does not prevent viral access to the SCG over axonal pathways.

Both surgical post-ganglionic neurectomy and systemic treatment with 6-hydroxydopamine (6-OHDA), 250 mg/kg, led to a marked increase in viral replication in the SCG with peak viral titers exceeding 10^4 PFU per ganglion compared to titers of 10 PFU or less in sham-operated or injected controls. In contrast to autonomic infection, virus replication in the trigeminal ganglion and inoculated eye was not significantly influenced by these procedures. Augmented viral replication in the 6-OHDA-treated animals was unaffected by prior preganglionic nerve section, but was blocked by pretreatment with desmethylimipramine. In addition, latent HSV infection (assessed by explantation-cocultivation of ganglia several weeks after viral inoculation) was reduced in inoculated mice subjected to surgical or chemical neurectomy.

These studies suggest that among the metabolic alterations accompanying the axon reaction is a change in the permissiveness of ganglion cells for viral replication. The hypothesis is offered that one or more of the metabolic changes induced by axotomy increases the probability that productive rather than latent HSV infection of the ganglion will occur. Exploitation of alterations in neuronal metabolism may represent a critical adaptive mechanism for HSV. Metabolic changes in neurons similar to those accompanying axotomy may be responsible for the switch from virus latency, a state which insures long-term persistence of virus in the human community, to reactivated productive infection which renders virus available for transmission to susceptible contacts.

1744 INTRACELLULAR MORPHOPHYSIOLOGICAL STUDIES OF CORTICAL NEURONS IN FELINE GM_1-and GM_2-GANGLIOSIDOSIS. D.P.Purpura, S. Highstein, A.B. Karabelas*, AND S.U. Walkley. Dept. of Neuroscience, Rose F. Kennedy Ctr., Albert Einstein College of Medicine, Bronx, N.Y. 10461

The gangliosidoses are inherited lysosomal hydrolase deficiency diseases characterized by progressive intraneuronal accumulation of uncatabolized glycolipids in various complex forms. Neurobehavioral deterioration in both human and feline GM_1 and GM_2-gangliosidoses occurs after an initial but variable period of normal postnatal development. Although the pathophysiological basis for the onset of neuronal dysfunction in the gangliosidoses is unknown it is widely held that such dysfunction is directly linked to the "cytotoxic" action of uncatabolized glycolipids and membranous cytoplasmic bodies (MCBs) whose accumulation presumably compromises normal intracellular organelles. We have attempted to test this "cytotoxicity" hypothesis by examining the functional status of cortical neurons in feline mutants with confirmed GM_1 or GM_2-gangliosidosis. Micropipettes for intracellular recording were filled with HRP for subsequent identification of impaled neurons. Cells encountered in motorsensory cortex were examined for antidromic (cerebral peduncle/pyramidal tract) or orthodromic (thalamic-VL) stimulation. Neurons impaled in feline mutants at various stages of clinical neurobehavioral deterioration exhibited membrane potentials and evoked PSP sequences qualitatively similar to those observed in normal animals of comparable ages. Although EPSP-spike discharge trains and spontaneous bursts were unremarkable the impression was gained that IPSPs evoked by thalamic stimulation were more prominent and were more frequently encountered in ganglioside-laden neurons than in neurons from normal cats. Several types of HRP-filled cells were recovered from preparations yielding satisfactory intracellular recordings. Neuron cell bodies were generally 2-3X the diameter of normal cells and were literally packed with MCBs. Thus accumulation of uncatabolized glycolipids and large numbers of MCBs in greatly enlarged cell somas does not influence electrogenic properties of cortical neurons in the feline gangliosidoses.

1745 ATROPHY OF CEREBELLAR DEEP NUCLEI IN THE STAGGERER MUTANT MOUSE. S. Roffler-Tarlov & K. Herrup, Dept. Neuroscience, Children's Hosp. Med. Ctr., Boston, MA 02115 and Dept. Human Genet., Yale Medical School, New Haven, CT 06510

Staggerer (sg) is an autosomal recessive mutation in mice affecting the development of the cerebellum. The most prominent pathological characteristic is an almost complete loss of granule cells. A majority of large neurons in cerebellar cortex are also missing. This finding, along with that of reduced wet weight and protein content in sg deep cerebellar nuclei (dcn) led us to ask if the sg gene affects all of the neuronal derivatives of the germinal zone of the roof of the fourth ventricle. These include deep nuclear cells, Golgi II cells and Purkinje cells.

We have made a histological examination of dcn from 20-day-old sg and control cerebella using serial sagittal 10 µm sections stained with cresyl violet. Starting with the first midline section in which deep nuclear cells appeared, we made sketches of the entire section and outlined the area containing the dcn as well as the area which included cells and white matter core. We weighed the paper areas occupied by the deep neurons with and without the white matter core in each of the sections and graphed these numbers with regard to distance from midline to approximate the total area occupied by white matter and the total area occupied by deep nuclei in sg and controls. We counted the deep nuclear cells in mutant and control animals using sections 50-100 µm apart. We tabulated the numbers of all neurons in which nuclear and cytoplasmic material was visible and graphed them with respect to distance from the midline.

We found that the number of neurons in sg dcn is not markedly different from that in normal dcn. However, the staggerer deep neurons are atrophied; cross-sectional area of representative sections revealed reduction in cell body size of all populations of deep neurons. The diminished size of the sg dcn is also caused by a reduction in the areas occupied by both the nuclei themselves and the white matter surrounding them.

We conclude that 1) the sg gene acts uniquely on those derivatives of cells from the ventricular germinal layers which become Purkinje and Golgi II cells and, 2) transneuronal degeneration does not take place in staggerer deep nuclei. The atrophic changes are likely to be a secondary effect and preliminary observation of two sg/sg ↔ +/+ chimeras support this hypothesis. Shrinkage of staggerer deep nuclear cells may be the result of early denervation by Purkinje cell axons or the consequence of their never having been innervated.

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1746 MODIFICATION OF AMPHETAMINE TOXICITY BY HERPES SIMPLEX VIRUS INFECTION OF THE CENTRAL NERVOUS SYSTEM. Richard F. Seegal and John E. Hotchin. Div. Labs. and Research, NYS Dept. of Health, Albany, NY 12201.

Acute central nervous system (CNS) infection with herpes simplex virus type 1 (HSV) increases locomotor activity and aggressive behavior in mice and metabolism of catecholamines (Lycke, Norrby & Roos, J. Neurol. Sci. 22, 277, 1974). Using a model in which mice are immunized by peripheral inoculation two weeks prior to central infection, we (Seegal & Hotchin, Birth Defects, 13, 179, 1978, NeuroScience Abstracts 4, 407, 1978) noted decreased spontaneous & d-amp. induced locomotor activity. To further analyze this model, we have examined the effects of peripheral injections of d-amp. and other sympathomimetic agents on mortality in pre-immunized centrally infected HSV mice.

Percent Mortality in HSV Infected Mice (24h. after Inoculation)

DRUG	DOSE (mg/kg)	N	HSV	CON
d-amphetamine sulfate				
5 day post IC	50	24	50	79*
	100	24	88	83
14 day post IC	50	25	72	60
	100	25	92	84
Isoproterenol HCl	200	15	0	0
	400	15	20	33
Clonidine HCl	75	15	27	67*
	100	15	87	100
Apomorphine HCl	150	15	69	46
	200	15	92	82
Carbachol	4	12	75	83

*Significantly different from control values

Based on the above results, we conclude that: (1) the protective effect of HSV on d-amp. induced death is a temporary phenomenon; (2) the effect is restricted to adrenergic neurons (no differential mortality between HSV and control groups was noted for carbachol or apomorphine); and (3) the phenomenon involves a partial saturation of α adrenergic receptor sites (differential deaths were seen with clonidine but not isoproterenol, blockade was overcome by high d-amp. and clonidine).

The immunized centrally infected HSV mouse provides not only a means of examining long-term interactions between infection and pharmacological agents, but may also represent a model for neurological disorders of brain catecholamines.

1747 FORMATION OF EPHAPTIC CONNECTIONS AFTER PERIPHERAL NERVE INJURIES. Zeev J. Seltzer* and Marshall Devor (SPON: Donald Price). Neurobiol. Unit, Life Sci. Inst., Hebrew Univ., Jerusalem, Israel.

Injury to peripheral nerves breaks the isolation between adjacent axons, thus producing acute artificial synapses at the injury site. These, however, decay within minutes after the lesion. In contrast to this acute phenomenon, we found that starting 30 days after the lesion, persistent electrical cross-talk between pairs of axons can be detected. In adult rats we cut the sciatic nerve and prevented its regeneration, promoting the formation of a neuroma. At intervals thereafter, in acute preparations, we severed the L_{4-6} dorsal or ventral roots and delivered electrical stimuli (0.1 msec, 1.5 Hz, up to 4 mA) to their distal portion. In all animals that survived at least 30 days after the lesion, we recorded from ventral or dorsal root strands that were cut centrally. Each action potential responded at fixed threshold and latency (3.5-28.0 msec) to each stimulus pulse. The possibility that we were stimulating and recording from one continuous axon possessing a long recurrent sprout, can be ruled out for at least 75% of the fibers recorded at 30-35 days after the lesion, on the basis of response latency. There is, therefore, cross-talk between independent axons. The interaction must occur in the vicinity of the injury since local anesthetic block or section of the nerve just proximal to the neuroma eliminates it. Such interactions were found to exist between pairs of motor fibers, sensory fibers and sensory and motor fibers. In a few rats we estimated only the fraction of the sensory to motor ephapses. By stimulating the neuroma we counted the number of motor fibers in each recorded strand. The mean fraction of these being ephaptically connected to sensory fibers was 6.8%. This interaction is probably electrical and not chemical, as it is bidirectional and has a very high safety factor (absolute refractoriness as determined by the double pulse method at as little as 0.55 msec; some follow tetanus to 1000 Hz). Based on collisions of action potentials initiated either at the dorsal root or at the sciatic nerve juxtaproximal to the neuroma, we could calculate the conduction velocities of the sensory and motor axons involved in each ephapse. Their conduction velocity histograms is the same as the distribution of dorsal and ventral root fibers in a neuroma. We repeated some of these experiments in rats that underwent sciatic section and immediate end to end resuture or after sciatic crush. In each of these we observed ephapses essentially identical to the neuroma ephapses. The occurrence of ephapses after peripheral injury lends support to longstanding hypothesis concerning their existance and possible contribution to various peripheral neuropathies.

1748 MULTIPLE SCLEROSIS: SENSITIZATION TO PERIPHERAL NERVE AND CNS MYELIN BASIC PROTEIN-A SERIAL STUDY. William Sheremata, AND Alan Sazant.* Dept. of Neurology, School of Medicine, U. of Miami, Miami, Fla 33101.

Numerous studies have documented immune responses to myelin, myelin constituents, and to oligodendrocytes in multiple sclerosis. However, prospective studies of sensitization to peripheral nerve have not been reported. We have therefore performed serial studies in 6 normals and in 20 patients with multiple sclerosis. Young patients, early in their clinical course were selected for this study. The Thor-Rocklin Indirect Macrophage Migration Inhibition Factor (MIF) assay was employed using human peripheral nerve and human central nervous system (CNS) myelin basic protein as antigens. A 10 mcgm./ml. concentration of basic protein and a 1/3000 dilution of a partially clarified homogenate of nerve was used for the lymphocyte cultures.

A group of normal controls gave a mean migration index of 96+ 6.5 (S.D.) with basic protein, and 101+11 with dilutions of 1/3000 peripheral nerve. All Multiple Sclerosis patients within 3 weeks of a exacerbation responded to myelin basic protein with signifigant results. Four, at the height of their exacerbations also responded to peripheral nerve. Occasional responses to peripheral nerve (2) and to basic protein (7) were seen apart from obvious clinical activity in these patients. Two controls had laboratory exposure to myelin basic protein and peripheral nerve. Lymphoblastic transformation with myelin basic protein gave positive results after, but not before such exposure, while MIF assays remained negative.

Sensitization to peripheral nerve may be a factor in producing some peripheral nervous system pathology as has been reported. Such results, however may only reflect the presence, in the in vitro preparation of peripheral nerve, of a second basic protein which is identical to CNS basic protein. Processing of the nerve may reveal antigenic sites not normally exposed. Limited studies using a purified basic (P2) protein preparation supplied by Dr. E.H. Eylar nevertheless closely parallel the results obtained using crude peripheral nerve. The positive MIF assays with CNS myelin basic protein in acute attacks ofmultiple sclerosis (in contrast to other assay systems) may reflect B cell depletion, or other alterations in lymphocyte subpopulations induced during cell separation procedures peculiar to the assay. (Sazant et al, in press).

This study was supported by a grant from the National Multiple Sclerosis Socelty

1749 ALTERATIONS IN MAZE PERFORMANCE ASSOCIATED WITH CHRONIC IMMUNE COMPLEX DISEASE. David W. Shucard, Steven A. Hoffman, Ronald J. Harbeck*, Andree A. Hoffman* and Hilary A. Brodie*. Dept. of Behavioral Sciences and Dept. of Medicine, National Jewish Hospital and Research Center, Denver, CO 80206.

Evidence is accumulating to suggest that immune complexes may be responsible for altering central nervous system (CNS) functioning in individuals with systemic lupus erythematosus (SLE). Studies from our laboratories and others, using animal models, have shown an array of CNS-associated effects of laboratory induced immune complex disease. These include immune complex deposition in the choroid plexus, changes in cerebrospinal fluid composition, and effects on behavior. In this investigation a rat model was used to study the effects of chronic immune complex disease on CNS functioning and on subsequent maze learning performance. Chronic immune complex disease was induced in rats by 3 initial subcutaneous injections of bovine serum albumin in Freund's incomplete adjuvant followed by biweekly or triweekly intravenous injections of bovine serum albumin. Behavioral measures of speed, errors and trials to criterion in a Lashley Maze were obtained. Proteinuria used as an index of the disease process was assessed over the course of the experiment. At the end of the study, the presence of immune complex deposits in the kidney and choroid plexus was determined by immunofluorescence microscopy. The results indicated that there were differences in maze performance associated with proteinuria levels. Experimental animals with elevated proteinuria produced significantly less errors and had fewer trials to criterion than either the experimental animals with low proteinuria or the controls. These same findings were obtained when animals were retested in the maze after a two week interval. Immunofluorescence data showed that none of the control animals but a majority of experimental animals showed evidence of immune complex deposits in both kidney and choroid plexus. In general, choroid plexus immunofluorescence was of lower intensity and more variable than that seen in the renal glomeruli. Furthermore, the data indicated that immune complex deposits in the choroid plexus may be related to performance in the maze. These results confirm and extend previous data from our laboratories reporting behavioral changes associated with the induction of chronic immune complex disease in rats. The types of behavioral alterations observed may be related to changes in attention-related mechanisms in the CNS. (Supported in part by USPHS Grant NS-12394.)

1750 MEMBRANE ELECTRICAL EVENTS ASSOCIATED WITH LYMPHOCYTE KILLING OF CULTURED NERVE CELLS. Cathy L. Stephens, Sandra Fitzgerald*, and Pierre A. Henkart*. NCI & NICHHD, NIH, Bethesda, MD 20205.

Intracellular microelectrode recordings were made of neuroblastoma-glioma hybrid cells (NG 108-15) while they were attacked by thymus derived (T) killer lymphocytes. Spleen cells from C57 Bl/6 strain ($H\text{-}2^b$) mice were sensitized in vitro in a standard 5-day culture against irradiated B10.A or A/J ($H\text{-}2^a$) mice in order to generate killer T cells which would recognize $H\text{-}2^a$, (the haplotype from which the neuroblastoma-glioma target cells were derived). Using the standard ^{51}Cr release assay with the neuroblastoma-glioma target cells, lysis was observed with such b anti-a lymphocytes but not with similarly sensitized a anti-b lymphocytes or with b lymphocytes co-cultured with irradiated b haplotype cells. Several lines of evidence indicate that killer T-lymphocytes induce channels in the target cell's membrane which allow ions to equilibrate across the membrane leading to colloid osmotic lysis. To investigate the membrane electrical events associated with this killing, intracellular microelectrode recordings were made in a neuroblastoma-glioma cell while adding activated T-lymphocytes and until the cell either completely lost its membrane potential or until the electrode was withdrawn after a long term recording (up to 4 hrs.). Prior to any treatment the resting potential of these cells was $\sim$ -60 mV; and the cells were typically electrically silent, but produced action potentials following intracellular stimulation. After lymphocytes were layered over the target cells, large reversible depolarizations (20-40 mV) lasting from 3-30 secs. were seen in the target cells. Between intermittent depolarizations the resting membrane potential remained constant.

Although all recorded cells maintained a normal resting membrane potential for over an hr after lymphocyte treatment, later (78-140 mins) 27% of these cells irreversibly lost their membrane potential following an abrupt depolarization from $\sim$ -60 to OmV.

All of the electrical changes described were seen using $H\text{-}2^b$ anti-$H\text{-}2^a$ lymphocytes; the control lymphocytes which did not show ^{51}Cr release from these target cells did not cause significant electrical changes in the target cell membrane. Our results suggest that while T-lymphocytes initially may transiently increase membrane permeability, progressive conic equilibration following the interaction of target cells and T-lymphocytes does not seem to occur.

1751 ETHANOL-INDUCED ALTERATIONS IN HIPPOCAMPAL AFTERDISCHARGES AND AFTERDISCHARGE THRESHOLDS. H.S. Swartzwelder, C.T. Johnson*, B.C. Cooley*, and R.S. Dyer. Psych. Dept. Towson State Univ., Baltimore, MD. and U.S. E.P.A., Research Triangle Park, NC.

Electrically induced hippocampal (HPC) afterdischarges (ADs) and their sequelae have been recommended as an index of neural function against which to measure toxicant-induced change. The properties of HPC ADs have been characterized both under normal conditions and following exposure to a number of toxicants. The present experiment was designed to assess the effects of acute systemic ethanol exposure upon some of these endpoints. Thirty male hooded rats were bilaterally implanted with bipolar stimulating and recording electrodes in the dorsal HPC. On test days animals were injected with either 0, 0.125, 0.25, 0.50, 2.0, or 3.0 g/kg of ethanol (IP) in a 20% (w/v) solution. Thirty minutes later AD thresholds were determined by a method similar to that described by Pinel et al., (1976). Each animal received each dose once in a counterbalanced order. Tail vein blood samples were taken immediately after the AD and assayed to determine blood-ethanol concentration. The most clear and profound effects were observed with the 3.0 g/kg dosage. Blood-ethanol concentrations at this dosage typically fell between 212 and 305 mg/dl. At this dosage the threshold for AD production was elevated, AD duration was decreased, and the frequency of spikes within ADs was decreased. These results parallel those obtained in previous investigations concerning sodium pentobarbital-induced alterations in HPC ADs and their sequelae, and suggest alterations in HPC ADs as an index of alcohol-induced neurophysiological depression. The results will be discussed in light of a dose response study on ethanol-induced changes in single unit activity in the hippocampus (Neuropharmac. 18: 63, 1979).

1752 ALUMINUM ENCEPHALOPATHY-INHIBITION OF HEXOKINASE BY ALUMINUM. George A. Trapp* (SPON: P.C. Jobe). Research Service, Veterans Administration Hospital and Dept. Psychiatry, LSU School of Medicine, Shreveport, La. 71130.

The aluminum encephalopathy model of aluminum neurotoxicity and neurofibrillary degeneration (NFD) has been studied in experimental animals and may have some relationship to human renal dialysis encephalopathy and also to a variety of conditions which lead to NFD, including Alzheimer's Disease. In the absence of specific information about aluminum function, we considered the possibility that aluminum exerts toxic effects by interference with well-known metal-activated reactions. We selected magnesium-dependent phosphoryl transfer catalyzed by yeast hexokinase as a convenient example for study.

Aluminum-ATP complex gives kinetics of linear competetive inhibition against magnesium-ATP as the varied substrate (K_m= 8×10^{-4} M, $K_i = 4 \times 10^{-4}$ M), and is non-competetive against glucose ($K_m = 3 \times 10^{-4}$ M). Aluminum-ATP was not a phosphoryl donor in this reaction and velocity is zero in absence of magnesium. The stability constant (K_s) for aluminum-ATP was 7.4×10^5 M and K_s for magnesium-ATP was 1.9×10^4M. Chromium-ATP, a known inhibitor of HK had K_s 2.5×10^5 M and gallium-ATP had K_s of 2.3×10^6 M.

We conclude that aluminum ion is a dead-end inhibitor of hexokinase. K_i is in the range of aluminum concentrations seen in experimental animal models of aluminum toxicity in brains. Inhibition of this or some other magnesium-ATP enzyme may be an important step in the genesis of NFD in animals or dialysis encephalopathy in man.

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1753 DEVELOPMENT OF ABNORMAL NEURON MORPHOLOGY IN FELINE GANGLIOSIDOSIS. Steven U. Walkley, Dominick P. Purpura and Henry J. Baker*. Dept. Neuroscience, Albert Einstein College of Medicine, Rose F. Kennedy Center for Research in Mental Retardation and Human Development, Bronx, N.Y. 10461.

Recent studies strongly suggest that the pathophysiology of ganglioside storage disease is intimately linked to progressive changes in neuron shape and synapse distribution rather than to simple cytotoxic effects of ganglioside accumulation (Brain Res. 116: 1-21, 1976). Of primary interest is the formation of abnormalities termed meganeurites, which are enlargements at the juncture of neuron soma and axon possessing synaptic contacts of unknown origin. Preliminary studies of feline GM_1 gangliosidosis have revealed similar morphological changes present in various types of neurons in the CNS (Brain Res. 143: 13-26, 1977).

The availability of colony-reared cats with GM_1 and more recently GM_2 gangliosidosis, which bear remarkable similarity to the disorders seen in children (Science 174: 838-839, 1971; Science 196: 1014-1017, 1977), has made possible a systematic study of the development of these morphological changes in the face of progressive neurological deterioration. Although clinical signs are similar in these two types of feline gangliosidosis, the neurobehavioral deterioration progresses at different rates. The earliest clinical signs, hind-limb weakness and head tremor, are seen at 6-8 weeks of age in GM_2 mutants and 12-15 weeks in those with GM_1 gangliosidosis. Similarly, onset of recumbency is more rapid in GM_2 cats, occurring at 4 months as opposed to 6-7 months in GM_1 mutants. Terminal states are reached by 6 months in GM_2 cats and 12 months in GM_1 cats.

Progressive morphological alterations can be demonstrated in Golgi preparations of neurons during the course of clinically evident neurological deterioration. Changes in individual neurons are type-specific and some types (e.g. Purkinje cells of cerebellum) demonstrate no abnormalities. Examples of affected neurons include pyramidal cells of cerebral cortex, medium spiny cells of caudate and putamen, apparent intrinsic neurons of thalamus, and stellate cells of cerebellum. Examples of alterations occurring in particular neurons include simple enlargement of the soma (nonprogressive), pre-meganeurite structures progressing to meganeurites, variable focal enlargements in dendritic trees, and secondary neurite formation, primarily from abberant sites such as meganeurites. Changes seen in any one neuron type are both specific and consistent, however, especially in pre-terminal stages, apparent normal neurons of a particular kind can be demonstrated in proximity to those showing morphological abnormalities. (Supported by NS-07512 and NS-10967.)

1754 CHARACTERISATION OF BRAIN TISSUE IN PEDIATRIC BRAIN TUMOR PATIENTS USING COMPUTERIZED TOMOGRAPHY. P. WEISS*, M. CERRONI*, D. DE MICHELE*, L. SINKS*, D. MC CULLOUGH*, D. ROBERTSON*, B. HAMILTON, W. NORMAN* D. SCHELINGER*, H. MANZ*, P. CRISP, AND J. MAZZIOTTA*. (SPON: P. CRISP) GEORGETOWN UNIVERSITY MEDICAL CENTER WASHINGTON D.C. 20007, AND U.C.L.A. MEDICAL CENTER, LOS ANGELES, CA 90024.

A system for monitoring glioma development and response to multimodal therapy utilizing Computerized Tomography (CT) has been developed at Georgetown University Medical Center. In implementing this program, we have found that in addition to compilation of ventricular, lesion, and total brain volumes in our patients, it has become within our reach to examine various intra cranial tissues through CT generated data. This is accomplished by on line data processing of scans based on a software package that produces frequency distribution histograms of CT density coefficient numbers and computes ventricular, lesion, and total brain volume and mass. CT histogram analysis permits us to separate ventricular system and contents from surrounding brain tissue, and normal from pathological tissues. This has value in that it allows us to check the accuracy of lesion, ventricular, and total brain volume determinations by summation of points represented under histogram tissue curves. Secondly, and equally important, we can distinguish between frank edema and tumor seeding in the absence of visible gross lesion in a scan series. We believe this program has great promise in that it enables us for the first time to follow the course of intra cranial disease in an exacting fashion that has been previously unavailable. This has optimal utility because we can now tailor therapy for each patient rather than make clinical judgements based on hindsight. Finally, we are hopeful that once a sufficient data base has been assembled on different types of tumors, it may be possible to employ this data diagnostically prior to craniotomy and biopsy.

1755 PATHOLOGY OF TAPETUM AND RETINA OF TAURINE-DEPLETED CATS. G. Y. Wen, H. M. Wisniewski*, J. A. Sturman, A. A. Lidsky, A. C. Cornwell* and K. C. Hayes*. NYS Institute for Basic Res. in Mental Retardation, Staten Island, NY 10314. Montefiore Hospital and Med. Center, Bronx, NY 19467. Harvard School of Public Health, Boston, MA 92115.

Taurine is widely distributed in animal tissues and is present in especially large amounts in the retina. Within the retina, taurine is present in greatest concentration in the photoreceptors. A series of studies demonstrated that cats fed a synthetic diet with casein as the sole source of protein become taurine-depleted and suffer central retinal degeneration in which the photoreceptors become damaged and eventually are lost completely. In this paper we report disorganization and degeneration of tapetal cells from taurine-depleted cats in addition to photoreceptor damage. Adult cats were fed a synthetic (taurine-free) diet or the same diet supplemented with 0.4% taurine for 18 months. Animals were sacrificed by perfusion. The eyes were removed and hemisected. The posterior half of eye was mapped and divided into 18 areas. There was an approximately 50% reduction in size of the tapetum in the taurine-depleted cats. In the center region of tapetum, the number of layers of tapetal cells was reduced from 12-16 to 2-3. Electron microscopic examination revealed varying degrees of disorganization and disruption of the tapetal rods in the taurine-depleted cats. In some cells, the tapetal rods, when present, were shorter, thicker and more electron-dense than in tapetal cells of taurine-supplemented cats. Some of the tapetal rods appeared broken. These changes apparently lead to the formation of electron-dense droplets reminiscent of pigment granules. In other tapetal cells from taurine-depleted cats the normal lattice arrangement of tapetal rods was apparently retained. Dietary deficiency of taurine resulted in the degeneration of photoreceptors and depigmentation in the retinal epithelium and choroid. The disk membranes of photoreceptors were disoriented, vesiculated and disrupted. The cell bodies of photoreceptors also underwent pyknotic degeneration. Both electroretinogram responses and visual evoked potentials of the taurine-depleted cats were greatly reduced. Amino acid analysis showed a severe depletion of taurine in the retinae and other tissues of these cats when not supplemented with taurine. This study demonstrated that the dietary deficiency of taurine in cats induces the pathological, biochemical and electrophysiological changes in the retinae. (This study was supported by the Office of Mental Retardation and Developmental Disabilities of the State of New York and by Public Health Service grants HD-11129 and EY-00631 from NIH).

1756 ACTIVATION OF "AUTOMATIC" SWALLOWING BY SEROTONIN AGONISTS IN RATS WITH EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS. Susan R. White and Detlef Bieger. Fac. Med. Memorial Univ. Nfld., St. John's, Nfld., A1B 3V6.

Depressed neuronal sensitivity to serotonin has been found in both the peripheral nervous system (Weinstock, et al., Brain Res. 125, 1977, 192) and in the lumbar spinal cord (White, Brain Res., in press) of animals with paraplegia resulting from experimental allergic encephalomyelitis (EAE). The purpose of the present study was to determine whether EAE also impaired serotonergic responsivity at the brainstem level. A convenient index of this activity is the periodic swallowing displayed by rats under urethane anesthesia (Bieger, et al., Neuropharm. 16, 1977, 245) which has been shown to be mediated, in part, by a serotonergic mechanism in the lower brainstem (Bieger, et al., Eur. J. Pharmacol. 18, 1972, 128). In the present experiment we examined "automatic" swallowing activity in EAE rats exhibiting complete hindlimb paralysis. The EAE rats displayed periodic swallowing under urethane anesthesia at rates and forces comparable to control rats. Intravenous administration of the serotonin precursor, 5-hydroxytryptophan, or the serotonin agonist, Quipazine, enhanced the rate of swallowing in both EAE and control rats. Neither of these drugs, however, increased the amplitude of lumbar monosynaptic reflexes in EAE rats, while both had marked facilitatory effects in control rats. These results suggest that serotonin sensitivity is depressed at levels of the neuraxis manifesting motor disability during the disease but is preserved at levels where motor impairment is not evident.

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NEUROPEPTIDES

1757 NALOXONE ALTERS LOCOMOTION AND INTERACTION WITH ENVIRONMENTAL STIMULI. Amy T. Arnsten* and David S. Segal. Dept. Psychiatry, Sch. Med., UCSD, La Jolla, CA 92093.

Effects of the opiate antagonist naloxone were examined on the behavioral response pattern of rats in a novel environment. After injection with saline or naloxone (0.5, 5.0 or 25 mg/kg, s.c.), the rats were monitored for locomotion and both frequency and duration of contact with stimuli in a multicompartment exploratory chamber. Naloxone produced a dose-related reduction in locomotion and in frequency of contact with stimuli. At the lowest dose tested this reduction was accompanied by an increase in total duration of contact and in time spent per contact with the stimuli. In contrast, the highest dose of naloxone decreased the duration of contact with stimuli and induced prolonged periods of inactivity. An intermediate response was observed with 5.0 mg/kg naloxone. These findings indicate that lower doses of naloxone may enhance interaction with environmental stimuli while the predominant effect of higher doses is a general suppression in behavioral activity.

1758 EFFECT OF ADH ON CALCIUM-DEPENDENT RELEASE OF SEROTONIN AND ENDOGENOUS LEVELS OF SEROTONIN FROM HIPPOCAMPAL SLICES. Sid Auerbach* and Peter Lipton (Spon. Peter Lalley) Dept. Physiol, U of Wis., Madison Wis. 53706

DeWied has reported that intracerebroventricular injection of ADH corrected learning deficits in rats lacking the ability to synthesize ADH (Brain Res. (1975)85,152). Rigter has shown that administration of ADH can reverse CO_2-induced amnesia of a learning task and that an increase in endogenous hippocampal 5HT was associated with this reversal (Brain Res. (1977)120,485). The data is consistent with the hypothesis that ADH may affect behavior by a direct or indirect effect on 5HT turnover in the hippocampus. We have used hippocampal slices as an *in vitro* model to study the effect of ADH on 5HT.

Thin slices of rat hippocampus were incubated in oxygenated buffer at 37°C. Endogenous levels of 5HT were measured by a radioenzymatic method (Fed. Proc. (1977)36,2134). 5HT levels were 2.59±.11 ng/mg prot. after a 30 minute incubation in normal buffer followed by a 10 minute period of incubation in high K^+ (63mm) buffer as compared to 4.42±.31 when Ca^{++} was omitted from the high K^+ buffer. This Ca^{++}-dependent decrease in 5HT is interpretable as equal to 5HT release as a result of depolarization-induced exocytosis. If 10^{-7}M ADH was included in the incubation medium during the exposure to high K^+, the levels of 5HT after 10 minutes was 3.31±.28. The difference in 5HT levels in the presence of ADH compared to control was significant ($P<.05$) and amounts to a 31% inhibition of 5HT depletion by the ADH.

In a second series of experiments, slices were preincubated with ^{3}H-5HT, and Ca^{++}-dependent, high K^+ induced release of radioactivity was measured during a 10 minute period. Release of radioactivity into the medium was decreased from 52.6%±1.6 of total radioactivity in slices to 49.6%±1.7 by ADH (10^{-8}-10^{-6}M).

The data is consistent with the hypothesis that ADH is acting in the hippocampus to inhibit Ca^{++}-dependent release of 5HT. The results could also be explained by postulating that ADH increases 5HT synthesis. Increased synthesis would decrease the specific activity of ^{3}H-5HT and result in an apparent decrease in release and in the increased endogenous levels of 5HT that we observed. The observed effect of ADH must be on serotonergic nerve terminals as 5HT containing cell bodies are not found in the hippocampus. The hippocampus, as a part of the limbic system may be involved in learning behavior. An effect of ADH on 5HT metabolism in hippocampus may be a partial basis for reported effects of ADH on behavior. Partially supported by NIH 1RO1N514175.01

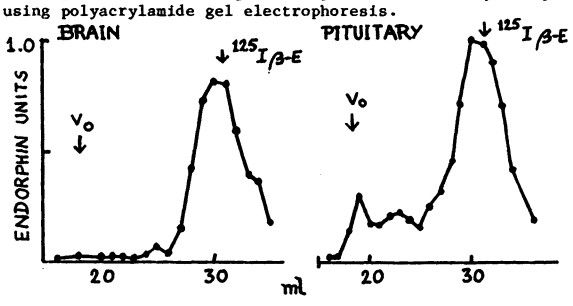
1759 INTRACELLULAR PROCESSING OF α-MSH AND ACTH IN HYPOTHALAMIC NEURONS: A PRELIMINARY STUDY. A. Barnea, G. Cho*, and J. C. Porter, Depts Ob/Gyn & Physiol, Green Ctr for Reprod Biol Sci, Southwestern Med Schl, Dallas, TX 75235.

Immunoreactive α-MSH (α-MSH$_i$) (Oliver and Porter, Endocrinology 102: 697, 1978) and ACTH (ACTH$_i$) (Krieger et al., Proc Natl Acad Sci 74: 648, 1977) are present in hypothalamic tissue. The current view is that in the pars intermedia of the hypophysis of the rat, α-MSH is derived from ACTH, and ACTH in turn is derived from a large (31K) precursor by a process involving a series of cleavage reactions. In addition to α-MSH$_i$ and ACTH$_i$, hypophysial cells contain significant amounts of the 31K precursor as well as intermediate forms between the 31K precursor and ACTH. We have addressed the question whether the processing of ACTH$_i$ and α-MSH$_i$ in the hypothalamic cells proceeds by a mechanism similar to that operating in the cells of the pars intermedia. We have analyzed the molecular forms of ACTH$_i$ in extracts of the hypothalamus and pars intermedia and calculated the amounts of the various forms of ACTH$_i$ and α-MSH$_i$. ACTH$_i$ and α-MSH$_i$ were extracted from hypothalami and neurointermediate lobes of adult male rats with 5M acetic acid (Eipper and Mains, Biochemistry 14: 3836, 1975). The various forms of ACTH$_i$ were separated by means of gel filtration using columns of Sephadex G-50 (superfine) under denaturing conditions (8M urea containing 0.5% 2-mercaptoethanol). ACTH and α-MSH were quantified by RIA. The recognition site of the ACTH antibody was contained within the amino acid sequence 11-16 of ACTH, and the antibody had no apparent cross-reactivity with α-MSH. The antibody to α-MSH had no apparent cross-reactivity with ACTH. The elution profiles of hypothalamic ACTH$_i$ and pars intermedia ACTH$_i$ were identical, and were indicative of the presence of three species of molecules crossreacting with the antibodies to ACTH: a large ACTH$_i$ which was eluted with the void volume, an intermediate ACTH$_i$, and a small ACTH$_i$ which co-eluted with hACTH$_{1-39}$. The K_d for large ACTH$_i$ was 0; for intermediate ACTH$_i$, 0.21 ± 0.007 (mean ± SD, N=8); for small ACTH$_i$, 0.32 ± 0.007; and for α-MSH$_i$, 0.72 ± 0.026. Of the total ACTH$_i$ in the hypothalamus, 60% was associated with large ACTH$_i$, 10% with intermediate ACTH$_i$, and 30% with small ACTH$_i$. Of the total ACTH$_i$ in the pars intermedia, 70% was associated with large ACTH$_i$, 10% with intermediate ACTH$_i$, and 20% with small ACTH$_i$. Based on weight, the ratio of α-MSH$_i$ to total ACTH$_i$ in extracts of the pars intermedia was significantly ($P<0.001$) higher than that in extracts of the hypothalamus: 13.2 ± 1.85 (mean ± SE, N=10) and 2.1 ± 2 (N=10), respectively. On the basis of these findings, it would appear that ACTH and its possible precursors are present in the rat hypothalamus and that the processing of ACTH in hypothalamic tissue may proceed in a manner similar to that in the pars intermedia. However, on the basis of the marked difference between the ratio of α-MSH$_i$ to ACTH$_i$ in the hypothalamus and in the pars intermedia, we suggest that the mechanisms of processing and/or storage of α-MSH in the hypothalamic cells may differ from those operating in the cells of the pars intermedia.

1760 ENDORPHIN IMMUNOREACTIVE MATERIAL IN THE BRAIN AND PITUITARY OF THE RAT EMBRYO. Alejandro Bayon, Wm. J. Shoemaker, Robert J. Milner*, Raana Azad*, and Floyd E. Bloom.. A.V. Davis Ctr. for Behav. Neurobiology, The Salk Inst., La Jolla, CA 92037.

We have previously shown that endorphin immunoreactive material appears in the rat brain early in ontogenetic development. This work is an initial attempt to characterize this material. Embryonic day 16 rat brains and pituitaries were extracted in boiling 0.2 N HCl. Extracts from several animals were pooled, centrifuged, neutralized, and lyophilized. The reconstituted samples were analyzed by permeation chromatography and fractions assayed for endorphin immunoreactivity as indicated below.

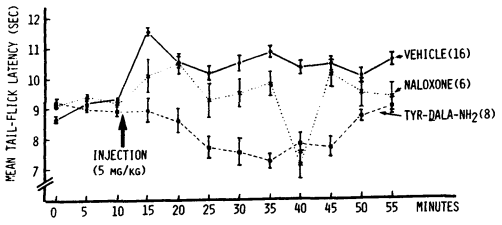
A single peak, co-eluting with ^{125}I-β-endorphin, was observed in extracts of embryonic brain, indicating that in the embryonic brain, as in the adult, very small amounts - if any - of the higher molecular weight endorphin are present. In contrast, embryonic pituitary extracts showed a substantial amount of larger endorphin-like material (possibly β-LPH and 31K protein). However, the relative contribution of these peaks to the total pituitary endorphin is much lower than in the adult where β-LPH is the predominant species. This difference might indicate different physiological roles for β-endorphin and β-LPH in prenatal and postnatal life. Currently, we are characterizing the higher molecular weight endorphins in the embryonic pituitary using polyacrylamide gel electrophoresis.



The endorphin RIA is equally sensitive, on a molar basis, to β-endorphin and β-LPH (1 unit = 1 ng β-endorphin immunoequivalents). Extracts obtained from 10 embryos were run through controlled pore glass-glycophase 200 columns (1 x 50 cm) in 0.1 M ammonium acetate, 0.1% triton-X 100. Recovery from the column was >90%.

1761 HYPERALGESIA AND REVERSAL OF ENKEPHALIN- AND STIMULATION-INDUCED ANALGESIA BY THE DIPEPTIDE TYR-DALA-NH_2. James D. Belluzzi, William H. McGregor and Larry Stein. Wyeth Laboratories, Philadelphia, Pennsylvania 19101.

Tyr-DAla-NH_2 has weak, naloxone-reversable, analgesic activity at an intraventricular dose of 100µg (McGregor, Stein & Belluzzi, 1978). We report here that, at 6µg centrally, or after systemic administration (5 mg/kg, s.c.), the dipeptide has the opposite effect, producing hyperalgesia and reversal of opiate- and stimulation-induced analgesia. The pain sensitivity of rats was assayed in the D'Amour-Smith tail-flick test. In a first experiment, analagesia was induced by morphine sulfate (2.5 mg/kg, s.c.) or the enkephalin analog DAla^2, DLeu^5-NH_2 (1µg, intraventricularly). Administration of the dipeptide 15 minutes after the onset of analgesia significantly shortened the duration of analgesic action of both compounds. In a second experiment using a weak heat stimulus, the effects of the dipeptide alone on normal pain sensitivity were examined. Tyr-DAla-NH_2 produced a significant hyperalgesia compared to controls (see Figure). Naloxone had smaller insignificant effects at doses of 1.25-20 mg/kg, s.c. If the pain threshold is normally elevated by circulating endorphins, these results suggest that Tyr-DAla-NH_2 is more effective than naloxone in reversing the action of endogenous endorphins. In a third experiment, profound analgesia was induced by electrical stimulation of the midbrain central gray. The dipeptide reversed this analgesia intermittently for approximately 30 minutes. Tyr-DAla-NH_2 thus lowers normal pain thresholds and antagonizes the analgesic action of opiate peptides and central gray stimulation. The mechanism of action of the dipeptide is presently unclear. Some presynaptic effect (such as inhibition of endorphin release or synthesis) seems more likely than a postsynaptic action, however, since the dipeptide has only weak affinity for the opiate receptor.



Hyperalgesia produced by Tyr-DAla-NH_2

1762 EFFECTS OF β-ENDORPHIN ON BODY TEMPERATURE IN THE MOUSE. Alan S. Bloom and Liang-Fu Tseng*, Department of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, Wisconsin 53226.

The effects of intracerebroventricular (ICV) injection of β-endorphin (β-END) on temperature in mice was studied at ambient temperatures of 10°, 20° and 31° C. Male Swiss mice were injected ICV with β-END in 5 µl of sterile saline. The observed results were dependent on both the dose used and the ambient temperature. Doses of β-END between 0.03 and 1.0 µg produce a dose related hyperthermia at an ambient temperature of 20° C. At this same ambient temperature, a 10 µg dose produced significant hyperthermia (-6.11° C). In mice studied at 31° C hyperthermia was produced by all doses (0.03-10.0 µg) tested. At an ambient of 10° C hyperthermia was produced by the lower doses of β-END and hypothermia by the higher doses (3 and 10 µg). These data demonstrate that the ICV administration of low doses of β-END produce an increase in body temperature whereas higher doses may cause the animal to become poikilothermic.

EFFECTS OF β-END ON BODY TEMPERATURE

Dose (µg)	31°	20°	10°
0.03	0.78 ± .23	0.96 ± .15	0.01 ± .16
0.1	0.90 ± .07	1.16 ± .16	0.41 ± .16
0.3	0.96 ± .13	1.30 ± .14	0.61 ± .17
1.0	1.48 ± .16	1.35 ± .23	0.48 ± .32
3.0	1.04 ± .16	0.32 ± .46	-5.22 ± .97
10.0	1.40 ± .14	-5.30 ± .75	-14.01 ± .54

Values shown are the difference in effect between drug treatment and saline control groups at 60 minutes after injection (N=8 to 10).

The effect of naloxone (1 mg/kg, sc) on both the low dose (0.3 µg) and high dose (10 µg) effects of β-END was also examined. Pretreatment with naloxone significantly blocked the hypothermia produced by 10 µg of β-END at 20° and 10° C. Naloxone did not block the hyperthermia produced at 31° C by the 10 µg dose. Similarly, the hyperthermia produced by the 0.3 µg dose of β-END was not blocked by naloxone at any ambient temperature. These data suggest that β-END may produce its hyperthermia and poikilothermic effects at different sites. (Supported in part by USPHS Grant # DA 00124).

1763 IMMUNO- AND ENZYME CYTOCHEMISTRY OF NEUROSECRETORY NEURONS IN NORMAL AND SALT-TREATED MICE. R. D. Broadwell, C. Oliver* and M. W. Brightman*. NINCDS and NIDR, NIH, Bethesda, MD 20205.

Supraoptic neurons of the hypothalamo-neurohypophysial system produce and secrete the octapeptides oxytocin and vasopressin. These neurons respond to alterations in osmolarity of the extracellular fluid and, therefore, can serve as a model for correlating morphological and physiological events associated with hyperosmotic stress. Supraoptic perikarya from control mice and mice given 2% salt water to drink for 5-8 days have been studied using immuno- and enzyme cytochemical techniques. Antibody directed against neurophysin, the carrier protein for the octapeptides, was used in conjunction with the peroxidase-anti-peroxidase (PAP) technique to stain, immunologically, organelles associated with production and packaging of the octapeptides. Thiamine pyrophosphatase (TPPase) activity was used as a marker for Golgi saccules. Acid phosphatase (ACPase) activity in Golgi associated Endoplasmic Reticulum from which Lysosomes arise (GERL) was demonstrated using ß-glycerophosphate and cytidine-5'-monophosphate as substrates.

With light microscopic immunocytochemical methods, supraoptic cell bodies stain positively for anti-neurophysin, indicating that these cells synthesize the associated octapeptides. PAP reaction product for neurophysin immunoreactivity was localized ultrastructually in the nuclear envelope, rough endoplasmic reticulum, Golgi saccules, secretory granules, and secondary lysosomes. Under normal conditions, TPPase activity was restricted to the innermost 1 or 2 Golgi saccules, whereas ACPase activity was confined to GERL and secondary lysosomes. Neurosecretory granules arose predominantly from GERL. When the animals were hyperosmotically stressed by salt loading, nearly all Golgi saccules and GERL were TPPase reactive. Secretory granules were now formed from all Golgi saccules as well as GERL. Little or no ACPase activity could be demonstrated in GERL. When the animals were allowed to recover for a period of 10 days after salt treatment, the ACPase activity in GERL returned to normal; however, the elevated TPPase activity and secretory granule formation seen in Golgi and GERL saccules with osmotic stress persisted.

These results reveal a morphological basis for metabolic responses and interrelationships among the Golgi apparatus, GERL, and secretory granules within peptidergic neurons of the hypothalamo-neuro hypophysial system and, thus, may be of value for considering similar morphological correlates in other peptidergic neurons.

1764 α-MELANOTROPIN-LIKE (α-MSH) IMMUNOREACTIVITY IN RAT AND HUMAN CEREBROSPINAL FLUID. Clivel G. Charlton*, Thomas L. O'Donohue*, Cinda J. Helke, Russel L. Miller* and David M. Jacobowitz (SPON) A. M. Laties). Lab. Clin. Sci., NIMH, Beth., Md. 20205 and Dept. of Pharm. Howard Univ., Washington, D.C. 20059.

An extensive α-MSH containing neuronal system has recently been described in the rat brain (PNAS 75, 1968). The perikarya of this system are located primarily in the arcuate nucleus lesion of which markedly depletes α-MSH concentrations throughout the brain.

In an attempt to measure the in vivo release of α-MSH, the lateral ventricle of chloralose-urethane anesthetized rats were cannulated and perfused with artificial cerebrospinal fluid (CSF). The perfusate was collected from an indwelling cannula in the cisterna magna at 20 min intervals. α-MSH concentrations in the CSF were determined by radioimmunoassay. Initially, approximately 180 pg of α-MSH/20 min interval was detected in the CSF. These concentrations gradually decreased and then stabilized. Rats hypophysectomized for 2-3 weeks had CSF α-MSH concentrations similar to controls.

In human CSF, an α-MSH-like immunoreactive compound has also been detected. Concentrations ranged from 4.0 to 73.3 pg/ml with mean of 22.9 pg/ml.

These data suggest that α-MSH present in CSF is not derived primarily from the pituitary gland but may represent neuronally released α-MSH.

1765 CHARACTERIZATION OF HIGH MOLECULAR WEIGHT ENKEPHALIN-LIKE IMMUNOREACTIVITY IN RAT BRAIN. Steven R. Childers and Solomon H. Snyder. Dept. Pharmacol., Sch. Med., Johns Hopkins Univ., Baltimore, MD 21205

In pituitary, as well as in hypothalamus, β-endorphin is formed from proteolytic breakdown of high molecular weight (MW) protein precursors. The relationship of these precursors to enkephalin is not clear, especially since β-endorphin and enkephalin possess different regional distributions in brain. In order to determine whether other high MW proteins may be enkephalin precursors, various preparations of rat brain were assayed for reactivity with enkephalin antisera. The antibodies were specific for met- or leu-enkephalin, and did not cross react with β-endorphin. The post-mitochondrial supernatant of rat brain was extensively filtered through Dia-Flo PM-10 membranes and eluted on a Sephacryl S-200 column. Two peaks, MW >100,000 and MW 40,000, of enkephalin immunoreactivity were observed. Neither peak non-specifically bound the ^{3}H-met-enkephalin ligand, nor appeared to bind endogenous enkephalin. The 100,000 MW peak, however, bound non-specifically to normal rabbit IgG, indicating the presence of an immunoreactive artifact. The 40,000 MW peak did not bind to normal rabbit IgG and, when prepared from microwave-irradiated brains to inactivate proteases and incubated with trypsin, formed lower MW enkephalin immunoreactivity as determined on P-10 columns. Further analysis revealed that the tryptic product was slightly larger than enkephalin, suggesting that trypsin-like enzymes are not the actual enkephalin-forming enzymes in the brain. However, unlike the high MW activity, the tryptic product did display reactivity in the opiate radioreceptor assay in addition to the enkephalin radioimmunoassay. These results suggest that enkephalin in brain may be formed from high MW protein precursors. (Supported by USPHS Grants DA-00266 and DA-01645.)

1766 IMMUNOCYTOCHEMICAL VISUALIZATION OF LUTEINIZING HORMONE RELEASING HORMONE (LHRH) IN VIBRATOME SECTIONED MURINE BRAIN. Claudia J. Clayton*and Gloria E. Hoffman (SPON: Bernard Weiss). Depts. Neurol. and Anat., Univ. of Rochester Sch. Med. and Dent., Rochester, NY 14642.

The unlabelled antibody-enzyme method of immunocytochemistry was used to visualize LHRH perikarya and fibers in vibratome sectioned rat and mouse brains. Adult male Sprague-Dawley rats and Swiss-Webster mice were fixed by cardiac perfusion of 10% neutral formalin or Zamboni's solution, and brains were removed and sectioned at 75 or 100um thickness. Immunocytochemical staining was performed using a modification of the method of Grzanna et al. (Proc. Nat. Acad. Sci. USA, 75: 2502-2506, 1978). Similar results were obtained with several anti-LHRH sera, including 743, 744 and 940 (Arimura), B4305 and B18 (Sternberger) and 4 (Silverman). No staining of perikarya or fibers was observed using primary antisera which had been preabsorbed with synthetic LHRH.

LHRH perikarya were not confined within traditional nuclear boundaries. Perikarya and fibers were found within and dorsal to the lateral olfactory tract, adjacent to the optic tract, within and lateral to the medial preoptic nucleus, within and dorsal to the OVLT, and in the septal and basal portions of the vertical limb of the diagonal band. Neurons extended rostrally from the medial septal nucleus and the nucleus triangularis to the medial olfactory peduncle, and a few scattered perikarya were seen in the main and accessory olfactory bulbs. LHRH perikarya and fibers also were found along the ventro-lateral border of the hypothalamus, with an occasional neuron in arcuate nucleus. Fibers were present in anterior hypothalamus, in subependymal and lateral hypothalamic areas, and within and ventral to the medial mammillary nuclei. The median eminence received fibers from both rostral and lateral directions. Positively stained perikarya and fibers were present in fornix and hippocampus, and in cortex adjacent to the rostrum of the corpus callosum. Longitudinally oriented LHRH fibers were present dorsal to the corpus callosum, with a few fibers branching dorsally. LHRH fibers also were observed in the subfornical organ, periventricular nucleus of the thalamus, medial habenular nucleus, fasciculus retroflexus, interpeduncular nucleus and mesencephalic central gray.

At the light microscopic level certain LHRH perikarya appeared to be contacted by LHRH fibers, indicating the possible existence of interconnections within the LHRH neuron system.

Supported by USPHS Fellowship HD 05668, NIH Grant NS 13725 and RCDA NS 00321.

1767 ELECTRON MICROSCOPIC LOCALIZATION OF NEUROPHYSIN-LIKE IMMUNOREACTIVITY IN TERMINALS OF THE RAT POSTERIOR PITUITARY: AN ALTERNATIVE METHOD USING FROZEN-DRIED, OsO_4 FIXED TISSUE. David Coulter and Robert Elde. Department of Anatomy, Medical School, University of Minnesota, Minneapolis, Minnesota 55455.

Radioimmunoassay studies of somatostatin remaining in dehydrated hypothalami indicate that approximately 90% of this neuropeptide is lost as a result of dehydration in an alcohol series, but that little or none is lost in tissue frozen-substituted in solvents such as 100% ethanol or acetone. Frozen-dried and resin infiltrated tissue was used in this study, and it likewise is expected to retain the neuropeptide.

The use of frozen-dried tissue for EM localization of neuropeptides permits two variables in fixation protocols: whether or not the tissue is prefixed by perfusion with paraformaldehyde before freeze-drying, and whether or not the frozen-dried tissue is post-fixed with OsO_4 vapor before infiltration. Prefixation with paraformaldehyde is advantageous for handling the tissue prior to freezing but results in some loss of immunoreactivity and compromises the morphological quality of the tissue. Postfixation of frozen-dried tissue with OsO_4 vapor does not seem to diminish immunoreactivity at all, at least for neurophysin and somatostatin, and has the advantage of yielding excellent and familiar images in the electron microscope.

Blocks of fresh or paraformaldehyde-fixed rat posterior pituitary were frozen on a metal surface at -196° C, dried in a temperature controlled environment for 2-3 days, fixed with OsO_4 vapor, and infiltrated with resin. Following polymerization of the plastic, serial ultrathin sections were cut and placed alternately on grids and glass slides. Slides were stained with anti-bovine neurophysin II (1:50) for 12 hours at 45° C, followed by fluorescein labeled protein A (100 ug/ml) for 2 hours. These were photographed using a 40 X oil immersion lens and yielded detail approaching the limits of resolution of the light microscope. Useful images were obtained even with sections 300 A in thickness. Some of the grids were stained with uranyl magnesium acetate and used only for electron micrography. Other grids were used first for electron micrography, then immunostained for neurophysin and photographed again in the fluorescent microscope, yielding images of terminals that could be enlarged and superimposed unambiguously on the electron micrographs.

This technique may prove to be of considerable value in the localization of neuropeptides in the nervous system. Maximum immunoreactivity is retained in the sections, the sensitivity of the immunofluorescence method is sufficient for the detection of small terminals in ultrathin sections, and these images can be correlated with electron micrographs having excellent morphology. Supported by NSF.

1768 PARTIAL ANTAGONISM OF STRESS INDUCED ANALGESIA BY ACTH PRETREATMENT. Hugh E. Criswell and Miriam David*. Dept. Psychol., Williams Coll., Williamstown, MA 01267.

If stress induced analgesia results from β-endorphin released concomitantly with ACTH, pretreatment with ACTH should block analgesia through feedback inhibition. To test this hypothesis we trained 56 rats for 10 consecutive days using the flinch-jump procedure to obtain stable jump thresholds. On day 11, the animals were divided into 3 groups. One group received no drug treatment and served as a control, one received 20 mg/kg of naloxone 1 hour prior to testing, and a third received 3 IU/Kg of ACTH 12 hours prior to testing. All drugs were administered SQ. Each of these groups was further divided into one group which received a cold water swim (2°C for 3 min) and another which received a warm water swim (29°C for 3 min) ½ hour prior to testing. An harmonic mean analysis of variance showed statistically reliable ($p < 0.01$) effects of drugs, swimming and water temperature as well as interactions between the drugs and swimming and drugs and water temperature. Individual t tests showed statistically reliable increases in jump thresholds for both hot and cold water animals in all drug groups. The increases were reliably smaller for the animals pretreated with naloxone and ACTH when compared to noninjected controls but the two groups were not different from each other ($p > 0.10$).

These results support previous work showing that cold water swimming produces analgesia which is partially reversed by naloxone. They further show that an equivalent blockade of stress induced analgesia can be produced by pretreating animals with large doses of ACTH. This suggests that feedback inhibition of ACTH release also blocks release of β-endorphin and opens the question of whether experimental effects previously attributed to changes in ACTH and glucocorticoid levels may be due to changes in β-endorphin levels.

1769 SOMATOSTATIN IN RAT CORTICAL NEURONS IN CELL CULTURE: SYNTHESIS AND PHYSIOLOGIC EFFECTS. John Delfs*, Marc Dichter, Richard Robbins*, James Connolly* and Seymour Reichlin* (SPON: Howard Blume). Dept. Neurology, Children's Hospital Medical Center, Boston, MA 02115.

Rat cortical neurons maintained in a dissociated cell culture system have been assayed for various neuropeptides. The cultures contained somatostatin (SS) and substance P; ACTH, LHRH, TRH, and ADH were not detected by standard radioimmunoassay techniques. SS concentration of both cells and media went from undectable at 0 to 5 days to nanogram levels after 3 weeks in culture.

Immunofluorescent staining showed SS positivity only in neurons and in no other cellular elements. Approximately 5% of the neurons contained somatostatin. Staining was prevented by prior adsorption of the anti-SS antibody with synthetic cyclic SS. SS-containing neurons had no definite morphologic appearance.

Direct application of SS by microperfusion (0.1 to 10 μM) to cortical neurons in these cultures during intracellular recording produced no significant change in resting membrane potential, membrane resistance, or action potential characteristics. SS did change the frequency of spontaneous background synaptic activity, usually increasing the rate of the postsynaptic potentials observed in the control state, whether these were inhibitory or excitatory.

These data support the hypothesis that SS acts as a neuromodulator in cortical synaptic transmission.

1770 CHARACTERIZATION OF PEPTIDERGIC NEURONS IN MONOLAYER CULTURES OF SPINAL CORD BY IMMUNOHISTOCHEMISTRY AND SCANNING ELECTRON MICROSCOPY. R. Elde, S.L. Erlandsen*, J.L. Barker and J.H. Neale. Dept. Anat., Univ. Minn. Med. Sch., Minneapolis, MN 55455; Lab. Neurophysiol., NINCDS, Bethesda, MD 20014; Dept. Biol. Georgetown Univ., Washington, D.C.

Mechanically dissociated fetal mouse spinal cord cells were grown in monolayer on collagen-coated glass coverslips. After varying periods of time, dissociated dorsal root ganglion (DRG) cells were added to some cultures. After 2-6 weeks, cultures were fixed with 4% paraformaldehyde at 4°C for 2 hrs. Fluorescence immunohistochemistry was accomplished using antisera to met-enkephalin (ME), somatostatin (SOM) and substance P (SP). Specificity of staining was established using antisera pretreated with an excess of the appropriate antigen prior to application on cultures. After observation and photography using fluorescence and phase contrast microscopy, the coverslips of cultured neurons were prepared for scanning electron microscopy (SEM) according to standard procedures.

Spinal cord cultures exhibited striking networks of varicose fibers, terminals and neuronal perikarya after incubation with ME antiserum. The fibers and terminals were frequently in close apposition to dendritic profiles and perikarya of unstained neurons. Immunofluorescence within neuronal perikarya often was restricted to a narrow zone of perinuclear cytoplasm. The perikarya containing ME immunoreactivity were predominantly multipolar and of small to medium diameter. A large increase in the number of immunoreactive SP and SOM fibers, terminals and perikarya was observed in cultures to which DRG had been added. These fibers and terminals, like those seen with ME antiserum, made frequent contact with unstained dendrites and perikarya. However, many SP and SOM immunofluorscent perikarya were small, smooth and bipolar and were identical to DRG neurons as identified by phase contrast microscopy. Examination of cultures with SEM revealed additional morphological details of the interaction of the varicose fibers and terminals with dendrites and perikarya. The morphological characteristics of the immunohistochemically identified peptidergic neurons in vitro resemble the morphology of the same population of peptidergic neurons in situ. Such findings suggest the utility of using cultured neurons as a model of neuronal architecture, connectivity and physiology. (Supported in part by a McKnight Scholars Award to R. E.).

1771 **DISTRIBUTION OF SOMATOSTATIN-CONTAINING NEURONS IN THE GUINEA PIG FOREBRAIN.** **S.C. Feldman*, A.-J. Silverman and E. Lichtenstein* (SPON. J.R. Currie) Dept. of Anatomy Columbia University, P&S, and Hospital for Joint Diseases and Medical Center, New York, NY 10032.**

Recent studies have suggested that somatostatin (SRIF) is contained in both neurosecretory and non-neurosecretory neurons and may function as a neurotransmitter as well as a neurohormone. We have undertaken a study of the distribution and morphology of SRIF-containing neurons in the guinea pig forebrain. SRIF was localized in 6 um paraffin sections by the unlabeled antibody enzyme technique of Sternberger using an antiserum to SRIF. Following immunocytochemical identification of SRIF, sections were counterstained in cresyl violet. Perikaryal diameter was determined by measuring the long and short axes using a 40x objective and 12.5 x ocular equipped with a measuring reticule. Neurons were included only if the nucleolus was visible. The diameters of SRIF-containing neurons were determined in the following areas (range and (mean)): Neocortex: 6.5-15 μm (10.2 μm); Caudate and Putamen: 9-13 μm (11.7 μm); Amygdala: 10-15μm (12.3 μm); Nucleus of the diagonal band: 8.5-15.5 μm (12.3 μm); Hippocampus: oriens layer, 11-18.5 μm (13.6 μm); molecular layer, 10-13.5 μm (11.4 μm); pyramidal layers (CA_1-CA_4), 11-12.5 μm (11.8 μm); Hypothalamus: anterior hypothalamus, 10-19 μm (14.2 μm); lateral hypothalamus, 10-12 μm (11.2 um), ventromedial nucleus, 10-12 μm (11.2 μm). In many cells of the forebrain SRIF was present in thick, unbranched processes, presumably dendrites, and in thin, beaded fibers which could be axons. SRIF-containing neurons in the neocortex appeard round or oval. Whereas most of these cells had only one immunoreactive process, generally beaded, some neurons had 3-4 thick SRIF-containing dendrites. In the caudate and putamen most SRIF neurons had dendrites and axons which extended for considerable distances (60-320 μm to 160-300 μm, respectively). Neurons in the oriens layer of the hippocampus were oval with 1-2 dendrites whereas in the molecular layer no immunoreactive processes could be traced to cells of origin. In the pyramidal layers, SRIF neurons showed only one, thin, process which branched at its distal end. In the lateral hypothalamus SRIF neurons had no visible processes whereas those in the anterior hypothalamus-periventricular zone had one to several immunoreactive processes. SRIF neurons in the ventromedial nucleus tended to be small with none or one immunoreactive process. Our results suggest that SRIF is synthesized in several different types of neurons; the functional implications of this remain to be determined. Supported by HD10665 to A.J. Silverman and Postdoctoral Training Grant 1-TK-32 GM07061 to S.C. Feldman.

1772 EXCITATORY ACTION OF OPIOID PEPTIDES ON CULTURED HIPPOCAMPAL PYRAMIDAL CELLS. B.H. Gähwiler. Pharmaceutical Division, Preclinical Research, SANDOZ Ltd., CH-4002 Basle, Switzerland.

Spontaneous activity was recorded intracellularly from rat hippocampal pyramidal cells maintained in explant cultures for several weeks. Pyramidal cells were recognized morphologically by their size and by their characteristic pattern of dendritic arborization following injection of horseradish peroxidase into single neurons.

Bath application of opioids or opioid peptides rapidly increased the amplitude of epsp's until large depolarization shifts and spike bursts occurred. Using the stable methionine enkephalin analogue, FK 33-824, excitatory effects were observed at concentrations as low as 10^{-8}M. The effects were dose-dependent, high concentrations of FK caused sustained depolarization of the membrane. Identical results were obtained with morphine (10^{-6} to 10^{-4}M), met-enkephalin (10^{-5}M) and leu-enkephalin (10^{-4}M). The effects of all these substances were shown to be reversible and naloxone-sensitive.

Our findings that these peptide effects were mimicked by blockade of GABA receptors with bicuculline methochloride and abolished by synaptic isolation with 8 mM Mg^{2+} suggest that the excitatory action of opioid peptides on hippocampal pyramidal cells comes about by removal of tonic gabaergic inhibition by basket cell interneurons (also Siggins et al., Soc. of Neuroscience, 8th Annual Meeting, St. Louis, 1978). Since iontophoretic application of enkephalins directly onto pyramidal cells in control solution unexpectedly produced similar excitatory effects, the possibility is raised that enkephalin receptors are located presynaptically on pyramidal cell afferent terminals.

1773 EFFECTS OF OPIATE AGONISTS AND AN ANTAGONIST ON AMINO ACID RESPONSES OF CULTURED SPINAL NEURONS. D.L. Gruol, J.L. Barker, and T.G. Smith*, LNP, NINCDS, NIH, Bethesda, Md. 20014.

The effects of several opiate agonists and an antagonist on the amino acid responses of spinal neurons was studied using cultured, dissociated, fetal mouse spinal neurons and conventional electrophysiological techniques. Intracellular recordings were made from large (25 to 50 μM) neurons which had been maintained in culture for 1 month or longer. The recording media consisted of Hank's BSS plus Hepes buffer (25 mM) and 10 mM $MgCl_2$. The putative amino acid neurotransmitters glycine (GLY), γ-aminobutyric acid (GABA), β-alanine (β-ALA) and glutamate (GLU) were applied extracellularly by iontophoresis. The opiate agonists and antagonist were applied by superfusion. All effects were dose-dependent and reversible.

When applied at 10 to 100 μM concentrations, the opiate agonist levorphanol (LP) depressed the inhibitory responses evoked by GLY and β-ALA but did not effect the inhibitory GABA response or the excitatory glutamate response. Dextrorphan (DP), the stereoisomer of LP, was ineffective or only slightly depressed the GLY response at these concentrations (effect on β-ALA not tested). Morphine (50 μM), another opiate agonist, also depressed the inhibitory GLY and β-ALA responses without altering the GABA response (effect on GLU not tested). The ability of naloxone (NAL), an opiate antagonist, to reverse the LP and morphine depressions is presently being tested to determine if these effects are mediated by opiate receptors. Leucine-enkephalin, an endogenous opioid peptide believed to act at opiate receptors, did not effect the amino acid responses of these neurons although such modulatory effects of leucine-enkephalin have been observed in other cultured spinal neurons.

(-)-NAL, the form of NAL which is active at opiate receptors, depressed the inhibitory GABA response when applied at 0.1 to 1 mM concentrations but did not depress the GLU response. The GLY response was slightly depressed at the higher (-)-NAL concentrations. This effect of (-)-NAL was not stereospecific since (+)-NAL, the stereoisomer of (-)-NAL, also blocked the GABA response. (+)-NAL did not alter the GABA or GLU response when applied at 10 to 100 μM concentrations but completely blocked the GLY response. The effect of (-)-NAL on GABA dose-response curves suggests that (-)-NAL acts as a competitive antagonist at the GABA receptor but the mechanism underlying the (+)-NAL depression of the GLY response has yet to be determined.

1774 MOUSE BRAIN ENKEPHALIN LEVELS: LACK OF EITHER DIURNAL RHYTHMICITY OR ALTERATION BY ACUTE OPIATE TREATMENT IN C57B1/6J AND DBA/2J MICE. G. Gwynn*, R.C. Frederickson and E.F. Domino. Dept. of Pharmacology, Univ. of Michigan, Ann Arbor, MI 48109 and Lilly Research Laboratories, Indianapolis, IN 46206.

The purpose of these studies was to examine the involvement of the enkephalin putative neurotransmitters (Met-ENK and Leu-ENK) in the genotype-dependent differences in behavioral responsiveness observed in C57B1/6J (C) and DBA/2J (D) inbred mouse strains.

A diurnal rhythm in hotplate (52°C) jump latency was observed in both C and D mice ($P < .01$). At both a.m. and p.m. timepoints D mice exhibited significantly longer latencies than C mice ($P < .001$). Acute p.m. administration of naloxone (NLX; 3.2 and 10 mg/kg s.c.) but not of 0.9% NaCl induced a significant lowering of jump latencies in both strains. The magnitude of this hyperalgesic effect was greater in D than C mice at both doses of NLX, resulting in similar post-NLX jump latencies in the two strains. After acute a.m. treatment with morphine (MRP; 1-100 mg/kg s.c.), D mice showed a biphasic depressant-stimulant pattern of locomotor activity (LMA) and potent antinociception while C mice exhibited solely a stimulant LMA pattern and relatively poor antinociception (Gwynn and Domino, Pharmacologist 19: 170, 1977).

Unrestrained mice were sacrificed by focused microwave irradiation (<0.4 sec.) and whole brain (minus cerebellum) ENK levels were determined by RIA. In both the Met-ENK and Leu-ENK RIA, curves generated by serial dilutions of mouse brain extracts and of standard ENK solutions were parallel. Interassay ENK cross-reactivity was less than 7%.

No evidence in either strain for a diurnal rhythm in Met-ENK or Leu-ENK was found. Both a.m. and p.m. Met-ENK, but not Leu-ENK, levels were significantly greater in D than C mice. Neither a.m. MRP (10 and 32 mg/kg s.c.) nor p.m. NLX (10 mg/kg s.c.) significantly altered levels of either ENK in C or D mice.

	Met-ENK (N=10) (pm/g)		Leu-ENK (N=8) (pm/g)	
	C57	DBA	C57	DBA
A.M.	725 ± 20	820 ± 25*	205 ± 14	200 ± 12
P.M.	715 ± 28	806 ± 22*	192 ± 14	186 ± 14

These data do not support a correlation between whole brain ENK levels and any of the strain differences in behavior noted above. The strain difference in brain Met-ENK may, however, speculatively, be involved in other established behavioral differences between C and D mice (Oliverio et al., Adv. Biochem. Psychopharmacol. 11, 411, 1974).

1775 IMMUNOHISTOCHEMICAL LOCALIZATION OF SUBSTANCE P AND OTHER PUTATIVE NEUROTRANSMITTERS IN OLD WORLD PRIMATES. Suzanne Haber* and Robert Elde (SPON: V. Seybold). Dept. Anat., Sch. Med., University of Minnesota, Minn. Minn. 55455.

The hypothesis that Substance P may serve as a neruotransmitter was greatly strengthened by immunohistochemical studies which revealed its immunoreactivity in discrete, but widespread neuronal circuites. Furthermore, these studies have shown sites where Substance P is morphologically related to other neuronal circuits containing identifiable putative transmitters. Since possible species differences may have important implications for interpreting results as models for the human brain, these studies were undertaken to study the distribution of Substance P and morphologically related neuromodulators in nonhuman primate brain.

Six monkeys were perfused normally or pretreated with colchicine 48 hours before perfusion for better localization of cell bodies. Groups of twenty-five consecutive 10u cryostat sections were cut every 750u: three were stained with cresyl violet; two sections were processed for immunofluorescence for each of five antibodies; one additional section for each antibody served as an absorption control; and the remaining sections were stored for future use.

Preliminary observations of Substance P immunoreactivity suggest a high density of fibers in the area of the globus pallidus, substantia nigra, and the substania gelatinosa of the spinal cord. Moderate fiber density was in many areas including the stria terminalis and the nucleus accumbens. Sparse to moderate innervation was seen in various parts of the hypothalamus.

The findings of this study should give a more representive indication of the distribution and morphological relationship between Substance P and other neuromodulators in the human brain.

1776 TRITIUM LABELING OF β_p-ENDORPHIN BY REDUCTIVE METHYLATION FOR *in vitro* BINDING TO RAT BRAIN MEMBRANES. R. Glenn Hammonds, Jr.*, Nicholas Ling*, and David Puett* (Spon: R. Kuczenski). Department of Biochemistry, Vanderbilt University, Nashville, TN 37232 and Laboratory for Neuroendocrinology, The Salk Institute, La Jolla, CA 92037.

A labeled endorphin derivative of sufficiently high specific radioactivity to be useful for *in vitro* binding assays has been prepared by reductive methylation of synthetic β_p-endorphin. The tritium labeled ε-N-dimethyl β_p-endorphin ($[^3H]$-Me β_p-EP), with a specific activity of 9.8 Ci/mmol, was homogeneous by gel exclusion chromatography and polyacrylamide gel electrophoresis; over 90% of the label was present as dimethyllysine. In equilibrium binding studies using a washed particulate fraction from rat brain, $[^3H]$-Me β_p-EP bound in a non-cooperative fashion and exhibited a dissociation constant of 0.3-0.6 nM; the receptor concentration was between 100 and 300 pmol/mg protein. Displacement of $[^3H]$-Me β_p-EP from brain membranes was the same using native and labeled peptide indicating that the tritium labeling did not alter the peptide-receptor affinity. The binding of $[^3H]$-Me β_p-EP was also decreased by several opiate alkaloids, enkephalins, and α and γ endorphin, but not by several other peptides including ACTH and the C-terminal fragments of β_p-endorphin corresponding to the sequences β_p-lipotropin 63-91 and 80-91. (Supported by NIH grants: AM 15838, AM 18811, HD 09690, and by the William Randolph Hearst Foundation.)

1777 β-ENDORPHIN INDUCED EPILEPTIFORM ACTIVITY INCREASES LOCAL CEREBRAL METABOLISM IN HIPPOCAMPUS, AMYGDALA AND SEPTUM. Steven J. Henriksen, Frederic Morrision, and Floyd E. Bloom. A.V. Davis Ctr., The Salk Inst., La Jolla, CA 92037.

The non-convulsive limbic EEG epileptiform activity elicited by intraventricularly administered β-endorphin (Henriksen et al.PNAS 75 (10) 5221-5225, 1978) was metabolically mapped using the ^{14}C-2-deoxyglucose technique (Sokoloff et al 1977,J. Neurochem. 28, 897-916). Rats were implanted with chronic jugular catheters, lateral ventricular cannulae, and electrodes for monitoring EEG activity in dorsal hippocampus and frontal cortex. Following a baseline EEG recording β-endorphin (15-17 μg in 15 μl of Ringer's solution), or an equal volume of Ringer's solution alone, was administered intraventricularly to rats and immediately followed by a intracardial bolus of ^{14}C-2-deoxyglucose. EEG was monitored continuously for 30 min after which animals were sacrificed and brains prepared for autoradiography. Rats exhibiting electrographic epileptiform activity (N=6) demonstrated significantly increased metabolic activity throughout the hippocampus compared to controls. Larger increases were observed in ventral compared to either dorsal hippocampus or the dentate gyrus as assessed by quantitative densitometry. The lateral septum showed increases consistent with the known anatomical projections to this nucleus from the hippocampal gyrus. Significantly increased metabolism was also observed in the medial nucleus of the amygdala while decreased metabolism occurred in the medial geniculate body. Animals administered β-endorphin but who exhibited only high voltage synchronous cortical slow waves with no epileptiform activity demonstrated metabolic activity similar to controls but with slight increases only in ventral hippocampus. These metabolic studies support our earlier electrographic observations that the epileptiform activity induced by intraventricularly administered β-endorphin is primarily limited to limbic brain areas. Furthermore since these limbic epileptiform events are induced with doses of β-endorphin that do not induce analgesia nor postural rigidity, this suggests a specific role for opioid mechanisms in the regulation of limbic excitability. Recent microiontophoretic studies (Siggins et al. Soc. N.S. Ab. 131, 1978) have demonstrated opioid induced excitations of hippocampal pyramidal neurons are primarily the result of opioid inhibitions of inhibitory interneurons. The role of other limbic areas in the induction of epileptiform events following intraventricular β-endorphin is presently being investigated. (Supported by DA-01785-03 and the Klingenstein Foundation.)

1778 **EVIDENCE FOR MULTIPLE OPIATE RECEPTORS IN BRAIN.** **William A. Hewlett,* Huda Akil[1] and J.D. Barchàs.** Dept. Psychiatry, Sch. Med., Stanford Univ., Stanford, CA 94305; [1]Ment. Health Res. Ctr., Univ. Mich., Ann Arbor, MI 48109.

Three populations of opiate receptors μ, κ and σ have been described by Martin et al. (J. Phamacol. Exp. Ther. 197:517-533, 1976). A number of morphines, morphinones, benzomorphans and oripavines have been pharmacologically characterized in terms of their interaction with each of these receptors. The abilities of opioid peptides and certain representatives of the above classes to displace the binding of ^{3}H-naloxone, ^{3}H-morphine, ^{3}H-cyclazocine, ^{3}H-UM1071,* ^{3}H-Leucine-enkephalin and ^{3}H-β-endorphin were examined in a washed membrane preparation of rat brain. Unlabled opiate compounds show marked differences in their ability to displace the binding of these tritiated opiates. This differential displacement is currently being investigated in brain regions. The significance of these studies will be discussed in relation to current models of multiple opiate receptors in brain.**

***Generous gift of Jim Woods**
****Generous gift of Choh Hao Li**

1779 LHRH PERIKARYA SEND AXONS TO THE OLFACTORY BULB IN THE HAMSTER. Gloria E. Hoffman, Barry J. Davis and Foteos Macrides. Dept. of Anatomy, Univ. of Rochester Med. Sch., Rochester, NY 14642 and Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

The relationship between luteinizing hormone-releasing hormone (LHRH) perikarya and axons in the forebrain and the olfactory bulb were studied in the hamster using the unlabelled antibody peroxidase method. The animals were anesthetized with pentobarbital, perfused with either neutral formalin or Zamboni's fixative and sectioned on a vibrating microtome. Two projections from LHRH perikarya in the forebrain to the olfactory bulb were observed. One projection could be traced from fusiform-shaped perikarya in the medial septal area and from perikarya within the septal and basal portions of the vertical limb of the diagonal band of Broca (DB). The axons coursed through the medial aspect of the olfactory peduncle directly into the granule cell layer of the caudal main olfactory bulb (MOB). A second population of immunoreactive axons could be traced into the olfactory bulb from a distinctive LHRH neuron population which was located in the superficial plexiform layer of the ventromedial surface of the brain. These LHRH perikarya extended rostrally from the level of the anterior hippocampus into the olfactory peduncle to the medial border between the anterior olfactory nucleus and the MOB. These neurons were frequently seen to be intermingled with axons arising from other LHRH perikarya of this group. In the bulb, the fibers coursed along the perimeter of the MOB and accessory olfactory bulb (AOB) within the superficial aspect of the glomerular layers. These axons entered the periglomerular region and external plexiform layers of both the MOB and AOB. HRP injections into the MOB and injections of tritiated amino acids into the medial aspect of the olfactory peduncle and in the medial septum/DB region have confirmed these direct projections to the olfactory bulb. These data will be compared to results in rat and mouse.

The presence of LHRH projections to the olfactory bulb suggests that LHRH may have functions different from its role in direct regulation of pituitary output of gonadotropins.

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1780 NALOXONE IN THE THERAPY OF SHOCK: STUDIES ON THE SITE AND MECHANISM OF ENDORPHIN INVOLVEMENT. John W. Holaday, Thomas P. Jacobs, and Alan I. Faden. Dept. of Medical Neurosciences, Walter Reed Army Inst. of Research, Washington, DC 20012.

The effects of parenterally administered naloxone in improving the cardiovascular pathophysiology of endotoxic, hypovolemic, and spinal shock in rats have been demonstrated in our laboratory (Nature, 275; Science, in press; submitted). In the present studies, we used these shock models to determine if the therapeutic effects of naloxone were centrally mediated and involved an antagonism of pituitary endorphins. Moreover, the effects of naloxone on the altered body temperature and respiratory rates in shock states were also examined.

Hypovolemic shock was studied in conscious, freely-moving male Sprague Dawley rats (250-300 g) previously prepared with indwelling cannulae in the external jugular vein and tail artery. Spinal shock was induced by acute transection of the cervical spinal cord at C6-C7 in anesthetized male rats weighing 500-800 g. In spinal shock studies, naloxone was injected parenterally through the external jugular vein cannula or intracerebroventricularly (ivt) into the lateral ventricle through an implanted guide tube. Injection of a dye solution ivt at the end of experiments was used to confirm accuracy of ventricular guide placement. Colonic temperature was obtained by means of a rectally inserted thermistor; respiratory rates were counted by observation. Hypophysectomized rats and sham-operated controls were purchased from Zivic-Miller Laboratory.

In spinal shock, cervical cord transection produced a transient bradycardia and hypertension followed by a loss of mean arterial pressure (MAP) to a value approximately 20 mmHg below control values minutes later. Intravenous naloxone HCl (10 mg/Kg) or ivt naloxone HCl (48 μg in 20 μl saline) restored MAP and pulse pressure (PP) to control values within 5 min; no significant effect on heart rate was observed. Respiratory rates and colonic temperature which were depressed by spinal transection, were significantly reversed by subsequent intravenous or ivt naloxone injections.

In pilot studies, hypovolemic shock was induced in conscious hypophysectomized and sham-operated rats by bleeding to 40 mmHg for 20 min. The effects of saline injections were compared to subsequent naloxone HCl injections (1 mg/Kg). Preliminary results indicate that hypophysectomized rats do not respond as well as sham-controls to the improvement in MAP and PP produced by naloxone.

Our results provide evidence that the decline in respiratory rates, colonic temperature, and blood pressure in spinal shock is mediated through a central mechanism that is naloxone sensitive and independent of sympathetic control. Preliminary evidence for the possible involvement of pituitary endorphins in hypovolemic shock was also obtained.

1781 RELEASE OF α-MELANOTROPIN (α-MSH) FROM RAT HYPOTHALAMIC SLICES IN VITRO. Gunnar E. Holmquist*, Thomas L. O'Donohue*, N. B. Thoa*, Terry W. Moody and David M. Jacobowitz (SPON: A. I. Leshner). Lab. Clin. Sci. and Biol. Psych. Branch, NIMH, Beth., Md. 20205 and Dept. of Pharm., Howard Univ., Washington, D.C. 20059.

An extensive system of α-MSH-containing nerves which originates primarily from the arcuate nucleus of the hypothalamus and projects to many forebrain, midbrain and some hindbrain sites has recently been described. A common feature of neurotransmitters is a depolarization-induced release mechanism. In this study, such a mechanism was investigated using an in vitro approach. Fresh rat hypothalami were sliced (approximately 225 μm) using a tissue chopper. Sections were suspended in 0.6 ml of a modified Krebs-bicarbonate buffer which was maintained at pH 7.4-7.6. α-MSH released into the medium was analyzed by radioimmunoassay.

High concentrations of α-MSH diffused from the slices into the medium immediately upon incubation. Rinsing the slices with buffer reduced the baseline release to 31 pg/2 minutes. Stimulation with K^+ (50 mM) resulted in a marked increase (250%) in α-MSH release. K^+ stimulation in the absence of calcium (1 mM EGTA) also resulted in α-MSH release but was signficantly attenuated compared to K^+ stimulated release in the presence of Ca^{++} (5 mM). Veratridine (100 μM) also markedly stimulated α-MSH release (380%). Once again this release was significantly depressed in the absence of Ca^{++}. These results suggest an α-MSH release process and support a neuroregulatory role for this peptide.

1782 REGULATION OF HIPPOCAMPAL [MET^5]ENKEPHALIN CONTENT FOLLOWING RECURRENT MOTOR SEIZURES. J.S. Hong, P.L. Wood, J.C. Gillin*, H.-Y. T. Yang* and E. Costa. Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hosp., Washington, D.C. 20032

Unilateral intrahippocampal injection of kainic acid (K.A.) (1 μg in 1 μl of 0.9% NaCl) produced recurrent motor seizures lasting about 5 hr and a selective change in the [met^5]enkephalin (ME) content of hippocampus. Six to 12 hr after injection, there was a small decrease in ME content in both injected and contralateral hippocampus, however within 24 hr after the injection the ME content was increased. The elevation in the injected hippocampus reached a maximal level (200%) after 2 or 3 days, this plateau lasted longer than 14 days. In contrast the ME content of the contralateral side increased by a greater extent reaching a peak at day 2 (300%) but returned to control level by day 14. The increase in ME content was greatly reduced when cycloheximide was administered intraventricularly 6 hr after intrahippocampal injection of K.A. This result suggests that the increase in ME content elicited by K.A. injection is probably due to an increase in the synthesis of ME rather than to a decrease in the efflux of ME from the hippocampus. The selective increase in hippocampal ME content was also elicited by intraseptal, intrastriatal or intraventricular injections of K.A. Since all the procedures caused recurrent motor seizures it is suggested that the change in hippocampal ME content after K.A. may be related to the recurrent seizures elicited by K.A. In fact the increase in ME content after intraseptal injection of K.A. was blocked when muscimol, an anticonvulsant, was injected together with K.A. Moreover, the increase in hippocampal ME 2 days after recurrent seizures was also induced by isoniazid or by repeated electroconvulsive shock (4 shocks given at 30 min intervals). Thus, it appears that prolonged recurrent seizures may cause a selective increase in the production of hippocampal ME.

1783 RAT BRAIN MEMBRANE BINDING AND SYNAPTOSOMAL UPTAKE OF ^{3}H-IMIPRAMINE AND ^{3}H-COCAINE. Robert B. Innis, Fernando M. A. Corrêa and Solomon H. Snyder. Dept. of Pharmacol., Sch. Med., Johns Hopkins Univ., Baltimore, MD 21205

The tricyclic antidepressants and cocaine are potent inhibitors of the synaptosomal reuptake of norepinephrine. In addition, the tricyclic antidepressants are potent antagonists of muscarinic cholinergic, α-noradrenergic and histamine receptors. These interactions may explain the therapeutic actions and side effects of these drugs. We have studied the binding of ^{3}H-imipramine (^{3}H-IMI), ^{3}H-desipramine (^{3}H-DMI) and ^{3}H-cocaine to rat brain membranes. The binding of ^{3}H-IMI to crude brain membrane fractions is saturable, and subcellular fractionation suggests a synaptosomal localization of the ^{3}H-IMI binding site(s). Imipramine (10^{-5}M) displaces 60-70% of total bound counts with an IC_{50} of approximately 80 nM. Scatchard analysis indicates binding to at least two separate sites with affinities of 4 and 20 nM. The detailed pharmacologic profile of ^{3}H-IMI binding will be described, and the possible interaction with the binding with ^{3}H-phencyclidine (^{3}H-PCP) will be discussed.

In order to investigate the mechanism of inhibition of norepinephrine uptake by the tricyclic antidepressants and cocaine, we have studied the synaptosomal uptake of ^{3}H-IMI and ^{3}H-cocaine. The uptake of both of these ligands appears dependent on time, temperature, and the integrity of the synaptosomal preparation. The data suggest that imipramine and cocaine inhibit the uptake of norepinephrine by being taken up themselves into synaptosomes. The detailed pharmacologic and kinetic parameters of synaptosomal uptake of ^{3}H-IMI and ^{3}H-cocaine will be described.

1784 A PEPTIDE RESEMBLING LUTEINIZING HORMONE RELEASING HORMONE AS A LIKELY TRANSMITTER IN SYMPATHETIC GANGLIA OF THE FROG. Yuh Nung Jan*, Lily Yeh Jan* and Stephen W. Kuffler. Dept. Neurobiology, Harvard Medical School, Boston, MA 02115

A peptide that resembles luteinizing hormone releasing hormone (LHRH) was found to be a likely transmitter for the late slow excitatory postsynaptic potential (epsp) that lasts for many minutes in the bullfrog sympathetic ganglia, because (1) Radioimmunoassays established that 100-800 pg of a LHRH-like substance is contained in the lumbar chain of sympathetic ganblia. The peptide is confined to those spinal nerves that contain a distinct group of preganglionic axons which initiate the late slow epsp, (2) Five days after ipsilateral preganglionic axons are cut, 95% of the LHRH-like material disappears from ganglia, while the LHRH immunoreactivity triples in the spinal nerves proximal to the cut region, (3) The LHRH-like substance is released by raising the external potassium concentration; this release is calcium dependent. (Proc. Natl. Acad. Sci. USA 76 1501-1505, 1979).

Recent electrophysiological experiments have shown that LHRH or two of its analogs, when applied by pressure from a micropipette to the cell surface, produce a depolarization of long latency and slow time course similar to that of the late slow epsp. The conductance changes during the peptide-induced depolarization are also comparable to those during the late slow epsp. Further, a potent antagonist of LHRH in mammalian systems completely blocks the nerve evoked late slow epsp as well as the LHRH-induced depolarization, suggesting that the late slow epsp is mediated by a LHRH-like peptide.

1785 SITE OF ENKEPHALIN ACTION IN THE HIPPOCAMPUS. Robert A. Jensen, Joe L. Martinez, Jr., Robert Craeger*, John Veliquette*, James L. McGaugh, and Gary Lynch. Department of Psychobiology, School of Biological Sciences, University of California, Irvine, CA 92717.

Evidence indicates that enkephalins exert a powerful modulating influence in the operation of the hippocampus. We now report data that points to the site and mode of action of enkephalin in the hippocampus. In these studies, the enkephalin analog D-Ala-D-Leu-enkephalin (H_2N-Tyr-D-Ala-Gly-Phe-D-Leu-OH) or naloxone was superfused over the in vitro hippocampal slice preparation and the electrical response to stimulation was recorded.

Hippocampal slices (600 μm) were placed in a recording chamber and were continuously perfused with fresh, warm (33°-35°C), oxygenated (95% O_2; 5% CO_2) medium. Bipolar stimulating electrodes were placed in the Schaffer-commissural projections extending to the pyramidal cell apical dendrites. Stimulation electrodes were also placed in the alveus, the fiber bundle that carries the axons of the pyramidal cells. These two electrode placements allowed for both orthodromic and antidromic activation of the pyramidal cells. One recording micropipette was placed in the regio superior dendritic zone to record extracellular activity resulting from Schaffer-commissural stimulation. A second micropipette was placed in the pyramidal cell body layer to record the "population spike" response of these cells to stimulation.

The "population spike" response of pyramidal cells to Schaffer-commissural stimulation was highly sensitive to the enkephalin and demonstrated dramatic increases in amplitude even with concentrations as low as 10 nanomolar. The onset of this effect was rapid and was reversed by perfusion with peptide-free medium. Naloxone, by itself, had no detectable effect on this response, but it blocked the effect of enkephalin. Additionally, even at concentrations in the micromolar range, enkephalin had no observable effect on synaptic potentials generated by Schaffer-commissural stimulation nor did it affect the antidromic potential recorded in the cell body layer to stimulation of the alveus.

Taken together, these findings suggest that enkephalin acts to attenuate the phasic inhibitory effects of a class of inhibitory interneurons (basket cells) that are brought into play by Schaffer-commissural stimulation. Experiments pairing antidromic with orthodromic stimulation have provided indirect evidence that these interneurons are not activated by the recurrent collateral interneurons and, instead, may be a category of interneurons involved in feed-forward inhibition. Supported by a grant from the McKnight foundation (J.L.McG.) and research grants MH 12526 (J.L.McG.), MH 19793 (G.L.), AG 00538 and BNS 76-17370 (J.L.McG. and G.L.).

1786 PHARMACOLOGICAL DISSOCIATION OF THYROTROPIN-RELEASING HORMONE (TRH) ANTAGONISM OF PENTOBARBITAL (PB) INDUCED NARCOSIS AND HYPOTHERMIA. Peter W. Kalivas* and Akira Horita* (SPON: Lawrence Halpern). Depts. Phcol. and Psychiatry, Sch. Med., Univ. of Wash., Seattle, WA 98195.

Microinjection of TRH into the lateral ventricle will significantly antagonize narcosis duration and hypothermia in male S.D. rats pretreated with PB. In addition, TRH will also induce shaking behavior resembling that seen during opiate withdrawal. To examine if these three effects of TRH are mediated by the same neurochemical substrate, we injected rats i.p. with a number of pharmacological agents either 10 min. prior to or 10 min. following i.p. administration of 40 mg/kg PB. Twenty min. following PB induced loss of righting reflex, animals received bilateral intracerebroventricular injections of either 1 μm TRH dissolved in 1 μl saline or saline alone. Colonic temperature was monitored at 10 min. intervals, shaking counted following TRH or saline, and narcosis duration determined by return of righting reflex. Pretreatment agents were dissolved in saline or distilled H_2O and included; 5 mg/kg atropine sulfate, 0.5 mg/kg muscimol, 300 mg/kg sodium salicylate, 1.0 mg/kg cyproheptadine or 0.5 mg/kg 6-chloro-2 (1-piperazinyl)-pyrazine (MK-212, 5-HT agonist). Sodium salycylate had no effect on any of the three TRH responses. Muscimol partially inhibited shaking and reversal of hypothermia, and while prolonging the duration of narcosis in animals receiving TRH, it also potentiated narcosis in animals receiving saline such that TRH was still determined to significantly antagonize narcosis duration. Atropine antagonized TRH reversal of narcosis, but was without effect on shaking and hypothermia. A similar response to atropine was observed with cyproheptadine indicating it may be acting in its capacity as an anticholinergic rather than as an antiserotonergic. MK-212 completely abolished all aspects of the TRH response. These data indicate that the capacity of TRH to antagonize PB narcosis can be pharmacologically distinguished from the shaking and temperature responses. Reversal of PB narcosis involves cholinergic systems, while activation of GABA systems will selectively antagonize the temperature and shaking responses. The sensitivity of all three responses to MK-212 is interesting, esp. in view of other data demonstrating that serotonergic agents will inhibit TSH release by TRH as well as inhibit electrically induced release of immunoreactive TRH from hypothalamic synaptosomes. (Supported by NIH Grant MH-29503)

1787 SUBSTANCE P-LIKE IMMUNOREACTIVITY (SPLI) AND TARGET ORGAN REPRESENTATION WITHIN THE NUCLEUS SOLITARIUS (nS) AND VAGAL MOTOR NUCLEUS (DMN X). David M. Katz and Harvey J. Karten, Depts. Biology (DMK) and Psychiatry and Anatomy (HJK), SUNY at Stony Brook, Stony Brook, New York 11794.

The distribution of SPLI within the nucleus solitarius and dorsal motor nucleus of the vagus nerve was compared with the representation of peripheral target organs within these two cell groups in pigeons. The distribution of SP was mapped by immunocytochemical techniques, using monoclonal antibodies to SP. Several cytoarchitecturally distinct regions within DMN X and nS contained extremely dense plexuses of immunoreactive fibers which were not seen in tissue stained with antibodies absorbed against synthetic SP. Two approaches were used to identify the peripheral organs represented in these regions.

First, horseradish peroxidase (HRP) labelling of specific vagal branches was used to identify the central projections of vagal sensory neurons to nS as well as the cells of origin of vagal motor fibers within DMN X (cf. Katz,D.M., and Karten,H.J., 1977,1978, Neurosci.Abs.). In a second set of experiments, target organ representation within DMN X and nS was mapped by studying the distribution of acetylcholinesterase (AChE) within these regions following selective axotomy of individual vagal branches. Both DMN X and nS contain abundant stainable AChE which is severly depleted following peripheral vagal axotomy. One week after transection of specific vagal branches, animals were perfused with 4% paraformaldehyde,and the brains were processed for AChE histochemistry. A very precise correlation was found between the results of the AChE depletion and HRP studies.

Those regions of DMN X which are richest in SPLI project to organs of the gastrointestinal tract: the esophagus, crop, and proventriculus. The crop is a food storage organ, and is responsible for the prolactin-stimulated production of "crop milk". The proventriculus, or glandular stomach, secretes pepsinogen and hydrochloric acid. Vagal sensory neurons which innervate these same organs project centrally to those regions of nS which stain most intensely for SP. Other regions of nS, such as those receiving aortic afferents (Katz,D.M. & Karten,H.J., 1979,Br.Res., in press), and of DMN X, exhibited some SPLI.

Our findings provide anatomical evidence for the involvement of SPL immunoreactive fibers within the central nervous system in autonomic pathways which mediate specific visceral functions. Some of these pathways may be involved in the neural regulation of gastrointestinal secretory functions, such as the vagally mediated release of pepsinogen and hydrochloric acid.

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1788 DOES NALOXONE SUPPRESS SELF-STIMULATION BY DECREASING REWARD OR INCREASING AVERSION? John E. Kelsey, James D. Belluzzi and Larry Stein. Wyeth Laboratories, Philadelphia, PA 19101.

The opiate-antagonist naloxone suppresses self-stimulation (SS) of enkephalin-rich brain regions, but interpretation of this effect is unclear. According to Belluzzi & Stein (1977), who assume that positive reinforcement is mediated in part at opiate receptors, naloxone acts to block endorphin-mediated reward. However, it is possible that naloxone decreases SS, not by blocking reward, but by increasing aversion. This alternative is plausible because naloxone blocks the aversion-reducing or analgesic effects of opiates and endorphins. The second alternative implies that naloxone's suppressive action should be greatest at SS sites where the stimulation also produces analgesia, and it should be least at SS sites where analgesia is not a factor. 53 rats had electrodes implanted in a region of midbrain central gray reported to yield both SS and stimulation-induced analgesia (SPA) (Mayer et al., 1971). Electrodes were assayed first for SPA (D'Amour-Smith tail-flick test) and then SS, and fell into 4 groups on the basis of positive (+) or negative (-) response in each test. 34 cases were positive for SS; these were evaluated for sensitivity to naloxone (2.5 mg/kg, s.c.) after stabilization of 1-hour SS rates. Surprisingly, self-stimulators that failed to exhibit analgesia (group + -) were suppressed by naloxone to at least the same extent as those that did display analgesia (group + +). Since naloxone failed to act preferentially at analgesic sites, the notion that naloxone suppresses SS merely by increasing aversion was not supported. Rather, the data are consistent with the hypothesis that naloxone blocks endorphin-mediated reward.

Group (SS:SPA)	N	Self-Stimulation (Mean ± S.E.M.)		Thresholds (Mean ± S.E.M.)	
		Control Rate (Responses)	Naloxone Rate (% Control)	SS (μA)	SPA (μA)
+ +	20	2983 ± 317	66.8 ± 6.5	89.0 ± 12.0	250.0 ± 21.5
+ -	14	2612 ± 281	56.2 ± 9.1	115.3 ± 13.3	-
- +	14	-	-	-	212.5 ± 24.3
- -	5	-	-	-	-

1789 METHIONINE-ENKEPHALIN-LIKE IMMUNOREACTIVITY IN FETAL RAT BRAIN CELLS IN AGGREGATING CULTURE AND IN MOUSE NEUROBLASTOMA CELLS. Elinor L. Knodel* and Elliott Richelson. Depts. of Psychiatry and of Pharmacology, Mayo Fdn., Rochester, MN 55901

Brains from fetal rats of 14-16 days gestation were mechanically dissociated and grown as aggregates in rotation culture. The aggregates were fixed in buffered 4% paraformaldehyde and 8 μm frozen sections were prepared. Sections were stained by the indirect immunofluorescence method using antibody to methionine-enkephalin (a gift from R. Elde). The specificity of the reaction was determined by staining with antiserum pre-incubated with 1 mg/ml methionine-enkephalin.

In aggregates cultured for 2 or 3 days, only background fluorescence was observed; however, by 6 days many aggregates contained specifically-labeled cells, which were generally scattered throughout the aggregate. Occasionally, fluorescent cells were clustered in groups in the central portion of an aggregate, known to be comprised mainly of neuronal cells in older aggregates.[1] After 4-5 weeks in culture very few aggregates had brightly fluorescing cells in their central areas. Both cell bodies, excluding nuclei, and cell processes were labeled. Incubation of the aggregates overnight with 10^{-4}M morphine had no effect on the fluorescence.

About 5% of mouse neuroblastoma cells (clone NIE-115) in stationary phase also contained methionine-enkephalin-like immunoreactivity, but the staining pattern differed from that seen in the fetal brain cells. Occasionally whole cells were labeled, including their nuclei. Most of the positively-staining neuroblastoma cells contained one or two brightly labeled inclusions, much larger in size than the storage vesicles for neurotransmitters. Thus, although these cultures of neuroblastoma and fetal rat brain cells contain few specific binding sites for opiates, the present results suggest that they may synthesize and store endogenous opiates.

(Supported by Mayo Foundation and USPHS Grants DA 1490 and MH 27692.)

[1]B. Trapp, et al., Brain Res. 160:117, 1979.

1790 HYPOTHALAMIC BRAIN SLICES: ANGIOTENSIN II SENSITIVE CELLS IN OVLT. W. Douglas Knowles and M. Ian Phillips. Dept. of Physiology, University of Iowa, Iowa City, IA 52242.

Angiotensin II (AII), when injected into the brain, has powerful biological effects including drinking, a pressor response, and vasopressin release. Low dose injection, lesion, and binding studies point to the organum vasculosum of the lamina terminalis (OVLT) as a receptor area mediating these effects. In a previous study, AII was applied microiontophoretically to this region and sensitive cells were found, but this was done in anesthetized animals. To study the effects of AII on the OVLT in the absence of anesthetics, possible modulatory effects of other brain regions, and uncontrolled physiological variables, the brain slice technique for the hypothalamus was developed in our laboratory.

Adult male albino rats were decapitated and blocks of their brains containing the hypothalamus and preoptic area were sectioned in the frontal plane at 300μ with a Vibratome while immersed in cold Yamamato's solution. The slices were mounted between two nylon nets in a chamber through which oxygenated (95% O_2, 5% CO_2) solution at 35±1°C flowed. Three-barrelled micropipettes (5 to 20 Megohms) were used for recording and iontophoresis. The barrels contained: recording- 3M NaCl and Fast Green dye, test- 1 mg/ml AII pH 4.5, control- 0.9% NaCl or 1 mg/ml Saralasin pH 4.5.

35 cells in 9 rats were tested. Of 32 cells in the OVLT, 91% responded to AII by increased firing rates, 3% had no response, and 6% were inhibited. Control applications of saline on 14 of the OVLT cells caused increased firing in 64%, but had no effect on 29%, and inhibited 7%. Saralasin (an AII antagonist with similar structure) ejections on 15 of the OVLT cells excited 60%, had no effect on 13%, and inhibited 27%. Statistical comparison ($p<.01$) of the frequency of firing during test and control applications to each of 22 OVLT cells showed that 64% of the cells fired at a higher frequency to AII than to control applications, 4% showed no response to AII or control ejections, and 32% showed the same response to AII and control applications. A dose response relationship of increased excitation with increased ejection of AII was seen in all of 9 cells tested.

These experiments demonstrate the recording from neurons in the hypothalamus in rat brain slices. The results show that the majority of cells tested in the OVLT are sensitive to AII. AII application increases the firing rate of cells in this region significantly more than control applications. These data support the hypothesis that the OVLT is a brain receptor area for AII. Supported by NINCDS Fellowship to WDK and NSF, NIMH, and RSCM grants to MIP.

1791 ACTH ACCELERATION OF REGENERATION OF CRUSHED PERIPHERAL NERVE AND DENERVATED SKELETAL MUSCLE. Ted T. Kung* and Fleur L. Strand. Schering Corp., N.J. and Biology Dept., N.Y.U., N.Y. 10003.

Recent studies have shown that ACTH has direct neurotrophic activity. This study was undertaken to determine whether ACTH treatment can accelerate the rate of regeneration of crushed peripheral nerve and denervated muscle in intact and adrenalectomized (adx.) rats. Animals were treated with 0.25 U of ACTH 1-39 daily following nerve crushing for periods of up to 40 days. The regeneration rate was measured by foot-flick response to pain, distance between 1st and 5th digit (d1-5), average length of regenerated axons, and incorporation rate of amino acids by the denervated muscles.

ACTH-treated adx animals recovered sensation and functional movements more rapidly than did untreated groups. D_{1-5} and foot-flick response to pain showed similar results. The average length of regenerated axons nine days after crushing for the adx-ACTH group was significantly longer (3.99 ± 0.47 mm) than that of adx-saline group (2.68 ± 0.36 mm). The incorporation rate of amino acids in adx-ACTH group, as measured 9-14 days after denervation, was increased significantly over that of untreated adx controls. Histological and histochemical examination of the muscles showed that ACTH has no effect on muscle weight, fiber diameter and fiber types as compared to their control groups. However, increase in number of enlarged endplates and more endplates with branched pre-terminal fibers were observed.

1792 NEUROTOXIC AGENTS REDUCE SOMATOSTATIN CONTENT OF THE RAT NEURAL RETINA. Norma Lake and Yogesh C. Patel*, Dept. of Research in Anaesthesia, and Fraser Labs, Dept. of Medicine, McGill University, Montreal, Quebec, Canada, H3G 1Y6.

Recent reports by ourselves and others have described the presence of somatostatin-like immunoreactive material (SLI) in the rat neural retina. In an attempt to localize this SLI we have utilized neurotoxic agents and have examined their effects on retinal SLI and morphology. Kainic acid is a glutamate analog which exerts neurotoxic effects on CNS neurones and, when given intraocularly to young chicks, causes marked degeneration of cells in the inner nuclear layer of the retina, particularly amacrine interneurones (Schwarcz and Coyle, 1977, Invest. Ophth. Vis. Sci. 16, 141). We have found similar degeneration in the retina of adult rats injected intraocularly 48 hours previously with 120 nmol of neutralized kainic acid. This was associated with a marked reduction of retinal SLI as measured by radioimmunoassay, from 1.24 ± 0.14 pg SLI/ μg protein in saline controls ($n = 7$) to 0.43 ± 0.05 pg SLI/ μg protein in kainic acid treated rats ($n = 8$). The second retinotoxic compound which we examined was monosodium glutamate (MSG) which we administered subcutaneously (2 mg/ g body weight) on a daily basis to rats for the first ten days of postnatal life. Histological examination of the retinas from 23-25 day old MSG-treated rats showed considerable degeneration in the inner plexiform layer and cell losses in the inner nuclear and ganglion cell layers. Concurrently there was a severe reduction of retinal SLI to 10% or less of control levels. These studies suggest the localization of retinal SLI within a population of neurones of the inner retina, perhaps within some amacrine and ganglion cells. SLI is present in sufficient quantities to suggest that it may have a neurotransmitter or neuromodulator role at these sites in the neural retina.

1793 IS THERE A ROLE FOR THE ENDOGENOUS OPIOID PEPTIDES IN THE REGULATION OF BLOOD GLUCOSE? Claudia Landau‡ Roberta Palmour*, and J.-K. Chang*. (SPON: E. Wm. Yund). Dept. Physiol. & Genet. U. California, Berkeley, CA 94720, Peninsula Labs, San Carlos, CA.

The presence, distribution and pharmacological properties of highly specific opiate receptors within the central nervous system and in anterior pituitary, gut and vas deferens have been the focus of intensive research. Several lines of evidence suggest that opioids and opiate receptors exist in other peripheral tissues. Preliminary evidence from this laboratory describes specific opioid binding sites in rat kidney and adrenal glands. While many of the reported effects of opioid peptides may be mediated through central or pituitary receptors, peripheral sites may also play some role in physiological regulation. Holaday et al. (PNAS 74:4628, 1977) found that adrenalectomized (Adx) mice responded to iv β-endorphin with severe respiratory depression, cyanosis and seizures, while intact mice did not. The suppression of these effects by pretreatment with dexamethasone, the similarity between these toxic effects and insulin shock, and the recent report of Ipp et al. (Nature 276:190, 1978) that β-endorphin stimulates insulin release in an in vitro pancreatic perfusion system all suggest a relationship between glucose regulation and opioid peptides.

We have tested the possibility that opioid peptides participate in blood glucose regulation using intact and adx rats. Saline, insulin, morphine, β-endorphin and the enkephalin analog tyr-D-ala-gly-N-me-phe-met(O)-ol (P8625) were administered iv to adx and intact rats. Blood was drawn at 0, 15, 30 and 60 min.; glucose, insulin, glucagon and endorphin levels were measured. Core body temperature, respiratory rate, blood pressure and other visible autonomic signs were monitored. Gross behavior and catalepsy were evaluated.

Adx rats given insulin iv exhibited signs of severe respiratory depression, peripheral cyanosis, hypothermia and flaccid immobility. Adx rats given morphine showed typical opioid effects--muscular rigidity, cataleptic immobility and peculiar postures. Adx rats treated with 2.5 mg/kg P8625 showed autonomic effects similar to, but more severe than those seen in insulin-treated animals. Following ip injection of 10 mg glucose, respiratory depression, cyanosis and hypothermia were reversed, unmasking typical opioid effects.

Comparison of the opioid and glucoregulatory effects of β-endorphin and P8625 will be discussed as will general physiological effects of these peptides.

1794 EFFECTS OF SOMATOSTATIN ON THE ATPase ACTIVITIES OF SYNAPTOSOMES OF RAT CEREBRAL CORTEX. Sheu L. Lee* and Viktor Havlicek, Dept. of Physiology, Faculty of Medicine, Univ. of Man., Winnipeg, Canada, R3E OW3.

Somatostatin has been found to be present in many structures of the rat brain including cerebral cortex. Synaptosomal pellet of the cerebral cortex has 40 times higher concentration of somatostatin than the whole cortex. Upon cell depolarization with high extracellular potassium ions tissue slices of the cerebral cortex show calcium dependent release of somatostatin. All these findings allow us to speculate that somatostatin is a putative neurotransmitter or neurohormone in the cerebral cortex (Havlicek and Friesen in Central Nervous System Effects of Hypothalamic Hormones and other Peptides, Ed. R. Collu, A. Barbeau et al., Raven Press, N.Y., 1979, 361-402). In the present study we show that somatostatin inhibits the activity of Na-K-ATPase (EC 3.6.1.3) in rat cerebral cortical synaptosomal fraction. The activity of Mg-ATPase was not significantly affected (Fig. 1) and there was no detectable effect on Ca-Mg-ATPase (for methods see Lee and Phillis, Can. J. Physiol. Pharmacol. 55, 961-969, 1977). Decrease of the ouabain-sensitive Na-K-ATPase of the synaptosomal membranes by somatostatin will result in depolarization of the membrane potential, which is in accord with out in vivo experiments in which somatostatin applied iontophoretically stimulated activity of cortical neurons in unanesthetised rabbits (Ioffe, Havlicek et al, Brain Research 153, 414-418, 1978) and caused membrane depolarization (see Havlicek and Friesen, 1979).

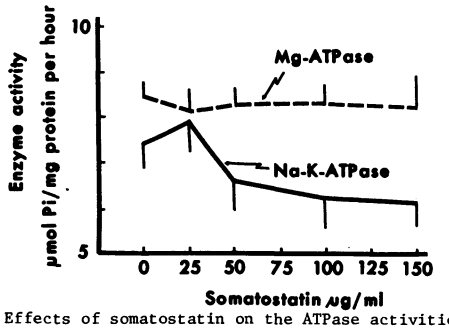


Fig. 1. Effects of somatostatin on the ATPase activities of synaptosomes of rat cerebral cortex (N=8-15; ± S.E.). (Supported by MRC of Canada)

1795 REGULATION OF PITUITARY β-ENDORPHIN BY PINEAL MELATONIN Robert M. Levy*, Huda Akil**, Stanley Watson** and Jack Barchas (SPON: L. Mathers). Nancy Pritzker Laboratory of Behavioral Neurochemistry, Department of Psychiatry and Behavioral Sciences, Stanford Medical School, Stanford, CA 94305 and **Mental Health Research Institute, University of Michigan School of Medicine, Ann Arbor, MI 48109.

Melatonin, N-acetyl-5-methoxy-tryptamine, a major product of the mammalian pineal gland, has long been implicated in the regulation of pituitary function. Changes in the levels, synthesis and release of several pituitary peptide hormones, including GH, prolactin, ACTH and MSH have been observed following a variety of manipulations involving the pineal gland. As both of these neuroendocrine organs lie outside the blood-brain barrier, the neurohumoral regulation of the pituitary gland by pineal melatonin exists as an interesting possibility. In light of the recent discovery that β-endorphin (β-END) shares with ACTH and β-MSH a common 31 K amino acid precursor (Mains, Eipper and Ling, PNAS, 74:3014-3018, 1977), we have attempted to determine whether this pituitary opiate peptide hormone is subject to physiological regulation by pineal melatonin.

To approach this problem, we have performed a number of manipulations known to alter the function of the pineal gland and measured the subsequent effects on the level of pituitary β-END by radioimmunoassay. These manipulations include exposure of animals to several altered lighting regimens known to alter melatonin synthesis and release, pinealectomy, intracerebroventricular and intravenous injection of varying doses of melatonin and the intravenous administration of highly specific antibodies raised against melatonin-BSA conjugates. Following the experimental manipulations, animals were sacrificed, pituitary glands were removed over Dry Ice, and β-END was extracted from the tissue. Radioimmunoassay of β-END from these extracts was performed as has been previously reported.

Preliminary results from these studies indicate that in those conditions where plasma and cerebrospinal fluid melatonin levels would be expected to be decreased, pituitary levels of β-END were significantly elevated. Melatonin administration, on the other hand, produced a significant decrease in pituitary β-END. These studies suggest a complex interaction between pineal melatonin and pituitary β-END and further implicate the pineal gland in the physiological regulation of pituitary peptide hormones.

1796 PEPTIDE ACTION ON INDIVIDUAL GIANT DOPAMINE NEURONS: ELECTROPHYSIOLOGICAL AND MICROFLUORIMETRIC ANALYSIS. W. Lichtensteiger and D. Felix*. Inst. Pharmacol. and Brain Res. Inst., Univ. Zürich, Zürich, Switzerland.

The giant dopamine neuron (GDN) in the left pedal ganglion of the snail Planorbis corneus provides a model system for studies of biophysical and histo- or biochemical parameters at the cellular level. Intracellular recordings were performed on GDN in vitro. The preparation was frozen to -195°C during electrical recording and processed for histochemical microfluorimetry of catecholamine fluorescence. Fluorescence intensity of GDN is correlated with their firing rate (Lichtensteiger, Felix and Hefti, Brain Research, in press), in analogy to the situation encountered in rat dopamine (DA) neurons.

We used this preparation in order to study cellular actions of representatives of two groups of centrally active peptides which affect the functional state of mammalian DA neurons. When administered to the bath (1 μM), lysine vasopressin (LVP) caused an acute rise in membrane resistance of GDN as checked by injection of depolarizing or hyperpolarizing current, with enhanced firing in response to depolarizing current. An analog with behavioral activity in mammals, desglycinamide-lysine vasopressin showed a similar but stronger action. In contrast membrane resistance was reduced after ACTH 4-10 (1-5 μM) in the majority of GDN. LVP exerted variable effects on spontaneous activity while ACTH 4-10 lowered activity and often induced a rhythmic pattern. The relation between cellular fluorescence intensity and spontaneous activity remained largely unchanged after LVP which suggests that transmitter changes were mainly related to the level of neuronal activity also after peptide administration. Injection of current and manipulation of cation transport by themselves affected fluorescence intensity. These effects are being further analyzed.

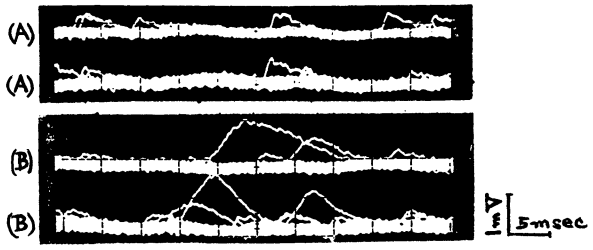
Our results illustrate the potential usefulness of this giant molluscan neuron for correlative studies of different aspects of peptide action at the level of individual neurons.

1797 α-L-ASPARTYL-L-ALANINE INCREASES MEPP AMPLITUDE. Ramon Lim, Shu Tim Cheung* and John W. Crayton, Brain Research Institute and Depts. of Surgery (Neurosurgery) and Psychiatry, University of Chicago, Chicago, Ill. 60637

α-L-Aspartyl-L-alanine (asp-ala) is a dipeptide we isolated from the pig brain (Cheung & Lim, Biochim. Biophys. Acta, in press). The biological activity of asp-ala is tested on a rat phrenic nerve-diaphragm preparation. At 10^{-5} M asp-ala enhances neuromuscular transmission by increasing the amplitude of MEPP by 35%. The increase is noticeable during the first 30 min. of intracellular recording and is sustained up to at least 120 min. in the presence of the drug (see table and tracings below). However, the effect is maintained even after washing out the drug following the first 30 min. The free amino acids L-aspartic acid and L-alanine do not show noticeable effects under similar conditions. Another dipeptide, α-L-aspartyl-glycine, which has been isolated from urine but not from the brain, also does not have any effect. The results suggest asp-ala as one of the neural peptides capable of modulating synaptic transmission. (Supported by USPHS grant No. NS-09228)

Time course of asp-ala effect on rat diaphragm

	No. of Diaphragms(n)	No. of Fibers	Amplitude of MEPP (mV)	Frequency of MEPP (No. per min.)
pre-drug (A)	9	90	.59 ± .042 (S.D.)	122 ± 39 (S.D.)
0-30' (B)	9	26	.80 ± .142 (p < .002)	108 ± 45 (N.S.)
30'-60'	9	38	.76 ± .132 (p < .003)	125 ± 39 (N.S.)
60'-90'	9	38	.78 ± .132 (p < .002)	123 ± 31 (N.S.)
90'-120'	9	38	.77 ± .122 (p < .002)	125 ± 44 (N.S.)



(Each tracing is a composite of 20 sweeps)

1798 CHARACTERIZATION OF MET- AND LEU-ENKEPHALIN RELEASE FROM RAT STRIATUM: INHIBITION BY ACETYLCHOLINE. Iris Lindberg* and June L. Dahl. Dept. Pharmacology, Univ. of Wisconsin Medical School, Madison, WI 53706.

The release of met- and leu-enkephalin from superfused striatal slices was studied. Pooled caudal striatal sections from five rats were chopped into 0.3 mm slices and divided among four superfusion filters. The slices were superfused for 15 min. with warm oxygenated Krebs-bicarbonate (containing 60 µg/ml bacitracin) at a rate of 0.35 ml/min. Fractions were collected at 2-min. intervals. After the collection of three baseline fractions, stimulated release was elicited by a 4-min. exposure to Krebs containing 50 mM potassium, followed by superfusion with normal Krebs. Enkephalins in slices and perfusates were measured by radioimmunoassay (leu-enkephalin antibody was a gift of Dr. Richard Miller; met-enkephalin antibody was purchased from Immunonuclear Corp.). Results are expressed as the percentage of the initial tissue enkephalin released per min.

The mean basal release rates ± SEM of met- and leu-enkephalin were 0.21 ± 0.02%/min. and 0.50 ± 0.07%/min., respectively. After subtraction of basal release rates, the net increase in leu-enkephalin release induced by exposure to 50 mM potassium was 1.36 ± 0.16%/min., whereas that of met-enkephalin was 1.22 ± 0.15%/min. No potassium-stimulated increase could be observed in the absence of calcium. When the calcium concentration was increased from 2 mM to 5 mM, potassium-stimulated rate of release of the enkephalins approximately doubled. The addition of 50 µM acetylcholine to the superfusion medium inhibited the basal release of leu- and met-enkephalin by 66 and 59%, respectively; net potassium-stimulated release was inhibited 79% (leu-enk) and 18% (met-enk). 100 µM acetylcholine virtually abolished potassium-stimulated leu-enkephalin release, while inhibiting basal release by 55%. At the higher concentration of acetylcholine, basal release of met-enkephalin was inhibited by 44%, while stimulated release was inhibited by 63%.

While a greater proportion of tissue leu- than met-enkephalin was released, the absolute amount of met-enkephalin released exceeded that of leu-enkephalin. The ratio of met- to leu-enkephalin released averaged 1.25 ± 0.10 in baseline fractions but rose to 2.23 ± 0.12 during potassium stimulation. The latter value more closely approximates the tissue ratio of the enkephalins found after perfusion (3.35 ± 0.10). It is likely that the lower ratio found in the superfusates is related to the differential degradation of the two peptides (Bayon *et al.* (1978) PNAS 75, 3503); this possibility is currently under investigation. (Supported by DA 00697; Iris Lindberg is an NIH Predoctoral Trainee supported by GM 07107.)

1799 DISTRIBUTION OF α-MELANOTROPIN (α-MSH) IN DISCRETE NUCLEI OF THE CAT BRAIN. V. John Massari*, Thomas L. O'Donohue*, Y. Tizabi*, and David M. Jacobowitz (SPON: G. M. Moolenaar). Dept. of Pharm., Howard Univ., College of Med., Washington, D.C. 20059 and Lab. Clin. Sci., NIMH, Bethesda, Maryland 20205.

Recently α-MSH has been localized within neurons in the arcuate nucleus, and in neuronal processes in the rat brain. In this study, α-MSH (or α-MSH-like immunoreactivity) has also been detected in cat brain. Five mongrel cats of mixed gender were sacrificed under Ketamine anesthesia and their brains were rapidly removed and frozen on dry ice. The brains were alternately sliced into 600 µm thick slabs and 60 µm sections in a cryostat at -8°C. Individual brain nuclei were microdissected from the tissue slices using needles having an internal diameter of 1000, 750, 500 or 300 µm essentially according to the method of Palkovits. The micropunches were placed in 100 µl of 2N acetic acid, boiled for 10 min. and homogenized by sonication. α-MSH was assayed by radioimmunoassay. The sensitivity of the assay is approximately 1.5 pg.

The highest concentrations of α-MSH were found in the arcuate nucleus, median eminence, and suprachiasmatic nucleus (≥ 25pg/µg). High levels of α-MSH were found in the medial preoptic nucleus, periventricular nucleus of the hypothalamus, and paraventricular nucleus of the thalamus (≥ 10 pg/µg). Very low levels of α-MSH were found in all cerebral cortical areas, neo- and paleostriatum, the geniculate nuclei, and the superior and inferior colliculi (≤ 1.0 pg/µg). Within the periaqueductal gray, successively greater concentrations of α-MSH were found in a rostral to caudal progression. Moderate amounts of α-MSH were also detected in the dorsal and median raphe nuclei.

These data indicate that α-MSH is differentially distributed within different nuclei of the cat brain and lend support to the hypothesis that α-MSH may be a CNS neurotransmitter or neuromodulator.

1800 IMMUNOCYTOCHEMICAL STUDIES ON NEURONAL PEPTIDES IN DISSOCIATED SPINAL CORD-DORSAL ROOT GANGLION CELL CULTURES. E. Matthew*, E.A. Neale, P.G. Nelson and E.A. Zimmerman. Dept. of Neurol., Columbia University, New York, NY 10032 and Lab. Devel. Neurobiol., NICHD, NIH, Bethesda, MD 20014.

Fetal mouse spinal cord (SC) and dorsal root ganglion (DRG) neurons grown in dissociated cell culture were examined by immunocytochemistry for endogenous neuronal peptides. Neurons of the intact mammalian spinal cord have been shown by others to contain immunoreactive substance P, enkephalin and neurotensin, while DRG neurons are reactive for substance P and somatostatin. Neurons grown in culture for 4-6 weeks were assayed for these peptides by an indirect immunoperoxidase technique using peroxidase-anti-peroxidase (PAP) complexes. Paraformaldehyde-fixed monolayers were reacted with primary antiserum directed against substance P, neurotensin (both from S. Leeman), leu-enkephalin (from S. Snyder) or somatostatin (from E. Lichtenstein). Specific immunoreactivity was tested by preincubation of each antiserum with synthetic homologous antigen. Reaction products to PAP were formed using 3,3'-diaminobenzidine tetrahydrochloride or 4-chloro-1-naphthol.

Complex networks of varicose fibers were observed in SC-DRG cultures after incubation with antiserum against substance P or enkephalin; reactivity was more extensive for enkephalin. Stained neuronal somata were small diameter cells, rounded or stellate in form, with a number of reactive neurites. The most intense staining was associated with 'beaded' neuronal processes, many of which contacted the processes and encrusted the somata of non-reactive cells. In DRG cultures (without SC cells) grown in NGF-containing medium, a large majority of cells displayed immunoreactivity for substance P, although no DRG cells were reactive for enkephalin. In SC cultures, each of these peptides was localized within a small number of neuronal somata. It appears, therefore, that some DRG and small SC neurons in dissociated cell culture contain substance P, while enkephalin can be demonstrated only in SC cells.

Immunoreactivity to somatostatin was seen in most neurons in NGF-treated cultures prepared from ganglia, and in occasional small neurons in combined SC-DRG cultures. Neurotensin immunoreactivity was detected in some fibers and in small stellate cell bodies in combined SC-DRG cultures.

The demonstration of endogenous neuronal peptides within neurons in dissociated SC-DRG cell cultures speaks for the development of specific chemical properties by these cells. The two-dimensional anatomy and ready accessibility of individual cells further advocate this system for studies of the morphologic and electrophysiologic interactions among peptidergic neurons.

1801 PEPTIDE MODULATION OF ADRENAL PARANEURONS. Fumio Mizobe*, Deanne M. Dean* and Bruce G. Livett, Division of Neurology, The Montreal General Hospital and McGill University, Montreal, Canada

Adrenal paraneurons provide a model tissue culture system of non-neoplastic cells of neural crest origin. We have previously shown that adrenal medullary chromaffin cells can be isolated in a highly purified form by retrograde perfusion with collagenase and density gradient centrifugation on Percoll™ gradients[1,2]. When plated on collagen coated plastic tissue culture dishes under conditions known to favor sympathetic neurite outgrowth in culture, these adrenal chromaffin cells produce long catecholamine-containing varicose processes and exhibit a Ca^{2+}-dependent release of ^{3}H-NE characteristic of noradrenergic neurons in culture[2]. Pharmacological studies have shown that the cells have nicotinic not muscarinic receptors[3]. The availability of this homogeneous adrenergic cell system has enabled a study to be made of the role of peptides as neuromodulators.

Substance P (10^{-8}-$5x10^{-5}$M; ID_{50} = $1.3x10^{-6}$M) and somatostatin (ID_{50} = $1.8x10^{-5}$M) produced a dose-dependent inhibition of the ACh ($5x10^{-5}$M) and nicotine ($5x10^{-6}$M) stimulated ^{3}H-NE release from these adrenal paraneurons. In contrast, the K^+ evoked release (56mM K^+) was not inhibited, and neither SP or somatostatin (10^{-8}-$5x10^{-5}$M) had any effect on the release of ^{3}H-NE in the absence of the nicotinic agonists. Other peptides known to be present in the adrenal medulla, beta-endorphin, leu-enkephalin and met-enkephalin inhibited the ACh ($5x10^{-5}$M) but not the K^+ (56mM) induced release only at peptide concentrations greater than 10^{-5}M (ID_{50} approximately $3x10^{-4}$M). Whether this pharmacological inhibition of the nicotinic response by these opioid peptides is physiologically significant is presently under investigation using peptidase inhibitors.

The results support a role for these peptides as putative neuromodulators in the nervous system.

1. Fenwick,E.M., Fajdiga,P.B., Howe,N.B.S. and Livett,B.G. (1978) J.Cell Biol.76: 12-30
2. Livett,B.G., Dean,D.M. and Bray,G.M. (1978). Society for Neuroscience Abstracts 4: 592
3. Livett,B.G., Kozousek,V., Mizobe,F. and Dean,D.M. (1979). Nature (Lond.) 278: 256-257

(Supported by MRC and MDAC)

1802 BOMBESIN-LIKE PEPTIDES: NEUROTRANSMITTERS IN MAMMALIAN BRAIN? Terry W. Moody, Nguyen B. Thoa*, Thomas L. O'Donohue*, David M. Jacobowitz, Agu Pert and Candace B. Pert. Biological Psychiatry Br. and Lab. of Clinical Science, NIMH, Bethesda, MD 20205

Bombesin, a tetradecapeptide isolated from frog skin, is active in the gastrointestinal tract and brain. In the central nervous system bombesin induces hyperglycemia and hypothermia with a well-defined structure activity relationship which corresponds to that required for receptor binding. Specific bombesin receptors, which show a markedly heterogeneous distribution in rat brain, are highly enriched in the periaqueductal gray and nucleus accumbens. Microinjections of bombesin (<1 µg) into these areas cause profound analgesia and enhanced locomotor activity, respectively.

Endogenous bombesin-like peptides have been detected using immunocytochemical techniques as well as by radioimmunoassay combined with microdissection techniques using an antibody which recognizes the C-terminal of bombesin. Bombesin-like immunoreactivity was observed in discrete varicose nerve fibers. The concentration of bombesin-like immunoreactivity was 30-fold greater in high regions (nucleus tractus solitarius, interpeduncular nucleus, median eminence, arcuate nucleus and substantia gelatinosa) than low regions (caudate, hippocampus and cingulate cortex). After intraventricular injection of vinblastin, neuronal cell bodies were observed in the preoptic, hypothalamic and mammallary nuclei as well as the mesencephalon. The distribution of bombesin-like peptides and substance P is strikingly coincidental, however, radioimmunoassay analysis of high-pressure liquid chromatographic fractions of brain extracts indicates that the two peptides are distinct.

Also, the subcellular distribution of bombesin receptors and bombesin-like peptides was investigated using our radioreceptor assay and radioimmunoassay. The greatest number of bombesin receptors and bombesin-like peptides is present in the synaptosomal fraction, suggesting an association with nerve terminals.

In addition, the release of bombesin-like peptides from rat hypothalamic slices was investigated. The amount released increased 2-3 fold when the brain slices were treated with depolarizing stimuli such as K^+ or veratridine in the presence of Ca^{++}. These results suggest that endogenous bombesin-like peptides may have a physiologic role in the synaptic function, either as neurotransmitters or neuromodulators.

1803 SOMATOSTATIN AND SUBSTANCE P LEVELS IN CULTURED SENSORY NEURONS ARE INFLUENCED BY ENVIRONMENTAL FACTORS. Anne W. Mudge* (SPON: E. Floor). Dept. of Physiol., Harvard Med. Sch., Boston, MA 02115

The peptides somatostatin (SOM) and substance P (SP) are present in separate populations of small diameter sensory fibers which project to the spinal cord dorsal horn. SP is thought to be involved in excitatory transmission in the nociceptive pathway. SOM on the other hand has generally inhibitory effects in the nervous system and its role in the nociceptive pathway is not clear. This abstract reports that cultured sensory neurons contain predominantly either SP or SOM depending on the conditions in which the neurons are grown.

Dorsal root ganglia from 9-10 day-old chicken embryos were dissociated and cells plated to yield three types of cultures--(1) neurons grown in the virtual absence of nonneuronal cells; (2) neurons together with ganglionic nonneuronal cells; or (3) ganglionic nonneuronal cells grown without neurons. The growth media contained nerve growth factor (NGF). Cultures were extracted in 2 M acetic acid and peptide content was measured by RIA. After four weeks, neuron-alone cultures contained 2049±227 fmol SP and 266±69 fmol of SOM; neurons plus ganglionic nonneuronal cell cultures contain 909±53 fmol SP and 5759±379 fmol of SOM; that is, the ratio of SOM/SP was increased from 0.13 to 6.25. The number of neurons was the same in both conditions. Cultures of nonneuronal cells alone do not contain detectable amounts of either peptide. Moreover, a similar result was obtained if neurons grown in the absence of nonneuronal cells were fed with medium which had been "conditioned" by incubation on ganglionic nonneuronal cells.

It is unlikely that an NGF-like material produced by the nonneuronal cells is the agent responsible for this change in SOM/SP ratio. First, the cultures were grown in saturating concentrations of NGF. Second, the addition of NGF to neuron-alone cultures at a dose sufficient to cause a 2-fold increase in the number of neurons surviving caused a 4-fold increase in the level of both SP and SOM with no change in the ratio of SOM/SP.

Extracts of CNS tissue added to the media produce the opposite effect: SOM/SP is decreased. Previous results in this laboratory have demonstrated that somatostatin (as well as enkephalin, 5-HT and NE) can inhibit Ca-action potentials in the cell soma as well as inhibit SP release from the neurons. The effects of these inhibitory compounds on the SOM/SP ratio is being investigated. (These experiments were carried out in the laboratories of G.D. Fischbach and S.E. Leeman, and were supported by NIH grants GM 00919 and AM 16510.)

1804 NEUROTOXIC ACTION OF CAPSAICIN ON SPINAL SUBSTANCE P NEURONS. J.I. Nagy*, S.R. Vincent, Wm.A. Staines*, H.C. Fibiger, T.D. Reisine, and H.I. Yamamura. Division of Neurological Sciences, University of British Columbia, Vancouver, B.C., Canada, V6T 1W5, and Department of Pharmacology, University of Arizona, Tucson, Arizona, USA, 85724.

Studies were made of the effects of neonatal injections of capsaicin on the ultrastructure of the neonatal spinal cord and some biochemical and behavioral parameters in the adult. Electron microscopic observations revealed degeneration and glial engulfment of boutons and unmyelinated axons in the dorsal horn 2 and 6 hours after subcutaneous neonatal injections. Capsaicin treatment had no effect of the activities of glutamic acid decarboxylase and choline acetyltransferase in the dorsal horn. Results of substance P measurements in the CNS showed no effect of capsaicin administration on striatal, hypothalamic or nigral substance P content, whereas spinal cord dorsal horn substance P levels were reduced by 46%. ^{3}H-Naloxone binding in dorsal horn tissue revealed a 37% decrease in opiate receptor levels in capsaicin-treated animals which kinetic analysis showed to be due to a change in receptor density rather than affinity. Capsaicin-treated animals showed significantly increased latencies to respond to a noxious thermal stimulus. Latencies to respond in the tail-flick and hot-plate tests were 166% and 144% respectively of control values.

The present values are consistent with the proposal that at least some primary sensory afferent neurons may utilize substance P as a neurotransmitter of nociception. Also supported by our findings is the emerging view of an intimate association between substance P and enkephalin neurons in pain mechanisms in the dorsal horn. If continued investigation shows the neurotoxicity of capsaicin in the neonate to be highly selective for the substance P neurons of the spinal ganglia, then it should prove to be a useful and important tool for the determination of the precise mechanisms by which substance P neurons participate in the transmission of specific sensory modalities.

1805 FURTHER STUDIES ON CENTRAL NERVOUS SYSTEM EFFECTS OF NEUROTENSIN, AN ENDOGENOUS PEPTIDE. C.B. Nemeroff, G.N.Ervin,* P.J. Manberg,* A.J. Osbahr, III,* S. Felts,* L.S. Birkemo* and A.J. Prange, Jr.* Biol. Sci. Res. Ctr., Depts. Psychiat., Pharmacol. and Psychol. and the Neurobiology Program, Univ. North Carolina, Sch. Med., Chapel Hill, NC 27514.

Neurotensin (NT), an endogenous tridecapeptide distributed heterogenously in the central nervous system of a variety of mammalian species, has previously been demonstrated to produce diminished locomotor activity, hypothermia, potentiation of barbiturate-induced sedation and antinociception after intracisternal (IC) administration in mice and rats. Because of similarities between certain of the properties of acknowledged neuroleptic aspects and NT, evaluation of the effects of the peptide in a variety of pharmacological screening procedures was performed. NT (3-100 µg IC in adult male mice), like neuroleptics, exhibited significant activity in the Julou-Courvoisier traction test, a measure of muscle relaxation. The simultaneous IC administration of the endogenous tripeptide thyrotropin-releasing hormone (TRH) abolished this effect of both neuroleptic agents and NT. The administration of either NT or haloperidol via bilateral nucleus accumbens cannulae in adult male rats blocks certain of the behavioral effects (↑locomotor activity and rearing) induced by d-amphetamine (2 mg/Kg IP). Further studies sought whether IC NT in mice blocks the behavioral effects of peripherally administered apomorphine in a paradigm designed to test striatal (and not mesolimbic) DA receptors (Psychopharmacol 50:1-6, 1976). NT showed no activity in this test. In the final series of experiments NT-induced antinociception was assessed and compared with the activity of ten other endogenous peptides. Adult male mice received IC injections of 1 µg NT (or another peptide in an equimolar dose) or vehicle and tested for antinociceptive activity by the Janssen method (measurement of latency to withdraw tail from 48°C water bath; Arzneitmittel-Forsch 13:502-507, 1963). β-endorphin was the most potent antinociceptive agent tested but NT was quite potent,possessing significantly greater activity than met-enkephalin, leu-enkephalin and morphine. Conclusions: (1) NT possesses activity in certain neuroleptic screening tests (e.g. Julou-Courvoisier test); (2) Direct n.accumbens injection of NT, like haloperidol, blocks certain effects of d-amphetamine, suggesting an interaction of NT at mesolimbic DA synapses; (3) in a test devised to measure blockade of striatal DA receptors, NT exhibited no activity; (4) TRH blocks certain behavioral effects of both neuroleptics and NT, (5) NT, unlike neuroleptics, possesses significant antinociceptive activity after IC administration. (Supported by NIMH MH22536, MH32316, and NICHHD HD-03110).

1806 SEPARATION, IDENTIFICATION AND QUANTITATION OF BIOLOGICALLY ACTIVE PEPTIDES BY HIGH PRESSURE LIQUID CHROMATOGRAPHY (HPLC): A PREREQUISITE FOR RADIOIMMUNOASSAY CHARACTERIZATION. Thomas L. O'Donohue*, Gunnar E. Holmquist* and David M. Jacobowitz. Lab. Clin. Sci., NIMH, Beth., Md. 20205 and Dept. of Pharm., Howard Univ., Washington, D.C. 20059.

HPLC provides a rapid means for resolving and quantitating mixtures of closely related peptides. For example, peptides differing by one amino acid, (e.g., Angiotensin II and Angiotensin III), or by only one molecular component (e.g., α-MSH, deacetylated α-MSH and deamidated α-MSH),were easily resolved by reverse phase (µBondapak/C_{18}, Waters Associates) gradient chromatography.

Using a solvent system composed of acetonitrile and trialkylammonium phosphate buffer at low pH, peptides from biological samples can be quantitated with precision and great reproducibility with minimal sample prepurification. Acid extracts from pituitary or brain are injected onto Sep-Pak C_{18} Cartridges (Waters Associates). Using buffers of high polarity,ions, impurities, and low molecular weight polar compounds can be rapidly removed. Peptides of interest are then eluted with an appropriate solvent of less polarity. These eluates are lyophylized, resuspended and chromatographed on the HPLC. Comparison of sample peak heights with known standard peak heights of identical retention times, yields a direct determination of peptide concentration in the tissue. For example, concentrations of α-MSH and vasopressin in the pituitary can be determined by absorbance at 280 nm.

HPLC also provides a rapid and accurate means for characterizing the specificity of antibodies. Antibodies which are determined to be specific by their recognition of the antigen but, lack of cross-reactivity with commercially available peptides, may recognize unidentified compounds in biological samples. We have recently seen that this is the case with antibodies to α-MSH, bombesin and substance P. These antibodies were deemed specific by both cross reactivity tests and by traditional column chromatographic techniques. HPLC, however, identified other tissue components which cross react with the antibodies. It is concluded that HPLC should be used as a minimum prerequisite for determining specificity of an antibody.

1807 SELF-ADMINISTRATION OF MORPHINE, LEVORPHANOL, AND DALA AT HYPOTHALAMIC AND NUCLEUS ACCUMBENS SELF-STIMULATION SITES. M.E. Olds* (SPON: A. Yuwiler). Division of Biology, California Institute of Technology, Pasadena, CA 91125

Adult male rats implanted chronically with a cannula guide and two electrodes glued to its shaft were tested for self-stimulation before and after self-administration tests for morphine, levorphanol, dextrophan and DALA.

For direct injection into the hypothalamus at sites yielding low to moderate rates self-stimulation, rats learned to discriminate between two pedals placed at opposite ends of a plexiglass test chamber, one that delivered 20 nl of morphine, 5 µg/µl for each depression of the lever, and the other that yielded nothing. The rates on the active lever were higher for morphine 5 µg/µl concentration than for the vehicle, which was artificial cerebrospinal fluid, and were higher for morphine 10 µg/µl than 5 µg/µl.

High-rate hypothalamic self-stimulators learned to reverse repeatedly when the active lever was changed from side to side in successive sessions and delivered 20 nl of morphine/press of 10 µg/µl. Responding was reduced on both levers and discrimination between active and inactive levers was reduced when naloxone (1-2 µg/µl) was mixed with morphine, or when it was given systemically (1 mg/kg). Extinction after morphine was slow and often produced higher rates of responding than during the acquisition session.

High-rate hypothalamic self-stimulators self-administered DALA (0.1 and 1 µg/µl concentration) but rates were generally lower than for morphine and reversal was more difficult to achieve. Responding was attenuated for a mixture of DALA and naloxone injected into the hypothalamus. Levorphanol was approximately as effective as morphine but dextrophan yielded lower self-administration response rates.

Moderate-rate nucleus accumbens self-stimulators self-administered morphine and levorphanol. Non-self-stimulators with probe and cannula in the caudate nucleus did not self-administer morphine or did so at very low rates.

Post-drug self-stimulation tests yielded rates of responding for the electrical reward comparable to those seen in pre-drug tests, a result indicating minimal functional damage to the elements responsible for self-stimulation behavior even after repeated self-administration tests.

The data are interpreted to indicate the need of both opiate receptors and high rates of self-stimulation behavior to obtain self-administration for intracerebral injection of opiates. This finding is viewed as supporting the notion that structures known to support self-stimulation behavior may mediate the reinforcing properties of morphine, levorphanol and DALA. (PHS DAO 1541).

1808 SOMATOSTATIN EFFECTS ON GLUTAMATE AND GABA TRANSPORT IN FROG SPINAL CORD. A.L. Padjen[1] & R.A. Davidoff, Depts. of Pharmacol. & Ther., McGill Univ., Montreal, Quebec H3G 1Y6 & Neurology Service V.A. Hospital & Dept. of Neurology, Univ. of Miami, School of Medicine, Miami, FL 33152.

Somatostatin is present in frog spinal cord, presumably in primary afferents (unpublished observations), as in mammals. However, effects of somatostatin on synaptic transmission do not favor its role as a primary afferent excitatory transmitter but suggest a possible "neuromodulatory" role (cf. Padjen, Neurosci. Abs. 3, 411, 1977). In concentrations of 10^{-6}-10^{-5}M somatostatin caused selective dose dependent depression of glutamate but not of GABA evoked responses on both motoneurons and primary afferents recorded by sucrose gap technique in isolated superfused frog spinal cord (synaptic transmission blocked by TTX or high Mg). These experiments were extended to an analysis of the effects of somatostatin on the uptake and release of (^{3}H) glutamate and (^{3}H) GABA in frog spinal cord slices (for methods see Davidoff & Adair, J. Neurochem. 24, 454, 1975; Brain Res. 118, 403, 1976). Somatostatin (10^{-5}M) caused a small but significant increase in (^{3}H) glutamate (10^{-7}M) high affinity uptake (+20%; control: 0.277 ± 0.031 SEM nmoles/g/min; with somatostatin: 0.333 ±0.045, n= 6) but no change in (^{3}H) GABA (10^{-7}M) high affinity uptake (+5%; control: 0.386 ± 0.061; with somatostatin: 0.404 ± 0.061; n = 6). On the other hand the increase in (^{3}H) glutamate release evoked by 40 mM K^+ was significantly depressed by the same concentration of somatostatin (no somatostatin: +156% ± 7.0 of resting efflux; with somatostatin: +114.3% ± 6.1; n = 6) without affecting K^+ evoked release of (^{3}H) GABA (no somatostatin: +157.3 ± 12.6; with somatostatin: +158.3 ± 7.8; n = 6).

These results suggest that somatostatin specifically interferes with glutamate transport in frog spinal cord. They contrast with somatostatin produced facilitation of ventral root potentials evoked by dorsal root stimulation, a pathway which is presumed to be mediated in part by glutamate.

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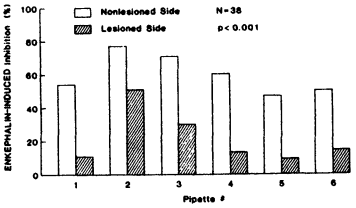
1809 CATECHOLAMINERGIC MODULATION OF ENKEPHALIN ACTION. M.R. Palmer, J. Stewart, M. Perlow†, W. Freed†, R. Wyatt†, B.J. Hoffer*. Depts. of Pharmacology and Biochemistry, University of Colorado Medical Center, Denver, Colorado, 80262, and †NIMH, St. Elizabeth's Hospital, Washington, D.C., 20032.

The interaction between iontophoretically applied D-Ala^2 methionine enkephalinamide (2dA) and catecholamine afferents to frontal cortex was investigated in rats. The depression caused by iontophoretically applied 2dA were similar to those observed after micropressure ejection from the same pipette, thus showing that the depressions are not artifacts of the iontophoretic technique. Antipsychotic agents known to block catecholamine receptors such as spiroperidol, α-fluphenthixol and (+) butaclamol, reversibly antagonized the enkephalin depressions when administered i.v. The biochemically inactive isomers of these antipsychotics, β-fluphenthixol and (-) butaclamol, had no such effects. Unilateral lesions of the catecholaminergic projections to frontal cortex produced by interstial injection of 6OHDA resulted in an ipsilateral decrease in 2dA efficacy, and in an elimination of the antagonism of 2dA depressions by antipsychotic agents.



The effects of dopamine and 2dA were similar only in deeper layers of the cortex; above 800 µm, any one neuron often showed differing responses to the two agents. The depression caused by 2dA were not antagonized by Mg^{++}, iontophoretically applied at levels high enough to reduce spontaneous activity. It is concluded that intact catecholaminergic transmission is required for the full expression of enkephalin depressions in frontal cortex, and that this mechanism probably does not involve a presynaptic release of catecholamines by enkephalin. Perhaps catecholamines act as postsynaptic modulators to augment neuronal responsiveness to enkephalins. (Supported by AA03527. M.P. has a fellowship from Pharmaceutical Manufacturers' Association, and a scholarship from the Reiger Educational Trust.)

1810 INVESTIGATION OF DES-TYROSINE^1-γ-ENDORPHIN ACTIVITY AT NEUROLEPTIC BINDING SITES IN RAT BRAIN AND OF OPIATE AND NEUROLEPTIC BINDING IN HUMAN SCHIZOPHRENIC BRAINS. N.W.Pedigo, T.D.Reisine, N.C.Ling[a], E.D.Bird[b], L.L.Iversen[b] and H.I.Yamamura, Dept. of Pharmacology, Univ. of Arizona, Tucson, AZ 85724, [a]Neuroendocrinology Lab, The Salk Institute, La Jolla, CA 92112, and [b]MRC Neurochemicalpharmacology Unit, Dept. of Pharmacology, Cambridge, England CB2-2QD.

Since the discovery of endorphins, endogenous morphine-like peptides, there has been considerable research directed towards understanding their possible role in psychiatric disorders. We have sought to contribute to this dynamic field of research by examining the possible activity of des-tyrosine^1-γ-endorphin (DTγE, βLPH_{62-77}), a proposed endogenous neuroleptic peptide, at ^{3}H-spiroperidol [^{3}H-S] binding sites in rat frontal cortex, corpus striatum and nucleus accumbens-olfactory tubercle. DTγE, in concentrations from 1 nM to 100 µM, did not inhibit ^{3}H-S binding in any of these brain regions, while control dose-response curves for haloperidol and (+)-butaclamol indicated 80-90% specific ^{3}H-S binding. The DTγE was not degraded during the binding assay as its final concentration was verified by high pressure liquid chromatography. The inability of DTγE to inhibit speicfic ^{3}H-S binding suggests that this peptide does not act at the neuroleptic receptor labeled by ^{3}H-S in these brain areas. It is possible that DTγE may be acting through mechanisms which require in vivo activation or, alternatively, DTγE may represent a new class of antipsychotic drugs with properties unlike other known neuroleptic agents.

We have also measured ^{3}H-S and ^{3}H-naloxone binding in the frontal cortex, caudate and putamen of brains obtained at autopsy from eleven schizophrenic patients. Eight of these patients had previously been treated with neuroleptic drugs. Binding of ^{3}H-S was significantly increase in the caudate (39%) and putamen (51%), but was not altered in the frontal cortex. These changes were due to an increased number of ^{3}H-S binding sites without any change in receptor affinity. In contrast, ^{3}H-naloxone binding was not significantly changed in the frontal cortex or putamen, but was reduced 43% ($p<.05$) in the caudate. Again this was due to a decrease in the number of opiate receptors rather than a change in their affinity. These receptor alterations could reflect neurochemical changes in schizophrenia or may in part represent drug-induced changes following long-term neuroleptic therapy.

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1811 ELECTROPHYSIOLOGICAL RESPONSES TO ANGIOTENSIN II AND LUTEINIZING RELEASING HORMONE. M. Ian Phillips and Dominik Felix*. Dept. Physiol. & Biophys., Univ. of Iowa, Iowa City, IA 52242 and Brain Res. Inst., Univ. of Zurich, Zurich, Switzerland.

Angiotensin II (AII) and luteinizing releasing hormone (LHRH) are neuropeptides which both produce behavioral effects when injected into the brain. Interest in the brain circumventricular organs as possible receptor sites for central effects of AII have focused on the subfornical organ (SFO) and the organum vasculosum of the lamina terminalis (OVLT). Previously, we have shown that the SFO in cat contains cells which are responsive to AII when it is applied directly by microiontophoresis. In the present study we have used a ventral approach to the OVLT of the rat for direct application by microiontophoresis of AII. Rats were anesthetized with urethane and the optic chiasm surgically revealed by microdissection. Extracellular action potentials were recorded through the central barrel of a 5-barrel glass micropipette. This contained 4 M NaCl and fast green dye. The tip diameter was approximately 4µm with a resistence of 4-12MΩ. LHRH (Beckman) 10mM, pH 5.5 angiotensin II (Calbiochem.), prepared as a 10^{-3} M solution in distilled water with a final pH of 4.5. Saralasin (Beckman) was prepared the same as AII. The criterion for a response to the peptides was a change of 20% or more in the firing rate. Only stable units were tested. The peptides were ejected with cationic currents. Cells were tested by application of LHRH or AII for 1-2 minutes with intervals between doses being several minutes to allow full recovery from testing. At the end of testing a rat, a fast green dye marker was made at the site of the last recorded unit. 87 units were tested in the OVLT or lamina terminalis. 55 were excited by AII, 25 not influenced and 7 were inhibited. Responses to AII lasted for the length of application and the latency of onset varied. Those cells which responded to AII were inhibited by Saralasin. When Saralasin was applied at the same time as AII, the response to AII was diminished or abolished. Saralasin alone diminished cell firing. Dose response curves to AII were established in several of the excited cells. Thus, cells in OVLT responded to AII with high sensitivity and specificity. LHRH produced inhibition in this region. 22 cells which responded to LHRH were all inhibited.

The results confirm other evidence that the OVLT may be an AII receptor site. They support the hypothesis that this region of the rat brain may be important in such basic functions as thirst and blood pressure control since these functions have been ascribed to AII. The inhibitory responses to LHRH suggest a negative feedback system and that this region may be involved in the control of sex behavior.

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1812 USE OF SUBSTANCE P PARTIAL FRAGMENTS TO CHARACTERIZE SUBSTANCE P RECEPTORS OF SPINAL CORD AND INTESTINE. M.F.Piercey, F.J. Einspahr* and L.A.Schroeder*. The Upjohn Company, CNS Research, Kalamazoo, MI 49001.

Several substance P (SP) partial fragments were tested for their effects on cat dorsal horn neurons and guinea pig ileum. Ileal segments were mounted in 5 ml baths of aerated Tyrode's solution (37°C). Potencies were measured as the doses required to cause half maximal contractions. C-terminal fragments as small as the hexapeptide (SP6) retained full SP potency. Potencies of the C-terminal penta- and tetrapeptides (SP5 and SP4) were much lower, while the C-terminal tripeptide (SP3) and the N-terminal decapeptide (SPN10) were inactive. SP tachyphylaxis completely eliminated the effects of SP and its analogs, but not those of non-SP receptor stimulants (acetylcholine, bradykinin, serotonin). Thus all SP fragments elicited their contractions via SP receptor stimulation.

Microiontophoresis was used to test the effects of the SP analogs on spinal cord dorsal horn neurons. Unanesthetized decerebrate cats with L1 spinal sections were used. Analogs (1 mM in 165 mM NaCl) were ejected electroosmotically from seven-barrelled microelectrodes onto dorsal horn neurons whose firing rates were monitored by a central recording barrel. Nearly all SP-excitable cells were excited by C-terminal fragments as small as SP6; smaller C-terminal fragments only occasionally excited these cells. Surprisingly, the N-terminal analog, SPN10, weakly excited about half the SP-excitable cells on which it was tested. The excitatory effects of SP fragments were slow, resembling that for SP. Thus, it is unlikely that SP's slow time course is due to its being broken down into a smaller, more rapidly acting fragment. None of the SP fragments excited SP-insensitive cells. Thus, it is concluded that the excitatory effects of all SP partial fragments were mediated through a common SP receptor located on specific dorsal horn neurons.

Because SP6 was the smallest SP fragment possessing full biological activity on both the ileum and the spinal cord, the SP receptors for both tissues must be quite similar. However, since SPN10 was weakly active in the dorsal horn, but not the ileum, these receptors cannot be totally identical.

1813 THE ACTIONS OF THE MOLLUSCAN NEUROPEPTIDE FMRFamide ON A MOLLUSCAN NEUROMUSCULAR JUNCTION. David A. Price. Dept. of Biological Sciences, Florida State University, Tallahassee, FL 32306.

The molluscan neuropeptide FMRFamide (phenylalanyl-methionyl-arginyl-phenylalanine amide) has excitatory actions on the isolated radula protractor muscle of Busycon contrarium. To determine what role, if any, the peptide might have in the normal function of this muscle, I examined the pharmacological interaction of FMRFamide with the presumed neurotransmitters, acetylcholine (ACh) and 5-hydroxytryptamine (5HT), and compared them to its effects on contractions elicited by nerve stimulation.

ACh causes contractures of the radula protractor which can be relaxed with 5HT, often with the simultaneous induction of spontaneous rhythmical activity. The main action of FMRFamide is ACh-like, though the FMRFamide induced contractures develop and decay more slowly than those induced by ACh. Since this action of FMRFamide is unaffected by ACh antagonists, it, presumably, represents a direct action of the peptide on the muscle and not an action on ACh-containing nerves. However, low concentrations of FMRFamide (less than 10nM) often cause a response resembling that seen with mixtures of ACh and 5HT. Since this oscillatory response is somewhat sensitive to ACh antagonists and is easily desensitized, it might be mediated through the nerves.

If the nerve trunk innervating the radula protractor is stimulated at low frequency (0.2/sec) with short (0.2 msec) pulses, a uniform train of distinct twitches is obtained. Low concentrations (less than 10nM) of FMRFamide in the bathing medium increase the twitch amplitude with no increase in the resting tone. ACh antagonists sharply decrease the twitch height. FMRFamide contractures developed in the absence of nerve stimulation relax when nerve stimulation is resumed.

These results support the supposition that ACh and 5HT are neurotransmitters at the radula protractor neuromuscular junction. Though FMRFamide has direct actions on the radula protractor, it is apparently not released by nerve stimulation, at least not in sufficient quantity to cause a twitch and so is likely not a neurotransmitter. It might be acting as a neuromodulator.

1814 INSULIN STIMULATES MACROMOLECULAR SYNTHESIS AND BINDS TO SPECIFIC RECEPTORS IN CELLS CULTURED FROM RAT BRAIN. Mohan K. Raizada* and Robert E. Fellows. Dept. of Physiology and Biophysics, The University of Iowa College of Medicine, Iowa City, Iowa 52242.

Although the effects of insulin on protein synthesis and cell growth have been characterized in non-neuronal tissues, they have not been studied extensively in brain. Insulin has been found in brain at levels which are higher than, and possibly independent of plasma insulin levels. Insulin immunoreactivity has also been detected in specific areas of brain, including olfactory bulb and brain stem. In order to extend our studies of the role of insulin in brain development, we have utilized primary cultures of fetal rat brain to investigate the effects of insulin on macromolecular synthesis, and to determine if specific insulin receptors are present in these cultures. Trypsin dissociated cells from rat brain, obtained at 20 days gestation, were grown in Dulbecco's modified Eagle's medium with 10% fetal bovine serum. On day 8, cultures consist of a background monolayer of glial and other cells, on top of which lie individual and clusters of "phase dark" cells, identified as neurons on the basis of morphological, electrophysiological and biochemical criteria. Incubation of cultures with insulin caused a time- and dose-dependent increase in ^{3}H-thymidine (TdR) and ^{3}H-uridine incorporation into TCA precipitable material. Maximum (2.2-fold) stimulation of TdR incorporation occurred 10 hr after incubation with 0.16 μM insulin. The same concentration of insulin caused a 2.0-fold increase in ^{3}H-uridine incorporation in 1 hr. Binding of ^{125}I-insulin to cultures was time- and pH-dependent and 85-90% specific. Porcine insulin competed for ^{125}I-insulin binding, with 35% inhibition at a concentration of 8 nM and 55-60% inhibition at 16 nM. Insulin analogs including desoctapeptide insulin, competed for ^{125}I-insulin binding in the order of their biological potency. Unrelated peptides, including glucagon and angiotensin II, did not compete for ^{125}I-insulin binding. The half-life of ^{125}I-insulin dissociation from receptors was 15 min. Scatchard analysis of binding data gave a curvilinear plot which could be resolved into two components representing high affinity ($Ka=2x10^{11}M^{-1}$) and low affinity ($Ka=4x10^{10}M^{-1}$) sites. Of the estimated $7.5x10^{4}$ total binding sites per cell, 28-30% represented high affinity sites. These studies demonstrate that cultured cells from rat brain contain specific insulin receptors which may mediate the action of insulin on macromolecular synthesis. Additional data suggest the effects of insulin on brain may be due at least in part to insulin synthesis within the central nervous system. Supported by NIH grant 1 RO1 HD11184-1.

1815 ENTRY OF OPIOID PEPTIDES INTO THE CENTRAL NERVOUS SYSTEM. S.I. Rapoport, W.A. Klee*, K.D. Pettigrew* and K. Ohno.* Nat'l Inst on Aging, Balto., MD 21224 and Nat'l Inst of Mental Health, Bethesda, MD 20014.

Cerebrovascular permeability of 4 modified opioid peptides -- [D-Ala^{2}]-methionine enkephalin amide, [D-Ala^{62},^{14}C-Homoarg^{69}]-β-lipotropin 61-69, [D-Ala^{2},^{14}C-Homoarg^{9}]-α-endorphin and [D-Ala^{2},^{14}C-Homoarg]-β-endorphin -- was measured in the conscious rat by the compartmental analysis method of Ohno et al., (Amer. J. Physiol., 253, H299, 1978). 5-10 μC$_i$ of radioactive peptide was injected i.v. as a bolus. Femoral artery plasma concentration, C_{plasma} dpm/ml, was followed until decapitation and fit by the following equation to give values of A_i and $\alpha_i(sec^{-1})$, where n = 2-4: $C_{plasma} = \sum_{i}^{n} A_i e^{-\alpha_i t}$. Parenchymal brain concentration C_{brain} dpm/g, calculated as net minus intravascular radioactivity, was represented as $dC_{brain}/dt = PA(rC_{plasma} - C_{brain}/V)$, where r = unbound tracer fraction in plasma, V = cerebral distribution volume of tracer, P = cerebrovascular permeability(cm sec^{-1}) and A = 240 cm^{-1}(capillary surface area/cm^{3} brain). Substitution of the first into the second equation and integrating gives brain concentration at time T of decapitation:

$$C_{brain}(T) = \sum_{i}^{n} \frac{rA_i\, PA}{PA/V - \alpha_i} \left(e^{-\alpha_i T} - e^{-PAT/V}\right).$$

The latter equation was fit to experiments with each tracer to give P and V. V = 0.32 for [D-Ala^{2},^{14}C-Homarg^{9}]-α-endorphin, demonstrating distribution in the brain extracellular space. P ranged from 1.2 x 10^{-6}cm $sec^{-1}$for [D-Ala^{2} ^{14}C-Homoarg^{9}]-α-endorphin to 3.9 x 10^{-6}cm sec for [D-Ala^{2}, ^{14}C-Homoarg]-β-endorphin, whereas the Brain Uptake Index technique indicates that peptides are impermeable at the cerebrovasculature (Cornford et al., Endocrinol. 103, 1297, 1978). Peptide permeability approximates the permeability of glycerol or thiourea, is 1/100 the permeability of caffeine or antipyrine, and 100 times the permeability of sucrose or erythritol. Permeability is sufficiently high to allow peptides into the brain extracellular space with a half-time of 4-12 min following a step rise in plasma concentration. The findings are consistent with observed central effects to some systemically administered opioid peptides, and suggest that feedback may operate, via the blood-brain barrier, between circulating peptides and brain sites that regulate their release into the systemic circulation.

1816 ENKEPHALIN NEURONS IN GOLDFISH HYPOTHALAMUS:PHYSIOLOGY & MORPHOLOGY STUDIED BY INTRACELLULAR RECORDING & LUCIFER YELLOW MARKING. T.A. Reaves, Jr. and J.N. Hayward. Dept. Neurology & Neurobiology Program, University of North Carolina, Chapel Hill, NC 27514.

Having described the presence of enkephalin (ENK)-containing neurons, with axons projecting to the pituitary gland, interspersed among the vasotocin (VT)- and isotocin (IT)-containing neurons of the goldfish preoptic nucleus (NPO; Reaves & Hayward, 1979), we now report the physiological and morphological characteristics of immunocytochemically-defined ENK neurons. We made intracellular recordings from antidromically identified NPO cells (Hayward, 1974) with glass micropipettes filled with the fluorescent dye, 3% Lucifer Yellow-CH (LY; Stewart, 1978). Having measured the cell's electrophysiological properties, we injected LY into the cell with 10-20 nA of anionic current for 1-5 min, perfused the brain, fixed it with Bouin's, prepared it for paraffin histology and examined the sections cut at 6-10 uM for LY fluorescence. We injected and located only one LY-marked cell for each side of the brain and traced them and all their processes with camera lucida. We stained, immunocytochemically, serial sections through the LY-marked soma to determine the peptidergic cell type. We identified chemically 30% of LY-filled cells as ENK neurons, the remaining 70% of the cells studied we identified chemically as VT or IT neurons. NPO ENK cells showed resting membrane potentials and action potentials similar to other neurons. Pituitary gland stimulation at threshold levels yielded long antidromic latencies while stepwise increases in the stimulus strength caused the invasion latency to shorten in an all-or-none fashion. Dye-marked ENK cells measured 14-42 uM with their processes projecting into the hypothalamic neuropil and to the pituitary gland. Where we saw extensive axonal branching, long sections of axons, axonal and dendritic processes and varicosities clearly, we also visualized three distinct ENK cell somata (unipolar, bipolar, multipolar) lying throughout the NPO, 50-250 uM deep to the ependymal lining of the preoptic recess of the third ventricle. In many cases, the physiological and morphological characteristics of LY-filled, chemically-defined VT and IT neurons resembled ENK cells closely. In summary, our data 1) support the one-neuron, one-peptide hypothesis; 2) illustrate the interspersed nature of complexly mixed groups of NPO peptidergic neurons; and 3) suggest that cell morphology, cell location within the NPO & cell electrophysiology are inadequate criteria for distinguishing ENK cell types. The three morphological ENK cell types (unipolar, bipolar, multipolar) may represent distinct functional subsets of this peptidergic opioid neuron.
(Supported, in part, by Grants # NS-13411 and NS-05696 from the USPHS)

1817 EFFECT OF CYCLO(LEU-GLY) ON MORPHINE DEPENDENCE AND MORPHINE-INDUCED DOPAMINE RECEPTOR SENSITIVITY

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The effect of cyclo(Leu-Gly) on the development of morphine tolerance and physical dependence as well as morphine-induced alterations in dopamine (DA) receptor sensitivity were assessed. Mice were rendered physically dependent on and tolerant to morphine by subcutaneous (S.C.) implantation of morphine (75 mg/kg free base) pellets for 3 days. A dose of 0.18 μmoles of cyclo (Leu-Gly) injected S.C. 2 hours prior to the pellet implant prevented the development of physical dependence as measured by changes in body temperature observed during either abrupt or naloxone-induced withdrawal. Cyclo(Leu-Gly) also inhibited the development of tolerance to morphine analgesia; however, the acute treatment did not modify morphine analgesia in tolerant mice.

The dose of the DA agonist apomorphine (APO) necessary to produce an increase in locomotor activity determined 24 hours after the removal of the pellets was lower in morphine-dependent mice relative to placebo controls. The pretreatment with cyclo(Leu-Gly) in the same manner which blocked the development of physical dependence and tolerance also prevented the increase in DA receptor sensitivity. Similar results were obtained using the hypothermic response to the DA agonist piribedil (20 mg/kg ip). Piribedil produced a greated hypothermic response (15 & 30min) in morphine dependent mice given cyclo(Leu-Gly) than in dependent mice given saline injection. Mice pretreated with cyclo(Leu-Gly) and chronic morphine treatment did not differ from morphine-naive mice in their response to piribedil. The injection of cyclo(Leu-Gly) on the third day of morphine treatment, at a time when physical dependence and tolerance could be demonstrated, did not alter either the signs of physical dependence and tolerance, or DA receptor sensitivity. These data indicate that cyclo(Leu-Gly) administered prior to morphine administration in mice prevents the increase in "DA receptor sensitivity" which accompanies the development of physical dependence and tolerance to morphine.

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1818 INTERACTION OF SUBSTANCE P WITH VARIOUS TREATMENTS MODIFYING CATECHOLAMINERGIC SYSTEMS.

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There is growing evidence that the undecapeptide substance P (SP), among other peptides having CNS effects, may functionally interact with the neurochemical mechanisms of the basal ganglia. In the present study, intraventricular injection of SP in a dose of .30 μg/rat increased motor activity in rats rendered hypokinetic by bilateral microinjections of 6-hydroxydopamine into the anterolateral hypothalamus. Behavioral observations indicated that grooming and not locomotion was mainly responsible for the greater activity scores. The reversal of the hypokinesia produced by administration of apomorphine, 1 mg/kg sc, to these 6-OHDA treated rats was not potentiated by SP.

Locomotion, grooming and rearing in a 16 squares open field (88 X 88 X 60 cm) was recorded in rats following intraventricular injection of SP in doses of .60 and 2.5 μg/rat. The lower dose of the peptide significantly increased locomotion and grooming. The effects of the same doses of the peptide on the hypokinesia induced by α-methyl-para-tyrosine, phenoxybenzamine and haloperidol were then examined. SP did not affect the behavioral depression produced by α-methyl-para-tyrosine (250mg/kg) and phenoxybenzamine (20 mg/kg). However, SP systematically reversed the decrease in locomotor activity induced by a relatively small dose of haloperidol (.1 mg/kg). On the other hand, SP did not counteract the hypokinesia and catalepsy resulting from the administration of a higher dose of this dopamine antagonist (3 mg/kg).

These results will be examined in light of the hypothesis that peptidergic pathways may exert a trophic modulation on catecholaminergic functions and may contribute to the pathogenesis of the extrapyramidal disorders.

(Supported by the Medical Research Council of Canada D.B.R. and F.B.J. are post-doctoral fellows from the MRC and CRSQ respectively. G.F. is from Dept. Psych., Concordia Univ., Montreal, Quebec, Canada).

1819 BOVINE ADRENALS CONTAIN HIGH LEVELS OF ENKEPHALINS AND EVEN HIGHER LEVELS OF SEVERAL PUTATIVE ENKEPHALIN PRECURSORS.

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Adrenal medullas from several species have been shown to contain enkephalin-like materials by radioimmunoassays and by immunohistology. Using HPLC and a fluorescamine detection system, we have now shown that bovine adrenals contain Met^5-enkephalin and Leu^5-enkephalin. One hour after sacrifice bovine adrenal medullas were homogenized in 1 N acetic acid, 20 mM HCl supplemented with 0.1% mercaptoethanol, 1 μg/ml PMSF and 1 μg/ml pepstatin. After gel filtration on a G-75 column, fractions with M_r below 1000 were analyzed by HPLC on Lichrosorb RP-18 eluted with gradients of 1-propanol in 0.5 M formic acid, 0.4 M pyridine, pH 4. Two peaks corresponding to Met^5-enkephalin and Leu^5-enkephalin were obtained. These peaks were characterized by radioreceptor assay, two different enkephalin radioimmunoassays and amino acid composition. A typical preparation from 3 adrenals (6.5 gm) contained 1.57 nmol/gm Met^5-enkephalin and 0.28 nmol/gm of Leu^5-enkephalin. Similar values are found in the rat striatum. We have also prepared chromaffin granules which were found to account for all the enkephalin content of the adrenals.

The existence of putative precursors for enkephalins in the adrenal was also investigated. Adrenal extracts were submitted to gel filtration on G-75 and each fraction was incubated with TPCK trypsin in order to generate active opiate peptides. In the absence of trypsin none of the fractions over M_r 5000 were found to contain opiate activity. Trypsin treatment produced no opiate activity in the void volume fractions but did so in all the fractions with M_r between 20,000 and 1,000. Treatment of these fractions with trypsin yielded a total of 3.14 nmol Leu^5-enkephalin equivalent/gm of adrenals in the radioreceptor assay, When a radioimmunoassay with a N-terminal Met^5-enkephalin serum (JR235) was used, a total value of 7.0 nmol/gm Met^5-equivalent was found. When a C-terminal Leu^5-enkephalin serum (RB92) was used, a total value of 0.13 nmol Leu^5-equivalent was found after trypsin. Trypsin digests of several fractions with M_r between 20,000 and 1,000 were analyzed on HPLC and found to yield two peaks of opiate activity neither of which corresponded to Met^5-or Leu^5-enkephalin, or β-LPH 61-69. Gel filtration of acid extracts of chromaffin granules displayed nearly the same pattern with one major difference: trypsin digest of the 20,000 M_r fractions yielded 3 times less opiate activity than similar fractions of the whole adrenal medulla. Adrenal medulla extracts contained no β-endorphin or its precursors, β-LPH and pro-opiocortin. All these findings are consistent with our previous reports that β-endorphin and the enkephalins arise via different pathways. J.R. is Charge de Recherche INSERM (France)

1820 INTERANIMAL TRANSFERABILITY OF LEARNED AVERSION TO .1% SACCHARIN.

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Interanimal transfer of brain extracts has been used in studies aimed at elucidating the role of neuromolecules in learning and memory. Problems associated with this approach have centered around the validity of the selected conditioned behaviors as representative of learning and the prevalence of confounding stressful variables. The selection of a well defined behavior and paradigm that keeps stress variables to a minimum is crucial. Taste aversion is a paradigm of this nature. The purpose of our investigation was to test the interanimal transferability of taste aversion learning in Wistar rats.

Experimental donors (ED) were injected intraperitoneally (IP) with lithium chloride (aversive) after 30 min access to .1% saccharin on 2 consecutive days. Two hrs after the second conditioning, ED were sacrificed by decapitation, brains homogenized, centrifuged and the supernatant lyophilized, all under cold conditions. Control donors (CD) underwent the same procedures except that they had access to tap water (Experiment 1) or were injected 90 min before access to .1% saccharin (sham conditioning)(Experiment 2 & 3). In each experiment (Experiment 3 within parentheses), recipients (ER & CR) were given 30 min access to .1% saccharin 12(24) hrs after the subdural (IP) injection. Recipients were given a lithium chloride injection IP 3 hrs after access to saccharin started. Twenty-eight hrs later and each subsequent day at the same time, recipients had simultaneous 6(1) hr access to .1% saccharin and tap water after taking licks from each solution.

Analysis of variance for the group X trial interaction indicated that ER had significantly lower saccharin consumption than CR (trials 1-22) in Experiment 1 ($p<.05$); no difference in Experiment 2; tended to drink less saccharin (trials 1-35) in Experiment 3 ($p<.1$); and had highly significant lower saccharin consumption (trials 1-22) in Experiments 1 and 3 combined ($p<.01$) with no differences due to procedure disparities.

The independent and pooled data from Experiments 1 and 3 suggest that the brain extracts from ED fostered an enhanced aversion to .1% saccharin in ER. Since saccharin consumption of ER and CR converge during later trials in these two experiments, the results appear to be related to learning rather than some permanent change. In Experiment 2, a convulsive and lethargic reaction was observed after subdural injection; this did not occur in either Experiment 1 or 3. Results from Experiment 2 may have been confounded by this reaction.

This paradigm has potential as a model for behavioral-neuromolecular investigations of the interanimal transfer of taste aversion (.1%, .5%, etc saccharin, maple, etc) and also those to purify and study mechanisms of action of such putative neurochemicals.

1821 PEPTIDE HORMONE EFFECTS IN THE ABDOMINAL GANGLION OF APLYSIA CALIFORNICA. Ronald L. Seaman†, Robert L. Moss and Martin J. Lynch*, Dept. of Physiology, University of Texas Health Science Center at Dallas 75235. †Present Address: Biomedical Research Group EME/STL, Engineering Experiment Station, Georgia Institute of Technology, Atlanta, Georgia 30332.

The abdominal ganglion of Aplysia californica was used to investigate the effects of peptides on the firing rates of invertebrate neurons. Abdominal ganglia from summer animals were studied in artificial seawater at 22 to 24°C. The peptides were applied at 1 μM in artificial seawater.

About one-half of the tested white-cell neurons (R3-R13) responded to luteinizing hormone-releasing hormone (LHRH, Ayerst). Responses to the bath applications of this peptide were increases in firing rate which lasted 25 to 80 minutes. The increased rate persisted if the bath was returned to normal seawater. In one instance, a second application of LHRH applied at 80 minutes after an initial application produced a second increase in firing rate. The absence of a change in firing rate by the remaining white-cell neurons suggests that, in terms of LHRH sensitivity, there may be two types of white cells.

The activity of other neurons in the abdominal ganglion changed in the presence of peptides. Application of LHRH altered the firing pattern of burster L2 but did not affect activity in bursters L10 and R15. The firing of a darkly pigmented neuron near R15 slowed and stopped in the presence of Substance P (Bachem). Activity did not resume for this cell when the bath was returned to normal seawater.

Some neurons in the abdominal ganglion of Aplysia californica are thus sensitive to the two peptide compounds tested. Interneurons may be involved in some of the responses, but since the white cells have no synaptic inputs, the effects on them are probably direct. The effects, when observed, lasted for several minutes and continued after the peptide in solution had been removed. Both LHRH and Substance P are present in mammalian nervous systems. Their actions on Aplysian neurons suggest these neurons as potential models for the membrane effects of these peptides. The sensitivity of invertebrate neurons to mammalian hormones may be more common than originally thought. (Supported by a Biomed. Res. Support Grant through UTHSCD to RLS, Program Project HD09988 to RLM, and NIH 4-S07-RR-05426-17.)

1822 PATHWAYS IN MOUSE AND RAT BRAIN CONTAINING VASOACTIVE INTESTINAL POLYPEPTIDE (VIP): AN IMMUNOCYTOCHEMICAL STUDY. Katherine B. Sims*, Donald L. Hoffman*, Earl A. Zimmerman, and Sami I. Said* (SPON: Virginia M. Tennyson), Dept. Neurol., College of P&S, Columbia Univ., NY 10032, and Dept. Med., Univ. Texas Health Science Center at Dallas, TX 75235.

Vasoactive intestinal polypeptide (VIP), a 28 amino acid residue peptide originally isolated from gut, is known to be present in brain by bioassay, radioimmunoassay and immunocytochemistry. By immunofluorescence it was previously demonstrated in cell bodies in cerebral cortex and amygdala and in nerve fibers innervating cerebral blood vessels and a number of nuclear regions including amygdala, accumbens, and suprachiasmatic hypothalamus and parabrachial brainstem. Although reactive perikarya were reported in arcuate region of mouse hypothalamus, they were not found in rat. We have studied both mouse and rat brains by immunoperoxidase technique using peroxidase-anti-peroxidase complexes (PAP) on 6 μm deparaffinized Bouins immersioned-fixed sections on glass slides and unmounted at 50 μm cryostat sections from animals perfused with paraformaldehyde-lysine-periodic acid and penetrated with Triton X-100. Preabsorption of anti-VIP with synthetic VIP abolished all reactivity while equimolar secretin, which shares structural homologies, had no effect. All previous immunocytochemical findings were confirmed except for the arcuate nucleus which was devoid of reactive cells. Instead, in both species, cell bodies containing VIP were found in the basal portions of the suprachiasmatic nucleus and their projections could be traced dorsally to the paraventricular, periventricular, and dorsomedial nuclei of the hypothalamus and on to the periventricular thalamus. In all other respects both species were identical as well. A much more extensive distribution of VIP was found than previously appreciated. Cell bodies were found to be scattered throughout the caudate, in central grey of the midbrain particularly ventral to the aqueduct and extending ventrally in the midline, in ventrolateral medulla in the nucleus of the tract of the trigeminal and extending dorsally and laterally into its substantia gelatinosa. A few cell bodies were also found in preoptic hypothalamus and bed nucleus of the stria terminalis. Many fibers were found in these and other regions including the entire length of the spinal cord. The most dense projections were found in bed nucleus, central nucleus of the amygdala, and nucleus of the solitary tract in the medulla. It is apparent that VIP, like substance P and enkephalin, is widely distributed in brain and spinal cord in multiple pathways, and like other peptides thus far studied does not appear to innervate cerebellum.

1823 IMMUNOREACTIVE THYROTROPIN RELEASING HORMONE (TRH) OUTISDE THE HYPOTHALAMUS REALLY IS TRH. Eliot Spindel* and Richard Wurtman (SPON: M. J. Fernstrom)

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Controversy exists concerning the identity of immunoreactive TRH in brain areas outside the hypothalamus. To establish its identity we analyzed regions of rat brain by thin layer chromatography (TLC), ion exchange chromatography, high pressure liquid chromatography (HPLC) and bioassay. By all methods of analysis, all TRH immunoreactivity corresponded exactly to that of synthetic TRH.

Tissue was extracted with either 2N acetic acid or 90% methanol; then subjected to one of three different HPLC separations or two different TLC separations. Ion exchange chromatography on SP sephadex was used to further purify acetic acid extracts prior to TLC or HPLC. For HPLC analysis, tissue extracts were lyophilized, resuspended in the HPLC solvent and injected into the HPLC. Timed fractions were collected, lyophilized, and reconstituted for TRH radioimmunoassay (RIA). Tritiated TRH or 3-methyl TRH was used for an interanl standard. The following three reverse phase HPLC systems were used at 30°C, 2 ml/min with a Waters microbondapak C18 column: 1)20% acetonitrile in 0.01 N NH4Ac, pH 4.0; 2) 0.5% acetonitrile in 0.01 N NH4Ac, pH 4.0; 3) 2.75% acetonitrile + 0.1% hexane sulfonic acid in 0.02 N acetic acid. The high acetonitrile content of system one would have eluted any large peptides which cross reacted with the TRH antibody. System three uses hexane sulfonic acid to form ion pairs with the imidazole proton of TRH and is extremely selective for small, positively charged peptides.

For TLC analysis, methanol extracts of brain tissue were spotted directly on the plates; acetic acid extracts were lyophilized (with or without preliminary ion exchange purification), resuspended in methanol and spotted. Two solvent systems were used: 1) methanol:methylene chloride (200:300) on Whatman K1 plates or 2) chloroform:methanol:NH4OH (60:25:5) on Whatman LK5D plates. After development, 0.5 cm sections of the plates were eluted directly into phosphosaline for TRH RIA. Tritiated TRH was used for an internal standard.

Analysis of extracts of hypothalamus, preoptic area, septum, brain stem, striatum, and pancreatic islets by the methods described above showed that all TRH immunoreactivity corresponded exactly and only to synthetic TRH. Thus despite the low molecular weight of TRH, the TRH RIA is highly specific.

1824 CORTICAL AND HIPPOCAMPAL SPREADING DEPRESSION INDUCED BY INJECTIONS OF MET- AND LEUENKEPHALIN AND BY D-ALA^2-METENKEPHALINAMIDE. U. Sprick*, K. Ornstein*, M.-S. Oitzl* and J.P. Huston (SPON: A. Borbély). Institute of Psychology, University of Düsseldorf, 4000 Düsseldorf, FRG.

Leuenkephalin, metenkephalin and d-ala^2-metenkephalinamide were microinjected into the hippocampus and neocortex of 250 rats to examine the effects on EEG and slow wave potentials. All substances were found to elicit spreading depression (SD) in both the neocortex and hippocampus. A dosage required to elicit spreading depression in at least 50% of the trials was determined by testing doses of 100μg/μl, 50μg/μl, 12.5μg/μl, 6.25μg/μl and 3.125μg/μl, always injected in 1μl of distilled water.

The following minimal doses were required for the induction of SD in at least 50% of the trials:

	Hippocampus	Neocortex
Leuenkephalin	12.5 μg	6.25 μg
Metenkephalin	100.0 μg	100.0 μg
D-Ala^2-Metenkephalinamide	12.5 μg	6.25 μg

Lower doses were required to induce hippocampal seizure activity in at least 50% of trials: leuenkephalin - 12.5 μg, metenkephalin - 50 μg, and d-ala^2-metenkephalinamide - 6.25 μg. Injection of naloxone prevented the elicitation of spreading depression.

The differences between the various enkephalins in terms of the effective dosages to induce spreading depression could be related to different receptor or acceptor densities.

It should be noted that some of the various reported behavioral effects of intracranial injections of the enkephalins could be artefacts of hippocampal and/or cortical spreading depression.

1825 DEMONSTRATION OF OPIATE BINDING IN AN INVERTEBRATE ORGANISM, M. EDULIS. George B. Stefano, Richard M. Kream*, R. Suzanne Zukin and Edward J. Catapane. Dept. Biochem. Albert Einstein Coll. of Med., Bronx, N.Y. 10461.

Opiate action has been investigated in the marine mollusc Mytilus edulis as a model invertebrate system. The opioid ligands, I-125-labelled-levallorphan (Lev) and FK 33-824 (a sulfoxide-carbinol derivative of methionine enkephalin with enhanced stability) bind stereospecifically and with high affinity (K_D's = 3.5 nM and 4.0 nM, respectively) to M. edulis pedal ganglia homogenates. Specific binding constitutes approximately 75% of total binding for both ligands at 4C for 1 nM Lev and FK 33 824. In addition, the ability of a series of enkephalin derivatives, including D-ala-met-enkephalin and FK 33 824, to alter dopamine levels in M. edulis was examined. All peptides examined produced an increase in dopamine levels of as much as 50%. D-ala and FK 33-824 increased dopamine levels at a much lower dose due to their resistance to proteolytic attack. Naloxone blocked the effects of the peptides on dopamine metabolism.

1826 EVIDENCE FOR ENDORPHIN MODULATION OF LOCUS COERULEUS

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

The locus coeruleus (LC) has been shown to contain a high density of opiate receptors. Systemically administered and ionotophoretically applied opiates exert a potent depressant action on coerulear cell firing rate. Anatomical and immunohistochemical studies have revealed the existence of β-endorphin containing axons emanating from cell bodies localized in or near the arcuate nucleus (Arc.N.) of the hypothalamus which project to various brain sites including LC. We have therefore investigated the effect of arcuate nucleus stimulation on LC activity.

Extracellular LC unit activity was recorded with metal microelectrodes in α-chloralose anesthetized cats. Brief conditioning trains (25-300 μA, 3 shocks, 100 Hz) delivered to the Arc.N. produced profound inhibition (average 1000 msec, N= 30) of spontaneous coerulear cell discharges. Intravenously administered naloxone (Nal) (2.5 - 5.0 mg/kg) reduced by 50% the period of arcuate-induced inhibition. In three instances Nal totally eliminated arcuate-elicited inhibition. Additionally, naloxone always increased spontaneous cell activity. In contrast to Nal systemically administered morphine (Mor) inhibited spontaneous LC unit activity in a dose related fashion and augmented the hypothalamic derived inhibition. Nal 5mg/kg reversed Mor activity to beyond pre-drug levels. To characterize the stereospecificity of the response, dextrorphan was administered. This inactive enantiomer failed to mimic morphine actions on LC cells. The results suggest that LC may be tonically influenced by the β-endorphin system originating from the arcuate nucleus.

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1827 INHIBITION OF EVOKED UNIT DISCHARGES IN THE PERIAQUEDUCTAL GRAY BY HYPOTHALAMIC STIMULATION

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Available anatomical and immunohistochemical evidence supports the concept of an endorphin containing neuronal system which has its origin in the hypothalamic arcuate nucleus (Arc.N.), and projects via long axons to various brain structures including the periaqueductal gray (PAG). That the PAG is an important structure with regard to opiate analgesia derives from studies demonstrating a high density of opiate receptors within this area and numerous investigations showing the PAG to be analgesically active when tested with microinjected opiates. Beta-endorphin is particularly active in this sense, inducing intense prolonged analgesia when applied to the PAG. We have therefore investigated the effect Arc.N. activation on PAG activity.

Single cell activity was recorded with stainless steel microelectrodes stereotaxically directed at the ventral-lateral aspects of the mesencephalic PAG in chloralose anesthetized, paralyzed, and artifically ventilated cats. Evoked discharges were incurred by strong electrical shocks (> 2.0 mA) applied to the sural nerve exposed in the popliteal fossa. Brief trains (100 Hz, 3 pulses, < 500 μA) of conditioning stimuli were applied to the Arc.N. ipsilateral to the recording side. Four units encountered displayed spontaneous activity. Arc.N. stimulation produced a period of total cessation of cell firing lasting 250 msec in one instance but failed to affect the 3 remaining units. Eighteen cells which discharged in response to sural shocks were tested with Arc.N. stimulation (C-T paradigm). Arc.N. suppressed sural evoked discharges by an average of 54% (range 3-100%). Intravenously administered naloxone (Nal) 2 to 5 mg/kg failed to markedly attenuate Arc.N. elicited inhibition and did not affect sural evoked activity. These results indicate that the Arc.N. is capable of inhibiting evoked PAG single unit activity, but unlike effects observed in locus coeruleus (this meeting) this inhibition is not changed by the narcotic antagonist Nal.

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1828 RADIOIMMUNOASSAY OF ENKEPHALIN: IMMUNOREACTIVE ENKEPHALIN LEVELS IN CAT SPINAL CORD. Tsung-ping Su*, Charles W. Gorodetzky and James A. Bell*. NIDA Addiction Research Center, Lexington, Ky. 40583

Antibody against methionine-enkephalin (Met-Enk) was produced in rabbits and used in radioimmunoassay (RIA). The antibody obtained gave a sensitive assay with 50% displacement of ^{3}H-Met-Enk at a concentration of 7 nM of unlabeled Met-Enk. The antibody was 1/35 as reactive to Leu-Enk and less than 1/1500 as reactive to β-endorphin as to Met-Enk.

In order to explore the involvement of opioid peptide in spinal neuronal function, the enkephalin RIA was used to determine the immunoreactive enkephalin content in cat spinal cord. The L_7-S_1 segments of the cat spinal cord were chosen for analysis of enkephalin levels, because intraspinal microinjection of Met-Enk into this area has been shown to depress the nociceptive C-fiber reflex recorded from the L_7 and S_1 ventral roots in the acute decerebrate spinal cat (Bell et al., Fed. Proc. 37, 763, 1978). The distribution of enkephalin in the dorsal and ventral sections of spinal cord was studied. The L_7-S_1 segments were dissected out as a unit, divided into dorsal and ventral sections at the level of the central canal, and processed for RIA. In agreement with an immunohistochemical report (Simantov et al., PNAS 74, 2167, 1977), our data indicate that the enkephalin content in the dorsal section of spinal cord is higher than that in the ventral section. The table below summarizes the levels of enkephalin found in dorsal and ventral sections of the spinal cord in three cats. Levels of enkephalin were expressed in terms of arbitrary enkephalin units (U-Enk; Rossier et al., PNAS, 74, 5162, 1977). One U-Enk = 1 f·mole Met-Enk, if sample contains no Leu-Enk.

Enkephalin Levels (U-Enk/mg wet tissue)

Cat #	1	2	3
Dorsal Section	412	515	442
Ventral Section	188	104.5	205.5

This study demonstrates a sensitive method for measuring spinal cord enkephalin levels and presents data which support a hypothesis that enkephalinergic neurons are present in the dorsal horn of the spinal cord of the cat.

1829 **Enkephalinase: Preliminary Characterization and Effect of Phosphorylation.** Sue Sullivan,* Joachim D. Raese,* Huda Akil,[1] Deborah Blacker* and Jack D. Barchas. Dept. Psychiatry, Sch. Med., Stanford Univ., Stanford, CA 94305; [1]Ment. Health Res. Ctr., Univ. Mich., Ann Arbor, MI 48109.

Metabolism of the enkephalins in brain tissue can occur via at least two different enzymatic routes. A soluble aminopeptidase can liberate the N-terminal tyrosine and a membrane-bound endopeptidase can cleave the gly-phe bond, thus liberating the tripeptide tyr-gly-gly (TGG) and phe-leu or phe-met. This endopeptidase (enkephalinase) has subcellular and regional brain distributions which correlate with the opiate receptor, whereas the amino peptidase does not. Enkephalinase is thought to be specific for the endogenous enkephalinergic system. It is a metalloenzyme which is completely inhibited by 1,10 phenanthroline (1mM) and partially inhibited by EDTA (1mM). EGTA has little effect on enzymatic activity indicating a lack of calcium dependence. The metal cofactor is apparently very strongly bound as extensive dialysis does not affect enzymatic activity. Enkephalinase is inhibited by various enzyme resistant analogues of the enkephalins and by the peptides tyr-gly, phe-leu and gly-gly-dlphe. Preliminary evidence suggests that enkephalinase is inhibited by phosphorylating conditions. Further experiments are in progress to elucidate the mechanism of this inhibition.

1830 EFFECTS OF NALOXONE ON COPULATION IN RATS AND THE ROLE OF ENDOGENOUS OPIATES IN A SPONTANEOUS REWARDING BEHAVIOR. Henry Szechtman*, Rabi Simantov* and Moshe Hershkowitz. Depts. Isotope Research and Genetics, The Weizmann Institute of Science, Rehovot, ISRAEL

Morphine has two well known behavioral effects: it induces analgesia and it induces euphoria. Endogenous opioid peptides, endorphins and enkephalins, compete with morphine at specific receptor sites in the brain and therefore should also possess these two behavioral effects. Indeed, it has been shown that injections of endorphins or enkephalins induce analgesia, and that rats self administer enkephalins indicating that the peptides have also rewarding effects. Yet in most behavioral paradigms designed to determine the physiological function of the endogenous opiates, there is no possibility for "analgesia" and "euphoria" (reward) to occur simultaneously. Both phenomena are part of copulation: there is profound analgesia before orgasm and hypersensitivity immediately afterwards; there are also many indications that coitus can be highly rewarding. Therefore we investigated whether mating behavior of male rats is altered by the specific opiate receptor antagonist, naloxone, and whether mating alters the content of opioid peptides in different brain regions and the pituitary. In 3 independent replications, naloxone (4 mg/kg, i.p., 5 minutes before test), altered significantly, only one parameter of copulatory performance: it extended the refractory period after an ejucalation (10-17% increase in the postejaculatory interval). This suggests that the blockade of opiate receptors may had extended the period of hypersensitivity following ejaculation. Measurement of opioid peptides (radioreceptor assay) indicated that there was a significant decrease in the content of opioid peptides in the midbrain but not in the hypothalamus, caudate or pituitary (compared to unmated controls). Thus opioid peptides may be released during sexual behavior producing simultaneously: a) a decrease in aversiveness of intense sexual stimulation, and b) an enhancement of its reward value.

1831 BOMBESIN DISRUPTS THERMOREGULATION IN RATS AT HIGH AND LOW ENVIRONMENTAL TEMPERATURES. Yvette Taché*, Quentin J. Pittman and Marvin Brown (SPON: W. Vale). Peptide Biology Laboratory, The Salk Institute, La Jolla, CA 92037.

Bombesin, a tetradecapeptide originally isolated from frog skin causes hypothermia when given intracisternally to rats at low ambient temperatures (T_A). The demonstration of bombesin-like immunoreactivity and bombesin-specific binding sites in rat brain raises the possibility that an endogenous bombesin-like peptide might play a role in thermoregulation in the rat. We have, therefore, undertaken the following studies to assess the action of bombesin on thermoregulation in unrestrained, unanesthetized rats.

Male Sprague-Dawley rats (250-300g) were implanted with 22 ga lateral ventricular cannulas. Following a minimum of 5 days recovery from surgery, rats were placed in individual cages in an environmental room where T_A could be precisely regulated. Rectal temperatures (T_R) were recorded at 30 min intervals from 60 min to a minimum of 90 min after injection. Bombesin, or an inactive analog were injected in a volume of 10µl of sterile, pyrogen-free artificial CSF made up fresh prior to injection.

The effects of intraventricular (ivt) bombesin (1ng-10µg) on T_R were tested at T_A's of 4°, 24°, 31°, 33° and 35°C. At all T_A's tested, control injections of the bombesin analogs [D-Trp^8]-bombesin or [D-Leu^{13}]-bombesin were without effect on T_R. At 4°C, bombesin caused a reduction in T_R with a return to normal T_R within 3.5 hr following the threshold dose of 50ng. Higher doses caused a more profound and longer lasting hypothermia with T_R decreasing by a maximum of 7°C at doses of 500ng-1µg. At T_A = 24°C, 1µg bombesin reduced T_R by 2°C, whereas administration of this amount at T_A's of 31 and 33°C was without effect on T_R. However, at T_A = 35.5°C, 1µg bombesin caused T_R to increase by 1.6°C (0.5-2.1°C, n = 11) with no further increase apparent at a dose of 10µg bombesin. The hyperthermia observed at high T_A could be reversed to a hypothermia by transferring the rats to a T_A of 4°C within 1 hr of drug administration.

These results confirm and extend previous findings by demonstrating a reversible hypothermic effect of bombesin administered ivt to the unanesthetized rat. Administration of bombesin to rats exposed to high T_A's revealed a hyperthermic action for this peptide. These findings demonstrate a disruptive effect of bombesin on thermoregulation at temperatures above and below thermoneutrality and suggest an inhibitory action on both heat production and heat loss pathways.

1832 EFFECT OF STRESS ON THE CONCENTRATION OF SOMATOSTATIN (SRIF) IN DISCRETE HYPOTHALAMIC AND EXTRAHYPOTHALAMIC REGIONS OF THE RAT. L. Cass Terry and William R. Crowley. Dept. Neurology & Pharmacology, Univ. Tenn. Center Health Sciences, Memphis, TN 38163.

Plasma growth hormone levels fall and remain low for several hours after stress in the rat. This effect is partially reversed by iv administration of antiserum to somatostatin. The present study was undertaken to determine the role of CNS SRIF in stress-induced suppression of growth hormone (rGH) secretion.

Adult male Sprague-Dawley rats kept on 14:10h light:dark cycle with food and water ad libitum were forced to swim for 30 minutes beginning at 1000h in a tank filled with water at 37.5°C. They were sacrificed immediately afterwards by decapitation. Their brains were snap-frozen and serum collected from trunk blood. Serum rGH was assayed using NIAMDD RIA kits. Somatostatin was determined in 10 microdissected, individual brain nuclei by a highly specific and sensitive radioimmunoassay.

Serum rGH was significantly ($p<.001$) lower in stressed animals compared to "nonstressed" controls (6.4± 1.0 vs 49.5± 12.5 ng/ml respectively). Swimming stress resulted in a significant reduction of SRIF in the median eminence*(ME)($p<.001$) and the medial portion of the caudate nucleus*(MCN)($p<.05$)(see table). There were no significant changes of SRIF in the periventricular n.(PVN), arcuate (ARC), ventromedial (VMN), medial preoptic (MPO), suprachiasmatic (SCN), accumbens (ACC), central amygdaloid (CAN) or interstitial stria terminalis (NIST) nuclear regions.

SRIF (pg/ug protein) + SEM

	ME	PVN	ARC	VMN	MPO
Stress	*362± 32.6	53.5± 5.5	56.9± 4.8	14.4± 1.6	10.1± 1.6
Control	679± 96.2	56.8± 2.8	67.5± 7.3	17.2± 1.5	11.9± 1.7

	SCN	NIST	MCN	ACC	CAN
Stress	20.5± 2.2	31.9± 3.1	*9.6± 0.5	22.4± 2.9	14.6± 1.6
Control	24.2± 2.6	31.8± 2.4	11.6± 0.9	24.8± 1.9	20.1± 3.2

Swimming stress resulted in a significant reduction of SRIF in two discrete brain regions, the median eminence and medial aspect of the caudate nucleus. These results suggest: (1) stress-induced suppression of growth hormone secretion in the rat is mediated by the release of somatostatin from nerve endings in the median eminence into the adenohypophyseal portal system to inhibit pituitary release and, (2) somatostatin-containing nerve fibers which innervate the caudate nucleus may influence extrapyramidal mechanisms associated with prolonged physical stress. (Supported by a New Investigator Grant from the Univ. Tenn.)

1833 IMMUNOHISTOCHEMICAL VISUALIZATION OF ENKEPHALIN AND CHOLECYSTOKININ FIBERS IN HIPPOCAMPUS FOLLOWING SELECTIVE DENERVATION. Donald R. Thorne*, Stephen W. Scheff, Dwight Hand*, Robert Elde and Carl W. Cotman. Dept. of Psychobiol., U.C., Irvine, CA 92717

Several immunohistochemical studies have reported the immunoreactivity of various peptides in hippocampal formation of the rat. Met-enkephalin (M-Enk) and cholecystokinin (CCK) are particularly prominent in the CA2 area of the hippocampus. It was of interest to determine whether these immunoreactive fibers were a) intrinsic to the hippocampus and b) would display a sprouting response similar to other neuronal afferents.

Young adult rats were subjected to a variety of surgical procedures which removed all or only part of the afferent pathways to the hippocampus. Unilateral lesions of the entorhinal cortex eliminated the major cortical inputs while an aspiration of the fimbria-fornix removed those inputs arising from the contralateral hippocampus and the septal nuclei. Additionally, some animals were given a transection of the dorsal psalterium, thus removing the crossed temporo-ammonic tract and an aspiration of the ventral, ipsilateral hippocampus was included. Still other animals were injected with 0.8 µg kainic acid unilaterally which totally destroyed regions CA3, CA4 and portions of CA1 pyramidal cell region. Antisera directed against either M-Enk or CCK were used for immunohistochemical peptide localization utilizing an immunoperoxidase staining technique on 10 µm thick frozen sections fixed with 4% paraformaldehyde in phosphate buffer.

Normal adult animals demonstrated positive staining for both M-Enk and CCK at all levels of the hippocampus with the staining restricted primarily to the CA2 pyramidal cell zone and with minimal staining observed in the subiculum. Adult, lesioned animals demonstrated staining patterns which were indistinguishable from sham-operated controls at the light microscopic level. In addition, no other area of the hippocampus was observed to contain positive staining for M-Enk or CCK following the surgical or chemical lesions. However, animals injected with kainic acid at 5 days of age and sacrificed as adults, demonstrated a marked deficit in positive staining for both CCK and M-Enk. Studies have also shown that immature rats (less than 30 days of age) demonstrate a striking difference in staining intensity from adult animals.

The present findings suggest that the immunoreactive fibers containing M-Enk and CCK are intrinsic to the hippocampus and are probably associated with small interneurons in the CA2 region. In addition, these peptide-containing fibers do not appear to sprout outside their normal terminal zones. (Grants MH19691)

1834 D-ALA^2-METHIONINE ENKEPHALINAMIDE AND MORPHINE ARE ANTICONVULSANT AFTER CENTRAL ADMINISTRATION IN RATS. F.C. Tortella, A. Cowan, and M.W. Adler, Dept. of Pharmacology, Temple University School of Medicine, Philadelphia, PA 19140.

Large subcutaneous doses of morphine and several other postulated µ receptor agonists raise the seizure threshold of rats to flurothyl, a volatile convulsant (Adler *et al*, JPET *198*:655, 1976; Cowan *et al*, Develop. in Neurosci. *4*:469, 1978). Yet, the central administration of enkephalins, β-endorphin, or morphine has been shown to produce an epileptiform EEG pattern that is not, however, associated with overt behavioral convulsions (Urca *et al*, Science *197*:83, 1977; Henriksen *et al*, Proc. Natl. Acad. Sci. *75*:5221, 1978; Tortella *et al*, JPET, in press). In our view, these findings may be simply another instance of a dissociation between EEG changes and behavioral events. In the present study, we administered morphine or D-ala^2-met enkephalinamide (D-ala) intracerebroventricularly (icv) to rats and now report that both compounds raise the seizure threshold in the flurothyl test.

Male, albino Sprague Dawley rats (initially 180-200 g) were implanted with the icv cannulae aimed at the right lateral ventricle 7 days prior to testing. Morphine (5 and 20 µg), D-ala (10, 40, and 80 µg), or sterile water was injected manually in a 5- or 10-µl volume over a 60-sec time period. Using dose-effect and time-course EEG data previously reported by Tortella *et al* (JPET, *206*:636, 1978), we monitored seizure threshold at the time of peak effect for both compounds: 20 min postinjection for D-ala (all doses) and 75 or 130 min postinjection for 5 and 20 µg morphine, respectively. With groups of 6-9 rats, both doses of morphine increased seizure threshold by 14% above the control range of 370-420 sec. A dose of 10 µg of D-ala produced a similar 14% rise in seizure threshold; 40 µg and 80 µg of D-ala raised seizure threshold above control by 49% and 67%, respectively. Time-effect studies may reveal even greater anticonvulsant effects. At the time of flurothyl challenge, both D-ala (40 and 80 µg) and morphine (20 µg) induced a *profound* state of behavioral stupor characterized by exophthalmos, immobility and generalized muscle rigidity. Wet-dog shakes occurred only after the administration of D-ala. Pretreatment with naloxone (0.1 and 1.0 mg/kg, s.c.), 15 min before testing, antagonized both the behavioral and anticonvulsant effects.

From these results, we conclude that both morphine and D-ala are anticonvulsant in the rat (when tested at the time of their peak drug effects on EEG and behavior) even though they produce an epileptiform EEG. D-ala is more effective than morphine in this respect.

This work was supported by Grant DA 00376 from NIDA.

1835 PEPTIDE HORMONE RELEASE OF STEREOTYPED MOTOR PROGRAMS IN AN INSECT: ROLE OF CYCLIC GMP. James W. Truman, Susanne M. Mumby*, and Susan K. Welch*. Dept. Zool., Univ. Washington, Seattle, WA 98195.

A peptide hormone, the eclosion hormone, triggers a 1.5 hour program of stereotyped behavior when injected into silkmoths. When the peptide is added to the isolated CNS of these animals, it evokes the corresponding program of motor activity (Truman, J.W., 1978, J. Exp. Biol. 74, 151-173).

A series of 3 methylxanthines were tested for their ability to evoke the behaviors in vivo. The relative potencies were isobutylmethylxanthine≫theophylline>caffeine; potencies which were similar to their ability to inhibit the breakdown of cyclic nucleotides in other systems. Also, protection of endogenous cyclic nucleotides by pretreatment of moths with low doses of phosphodiesterase inhibitors markedly enhanced the sensitivity of moths to injected peptide. Injection of cyclic nucleotides into moths caused the typical behavioral responses with cyclic GMP being more than 100 times more potent than cyclic AMP. Exogenous cyclic GMP also acted directly on the isolated CNS to evoke the characteristic motor programs.

Injection of the partially purified peptide into animals was followed by an increase in nervous system cyclic GMP but not cyclic AMP. The increase preceded the first behavioral response to the hormone. The use of various doses of hormone showed that the behavioral effectiveness of each dose was correlated with its ability to cause a cyclic GMP increase. It was concluded that the long term behavioral changes caused by the eclosion hormone are mediated through an increase in cyclic GMP in the nervous system of the moth.

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1836 EFFECT OF MORPHINE ON SUBSTANCE P NEURONS IN RAT SPINAL CORD AND BRAIN. Linda L. Vacca, Susan J. Abrahams* and N. Eric Naftchi*. Depts. of Path. & Anat., Med. Coll. GA, Augusta, GA 30912, Lab. Biochem. Pharmacol., IRM, NYU Med. Ctr., New York, NY 10016.

Adult male Sprague-Dawley rats were treated with morphine (3, 10, 40 mg/kg, s.c.) for 3 or 7 days. The effects on substance P-containing neurons were assessed by the Sternberger peroxidase anti-peroxidase (PAP) method and the double bridge PAP method of Vacca et al (J. Histochem. Cytochem. 26: 226, 1978) in the spinal cord and midbrain. Compared with saline and naloxone-treated controls, spinal cord from morphine-treated rats demonstrated increased amounts of immunoreactive substance P (SP) in three regions: substantia gelatinosa (including Rexed's lamina I), Rexed's lamina V, and the ventral horn. Certain midbrain regions, which included the interpeduncular nucleus and A10 area, also exhibited different amounts of immunoreactive SP. The immunoreactivity was found within presumed varicose processes cut in longitudinal and transverse section. The data suggest that morphine can affect the release of intraneuronal substance P. However, not all regions in the brain and spinal cord were affected in the same manner. SP-containing processes which surround the central canal and occur in the substantia nigra were not substantially influenced, and remained similar to those of controls. Such differential effects may reflect regional differences in the numbers and types of opiate receptors or in their pre- and post-synaptic locations. More detailed dose response and opiate binding studies are in progress. In addition, we are examining the effect of morphine on immunoreactive enkephalin-containing neurons. (Acknowledgements: We wish to thank Dr. Susan Leeman for providing us with rabbit anti-SP serum, and Dr. Margaret Kirby for her role in initiating the work at Med. Coll. GA. The work was supported by Med. Coll. GA Biomedical Research Grant 10-16-04-3611-23 and Edmond A. Guggenheim Research Endowment and DHEW-RSA Grant Nos. 16-P-56801/2 and 13-P-57975/2-01).

1837 IMMUNOCYTOCHEMICAL LOCALIZATION OF GASTRIN-CHOLECYSTOKININ-LIKE PEPTIDES IN THE BRAIN AND HYPOPHYSIS OF THE RAT. Jean-Jacques Vanderhaeghen * and Françoise Lotstra * (SPON: E. H. Cantor) Neuropathology Department, Brugmann University Hospital, Free University of Brussels, B-1020 Brussels, Belgium.

Peptidic material reacting with antigastrin 2-17 serum, but of lower weight than gastrin hexadecapeptide has been detected by radioimmunoassay in the central nervous system of various vertebrates (Nature 257 : 604-605, 1975). This material is also present in crude extracts of substance P (Naunyn-Schmiedeberg's Arch. Pharmacol. 305 : 189-190, 1978) and most of it consists in the sulphated COOH-terminal octapeptide of cholecystokinin (Proc. Natl. Acad. Sci. USA 75 : 524-528, 1978).

Using serum raised against sulphated COOH-terminal octapeptide of cholecystokinin, recognizing equally well gastrin heptadecapeptide or sulphated COOH-terminal octapeptide of cholecystokinin, the immunoenzyme histochemical localization of gastrin cholecystokinin-like peptides will be presented in the CNS and hypophysis of the rat, treated and untreated with colchicine.

Neuronal cell bodies and fibers are present in neocortex, hippocampus, amygdala, substantia nigra, nucleus supra opticus and nucleus paraventricularis, nucleus linearis, nucleus raphe dorsalis, nucleus parabrachialis and in A10 region. Fibers are present in thalamus, hypothalamus, in caudate putamen, in brain stem, in posterior horns of spinal cord and in neurohypophysis.

Controls with sera absorbed (solid phase absorption) with oxytocin, vasopressin, neurophysin I, neurophysin II, enkephalin and substance P are positive. Controls with sulphated COOH-terminal octapeptide of cholecystokinin or with gastrin heptadecapeptide are negative.

Studies will be presented using sera raised against enkephalin, substance P, somatostatine, oxytocin, vasopressin neurophysin I and neurophysin II in combination with sera against sulphated COOH-terminal octapeptide of cholecystokinin (double staining technique).

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1838 **MET- AND LEU-ENKEPHALINS AND MET-ENKEPHALIN-LIKE PEPTIDES IN THE ADRENAL MEDULLA: STUDIES ON STORAGE, SECRETION, AND SYNTHESIS.** **O.H. Viveros, E.J. Diliberto, Jr.*, E. Hazum*, and K.-J. Chang*. Dept. of Medicinal Biochemistry and Dept. of Molecular Biology, Wellcome Research Lab., Research Triangle Park, N.C. 27709**

Secretion of catecholamines from the adrenal medulla occurs by exocytosis. In this process, fusion of the chromaffin vesicle and plasma membranes is followed by extracellular release of the intravesicular contents. Vesicular proteins and peptides of unknown circulating extramedullary function are secreted along with catecholamines [Kirshner, et al., Mol. Pharmacol. 3: 254 (1967); Viveros, et al., Life Sci. 7: 609 (1968)]. Recently, in a continuing effort to explore the biological significance of these proteins and peptides, we have found enkephalin-like materials (ELM) in adrenomedullary chromaffin vesicles which are secreted by physiological stimuli. ELM, measured as met-enkephalin equivalents, were examined in acetic acid extracts of adrenals from nine mammalian species. The ELM content ranged from a low of 0.29 ± 0.03 nmol/g wet weight in the rat medulla to a high of 21.2 ± 1.2 nmol/g in the dog. These peptides were confined to the medulla of the adrenal gland. Storage in the chromaffin vesicles was indicated by a pattern identical to the catecholamines (CA) and dopamine-β-hydroxylase on differential and on continuous sucrose density gradient centrifugation. Separation of ELM on Sephadex G-50 and by HPLC indicated four peptide peaks of M_r below 2,000; two of these peaks correspond to met- and leu-enkephalin. The ELM are secreted from the perfused dog adrenal by the same stimuli that secrete CA and in the same proportion as contained within the gland. Furthermore, secretion is blocked by Ca^{2+} deprivation and by blockers of CA secretion. Increased splanchnic nerve discharge induced by insulin hypoglycemia depletes ELM and CA in parallel from the cat adrenal medulla. In guinea pigs and cats, low doses of reserpine deplete CA markedly with no immediate change in ELM levels. ELM are elevated three-fold by the second and third day after reserpine when CA are 20% below control. At 10 days, ELM remain elevated by two-fold when CA are still 40% below control. These results suggest that the CA content may be one of the factors regulating synthesis of ELM and that ELM storage in the vesicle precedes CA synthesis and storage. These findings strongly support a role for adrenomedullary enkephalins as neurohormones and suggest that enkephalins may exert important neuroendocrine functions outside the CNS.

1839 ENKEPHALIN IMMUNOREACTIVE SITES IN RAT FOREBRAIN: AN IMMUNOHISTOCHEMICAL STUDY. James K. Wamsley, W. Scott Young III and Michael J. Kuhar. Dept. Pharmacol. Expt. Ther., Johns Hopkins Univ., Sch. Med., Baltimore, Md. 21205.

Our laboratory has recently completed a detailed analysis of enkephalin immunoreactive areas in the rat forebrain. We employed untreated animals as well as animals pretreated (48 hrs) with colchicine (to interrupt axonal transport and thus cause immunoreactive cell bodies to become more apparent) administered by either cerebrointraventricular (50 µg) or peripheral subcutaneous (120-180 µg/gm body weight in two 24 hr periods) injections. The primary rabbit antisera used was directed against methionine-enkephalin, but is thought to also react with leucine-enkephalin containing neurons. The antigen-antibody complexes were visualized by using fluorescent and unlabeled (PAP) antibody techniques.

Our data agree well with the description of enkephalin containing areas reported by other laboratories. We have, however, identified many areas of enkephalin-like immunoreactivity which have not been previously reported. These areas include enkephalinergic cell perikarya located in the pars lateralis and medialis of the nucleus mamillaris medialis, nucleus ventralis corporis geniculati lateralis, nucleus parafascicularis, nucleus anterior and nucleus posterior hypothalami, nucleus periventricularis rotundocellularis and stellatocellularis, nucleus mamillaris prelateralis, nucleus tractus diagonalis (Broca), nucleus septalis fimbrialis, organum vasculosum lamina terminalis, subfornical organ, and the nucleus paratenialis, among others. Areas of immunoreactive fiber localization, not immediately associated with immunoreactive cell somata, were found in the subiculum of the hippocampal formation, cortex piriformis, cortex entorhinalis, ansa lenticularis, fasciculus mamillothalamicus, fasciculus mamillotegmentalis, dorsal and ventral commissura supraoptica dorsalis, the fibrae periventriculares thalami and hypothalami, and others.

None of these areas demonstrated the reaction product when either antisera preadsorbed with met-enkephalin or when normal rabbit sera was used. Many immunoreactive cell perikarya were found near non-reactive fiber pathways and many of the nuclei showing enkephalin immunoreactivity are known to be associated with various neuroendocrine functions. [Supported by USPHS grants DA00266, MH00053 (M.J.K.), MH07624 (W.S.Y.), and HD05739 (J.K.W.)].

1840 β-ENDORPHIN AND α-MSH: COMMON CELLS OF ORIGIN AND BINDING PROPERTIES. Stanley J. Watson and Huda Akil. Mental Health Res. Institute, Dept. Psych., Univ. Mich., Ann Arbor, MI 48109.

After the discovery of β-Lipotropin (β-LPH) and β-Endorphin (β-END) in mammalian pituitary, several studies indicated its common distribution with ACTH-like immunoreactivity (ACTH-LI) in corticotrophs and intermediate lobe cells. We and others reported the presence of β-LPH and β-END in rat brain. Since ACTH-LI was found along with these peptides in pituitary, brain was immunohistochemically studied for ACTH as well. We have shown that β-END, β-LPH and ACTH-LI were located in the same hypothalamic neurons. These data are seen as strongly supporting the notion of a common 31K precursor for ACTH-β-END proposed by Mains et al., and extending that hypothesis to include brain cells.

Although ACTH-LI was clearly demonstrated in brain, it was not clear whether ACTH-LI was actually ACTH or α-MSH, as both would react with the ACTH antisera used. We therefore have studied α-MSH-LI (antiserum a kind gift of Drs. Weber and Voigt, Ulm, Germany) and β-END-LI (antiserum a kind gift of Drs. R. Mains and B. Eipper, Univ. of Colorado) in serial 4µm sections of rat brain. Both antisera are blockable with 1µM authentic peptide. The β-END antibody is not blocked by α-MSH nor is the α-MSH antibody blocked by β-END. The β-END antibody is 100% cross-reactive with β-LPH, whereas the α-MSH antibody is not blocked by ACTH $_{1-24}$, $_{1-39}$ or $_{17-39}$. In all animals studied α-MSH was found in β-Endorphin cells. It is therefore concluded that the brain systems contain β-END/α-MSH immunoreactivity. Because this suggests that both peptides may be stored and released from the same neurons, we have investigated the possible existence of specific "receptors" for them. The binding properties of ACTH and its fragments and the interactions with β-Endorphin at the binding sites will be described. Parallels and differences between binding of ACTH/α-MSH and β-Endorphin will be discussed.

1841 IMMUNOCYTOCHEMICAL LOCALIZATION OF ANGIOTENSIN II IN THE CNS OF WISTAR KYOTO AND SPONTANEOUSLY HYPERTENSIVE RATS. James A. Weyhenmeyer* and M. Ian Phillips. Dept. of Physiology & Biophysics, University of Iowa College of Medicine, Iowa City, IA 52242.

Studies from several laboratories have suggested a physiological role for angiotensin II in drinking behavior and blood pressure response. In this communication we report localization of angiotensin II-like immunoreactivity, by the unlabelled antibody enzyme method, in specific structures or regions of brain. Since it has been shown that blood pressure in spontaneously hypertensive rats (SHR) can be lowered by central injections of angiotensin II antagonist, we have compared the presence of angiotensin II-like material in SHR and their normotensive controls from Wistar Kyoto (WKY) rats. Twelve young adult WKY and SH rats were anesthetized with diethyl ether and perfused via the left ventricle with saline followed by phosphate buffered picric acid-paraformaldehyde, pH 7.4. Vibratome sections, at 50μ and 100μ, were pretreated with triton X-100. The sections were incubated with a 1:1000 dilution of rabbit anti-angiotensin II, titered at 1:95,000 by RIA at 50% binding, for 24 hr at 24°C. The tissue was subsequently incubated with a 1:100 dilution of goat anti-rabbit IgG and a 1:200 dilution of rabbit peroxidase antiperoxidase. Staining was completely eliminated by the substitution of primary antiserum preabsorbed with angiotensin II. No staining was observed when pre-immune serum was substituted for the primary antiserum.

Angiotensin II-like immunoreactivity was demonstrated in both WKY and SH rats. Cell bodies were observed in supraoptic nucleus, hippocampus and cerebral cortex of both strains. Fiber tracts with darkly staining varicosities were found in the cortex, corpus callosum, septum, striae terminalis, amygdala, hippocampus, fimbria, medial preoptic area, hypothalamus, thalamus, striae medullaris, caudate, putamen, periaqueductal gray and reticular formation of both WKY and SH rats. These fibers, containing angiotensin II-like material, were found in isolation and not in bundles or tracts.

A significant increase in positively staining fibers in the SH rats compared with the WKY rats was demonstrated in the lamina terminalis surrounding the organum vasculosum. This finding, supported by receptor binding studies on angiotensin II previously reported from our laboratory, suggest that the organum vasculosum is an important site for the central regulation of blood pressure in the SH rats.

We are grateful to Detlev Ganten, University of Heidelberg, for supplying the angiotensin II antiserum. JAW is supported by a PMA Foundation Fellowship Award. Supported by NSF grant #BNS77-24415 and RSDA to MIP.

1842 INTRASEPTAL INJECTIONS OF PROLACTIN MODULATE DOPAMINERGIC REGULATION OF HIPPOCAMPAL ACh METABOLISM. Paul L. Wood, D.L. Cheney and E. Costa. Dept. of Pharmacol., Merck Frosst Labs., Dorval, Canada and Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hosp., Washington, D.C.

Prolactin has recently been reported to regulate the release of striatal dopamine (Perkins and Westfall, Neuroscience 3: 59, 1978). As a result of these observations, we have examined the actions of prolactin on the turnover rate of acetylcholine (TR_{ACh}) in several brain regions. The striatum and the hippocampus were chosen as areas in which dopaminergic terminals are known to regulate TR_{ACh} trans-synaptically while the frontal cortex and parietal cortex were examined as areas in which trans-synaptic dopaminergic regulation of TR_{ACh} does not appear to be operative. Prolactin administered intraventricularly (i.v.t.) reduced the TR_{ACh} in the striatum and hippocampus while the cortical areas were unaffected. Intraseptal injection of prolactin reproduced the action of the peptide injected i.v.t. on the TR_{ACh} in hippocampus but not in striatum. These data suggest that prolactin acts in the septum via changes in the activity of A_{10} neurons impinging on the cholinergic septal hippocampal cell bodies. In addition, the reduction in striatal and hippocampal TR_{ACh} induced by i.v.t. prolactin were nullified by 6-hydroxydopamine lesions of the A_9 and A_{10} dopaminergic cell bodies. In summary, our data suggest that prolactin decreases the TR_{ACh} in the hippocampus and striatum by augmenting the release of dopamine from dopaminergic terminals innervating these brain areas.

NEURO-PHARMACOLOGY

1843 EFFECT OF ACUTE ADMINISTRATION OF ACRYLAMIDE UPON DOPAMINE RECEPTORS IN THE RAT STRIATUM. A. K. Agrawal*, S. C. Bondy*, R. E. Squibb* (SPON: C. L. Mitchell). Lab. of Behav. and Neurol. Toxicol., Natl. Inst. of Environ. Hlth. Sci., Research Triangle Park NC 27709.

The binding of tritiated spiroperidol to membranes prepared from the striatal region of 6 week old male Sprague Dawley rats was studied. This region had considerably more specific binding activity than other region studied (cerebral cortex, hippocampus, cerebellum, hypothalamus, midbrain, pons-medulla). This interaction had high affinity characteristics. Binding equilibrium was reached within 10 min at 37° and the association was rapidly reversible. Stereospecificity of binding was demonstrated by use of d- and l-butaclamol. Scatchard plot analysis was performed using ^{3}H-spiroperidol. The dissociation constant (K_D) for this interaction in control rats was 0.56 x 10^{-9}M and the striatal binding density was 42 pmoles/100 mg protein. Twenty-four hours after treatment with acrylamide, administered orally by gavage, dopamine receptor binding was measured. Any binding of 10^{-9}M ^{3}H-spiroperidol in the presence of 10^{-6}M haloperidol was taken to be non-specific. There was a significant increase ($p<0.05$, analysis of variance followed by the Least Significant Difference test (two-tailed) in ^{3}H-spiroperidol binding at all acrylamide doses tested (50, 100, 200, and 250 mg/kg). There was clear dose dependent response up to 100 mg/kg. Since the highest dose of the neurotoxic agent caused pronounced mortality (62%), further kinetic analysis was carried out using an intermediate dose level (100 mg/kg). The dissociation constant for spiroperidol binding to striatal membranes of these animals was 0.40 x 10^{-9}M and the binding site density was 48 pmoles/100 mg protein. These data indicated that the effect of the acrylamide was largely to increase (40%) the affinity of binding of these receptors toward the labeled ligand while the number of receptors available for binding was not altered in such a pronounced manner (14%). Dopamine levels in striata of untreated and dosed animals were determined fluorimetrically. There was no significant change in dopamine concentration in animals dosed at 50, 100, and 200 mg/kg relative to control values.

1844 EFFECT OF PENTYLENETETRAZOL ON MAMMALIAN NEUROMUSCULAR JUNCTION: POSTSYNAPTICALLY MEDIATED DEPRESSION. B. J. Albala, C. G. Carlson* and C. G. Muniak*. Div. Biopsychology, Syracuse University and Dept. Physiology, Upstate Medical Center, Syracuse, N.Y. 13210

The synthetic analeptic drug pentylenetetrazol (PTZ) is a convulsant agent when administered peripherally in sufficient doses. The mechanism of action by which PTZ induces epileptic-like neural and motor activity has not been adequately elucidated. Research conducted by Eccles and his colleagues (*J. Physiol. 168:* 500, 1963) demonstrated that PTZ did not significantly affect either presynaptic or postsynaptic inhibition in spinal neurons. The present experiments will attempt to determine whether PTZ exerts any effect at the mammalian neuromuscular junction.

In vitro intracellular recordings from the diaphragm of young mice (17-23 days of age) were conducted utilizing 3 M KCl glass microelectrodes. PTZ was added to normal mouse Ringer's solution to achieve a final concentration of 25-100 mM. The addition of PTZ significantly lowered the amplitude (25-75 %) of the spontaneous miniature endplate potential (MEPP) and produced a slight increase in the frequency of MEPP discharge. Following the application of PTZ in higher concentrations, muscular contractions induced by low frequency phrenic nerve stimulation were abolished. During subsequent recording, small endplate potentials (EPP) were observed at a constant latency following each stimulating pulse to the phrenic nerve (i.e. no failures of transmission were observed). In similiar experiments in which cobalt was used to partially block the release of neurotransmitter, PTZ significantly lowered the amplitude of the average EPP, with a concurrent reduction of the unitary evoked potential. These results suggest that PTZ may be exerting a depressant neuromuscular action via some postsynaptic mechanism. Consistent with this interpretation, initial experiments indicate that PTZ reduces the endplate response to a constant pulse of iontophoretically applied acetylcholine. Throughout the course of these experiments the administration of PTZ did not significantly alter the resting potential. Furthermore, the PTZ-induced amplitude depression was reversed following the substitution of fresh Ringer's solution.

In summary, the results of these experiments indicate that PTZ has a postsynaptic depressive effect at the mammalian neuromuscular junction. The specific cellular mechanism by which PTZ exerts its depression at this nicotinic synapse is not presently clear.

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1845 GUANINE NUCLEOTIDES AND MAGNESIUM ION DO NOT ALTER AGONIST OR ANTAGONIST COMPETITION FOR RAT STRIATAL ^{3}H-SPIROPERIDOL BINDING SITES. A.C. Andorn*, S.Y. Cech*, S. Johnson* and M.E. Maguire (SPON: S.G. Younkin), Depts. of Pharmacology and Psychiatry, Case Western Reserve Univ., Sch. of Med., Cleveland, OH 44106.

Guanine nucleotides regulate β-adrenergic receptors (β-ARs) and other receptor types. In β-AR systems, this type of regulation appears to reflect the degree of receptor-adenylate cyclase coupling. We have recently shown (Bird and Maguire, J. Biol. Chem., 253: 8826, 1978) that Mg^{++} alters agonist affinity for β-AR in a manner opposite that of guanine nucleotides. We can now show that the guanine nucleotide effect on agonist affinity for β-ARs is dependent upon a pre-existing magnesium effect. In the absence of Mg^{++}, guanine nucleotides have no effect on ligand binding to β-ARs. We therefore examined the ability of Mg^{++} and guanine nucleotides to alter ligand binding to ^{3}H-spiroperidol (^{3}HSP) binding sites in rat striatum as such sites are proposed to be dopaminergic receptors. We report here that Mg^{++} and other ions have no effect on agonist or antagonist competition for either of the two ^{3}HSP binding sites routinely observed in rat striatum (Andorn and Maguire, Soc. for Neurosci. 1978, IV, 509). GTP, $GppCH_2p$ and ATP at concentrations from 1 μM to 10 mM have no effect on agonist or antagonist competition for the specific ^{3}HSP binding sites or on specific ^{3}HSP binding itself. This lack of effect is observed in the presence or absence of Mg^{++} (or sodium). There is a small effect of guanine nucleotide on nonspecific (and therefore total) binding of ^{3}HSP at relatively high nucleotide concentrations (0.1 - 1.0 mM). However, this does not result in any change of specific binding whether defined by the use of either agonist or antagonist ligands. There are two possible conclusions from these binding data: (1) neither ^{3}HSP binding site in rat striatum is coupled to adenylate cyclase, or (2) one or both of these sites may be poorly coupled to adenylate cyclase. This situation would be analogous to that observed in certain β-AR-cyclase systems (Maguire et al., Adv. Cyc. Nucl. Res. 8: 1, 1977. We obviously favor the latter conclusion in view of abundant biochemical evidence for the existence of a dopamine-sensitive adenylate cyclase. Since substantial data indicate that the general structure of the receptor-adenylate cyclase complex is invariant from system to system, we further suggest that detailed examination of the coupling mechanism per se will help elucidate the role of dopamine-sensitive adenylate cyclase in the striatum. (NSF PCM 77-24693 and NIH GRS RR05140A2)

1846 EFFECT OF CHRONIC ADMINISTRATION OF MONOACYLCADAVERINES ON SLEEP-WAKING BEHAVIOR IN MICE. Helen A. Baghdoyan* and Matej Stepita-Klauco. Dept. Biobehavioral Sciences, U. Conn., Storrs, Ct. 06268.

Monoacylcadaverines have been suggested recently as possible biological markers for schizophrenia (1). They were demonstrated to be formed from cadaverine in the rat brain (2). Endogenous cadaverine has been shown to be present in mammalian brain and blood (3), and is elevated in both tissues during behavioral sleep (4). The following study is being conducted in an attempt to assess the effects of chronic administration of these compounds on sleep-waking behavior.

Adult, C57BL/6 male mice were implanted with gold wire electrodes for recording the EEG and a modified EMG. Animals were recorded continuously for 6 days under each of the following conditions: baseline, control (administration of saline) and drug administration. A recovery period lasting 6-12 days was also obtained. Drugs in saline were administered via chronically implanted osmotic minipumps. Three behavioral states, waking, slow wave sleep (SWS) and desynchronized sleep were recognized.

The average duration of SWS was significantly decreased below both baseline and control durations ($p<.01$) in animals receiving monoacetylcadaverine at a dose of 100 ng/hr. This decrease in the mean duration of a SWS episode was observed during the lights-on period only, whereas night durations remained unchanged. The total amount of SWS was slightly but not significantly decreased. There was no change in the number of SWS episodes. Similar results were seen in animals receiving monoacetylcadaverine at a dose of 100 ug/hr, the major difference being that with the higher dose the effect was seen sooner after the onset of drug administration. Studies of the effects of cadaverine and monopropionylcadaverine on sleep-waking behavior are now in progress.

Supported by USPHS grant NS12482 and NSF grant BNS77-15323. 1)Dolezalova and Stepita-Klauco, J.Chromatog., 146(1978)67. 2) Salzman and Stepita-Klauco, Trans.Am.Soc.Neurochem., Abstract No.182, 1979. 3)Stepita-Klauco and Dolezalova,Nature 252(1974)158. 4)Dolezalova et al., Brain Res.,77(1974)166.

1847 ACTIONS OF IONTOPHORETICALLY APPLIED MORPHINE ON RAT HYPOTHALAMIC TEMPERATURE-SENSITIVE NEURONS. Frank Baldino, Jr.*, Alexander L. Beckman, and Martin W. Adler. Dept. Pharmacology, Temple Univ. Sch. Med., Philadelphia, PA 19140, and A.I. duPont Institute, Wilmington, DE 19899.

The acute administration of morphine in the rat produces a biphasic effect on body temperature (Tb). Morphine appears to exert this influence by acting in the preoptic/anterial hypothalamus (POA). The purpose of this study was to analyze the response of temperature-sensitive neurons in the POA to iontophoretic application of morphine (M) and naloxone (NX).

These experiments were conducted in male Sprague-Dawley rats (350g) that were anesthetized with Urethan^{R} (1g/kg i.p.) and placed in a stereotaxic instrument. Two bilateral water perfused thermodes, implanted rostral to the POA, were used during the experiments to vary hypothalamic temperature (Th) over a range of 10° C. Th was sensed by a bead thermistor inserted into an implanted stainless-steel re-entrant tube positioned to the left of midline, symmetrical with the POA recording site. Recording of single cell discharges and iontophoretic application of test compounds were made with 5-barrel glass micropipettes (tip diameter, 5 μ).

Sixty one cells were analyzed for their response to M and NX. Of the 60 cells, 34 were unresponsive to changes in Th within the range of 32-42°C and were classified as temperature-insensitive cells. Twenty cells responded with an increased firing rate (FR) to an elevation in Th and were therefore classified as warm-sensitive cells. Seven cells responded with a decrease in FR to increases in Th and were therefore classified as cold-sensitive cells.

M (50-100 nA) increased the FR of 12 warm-sensitive cells, the other 8 were unaffected. None were inhibited. The FR of 6 cold-sensitive cells was decreased after M application. One cell was unaffected and none were excited. The temperature-insensitive cells responded variably to M application. Four cells were excited, 19 inhibited, and 11 remained unaffected.

NX (≤15 nA) antagonized morphine's excitatory effect in 4 of 8 warm-sensitive cells. Low currents of NX (≤15 nA) had no direct effect, while higher currents produced inhibition. NX antagonized the inhibitory response of M on 1 of 2 cold-sensitive cells while having a direct inhibitory action on the other.

Our results demonstrate that M excited warm-sensitive cells which are assumed to mediate heat-dissipation responses, and inhibited cold-sensitivie cells, which are assumed to mediate heat-gain responses. These actions parallel morphine's hypothermic action in the intact animal, and therefore suggest that M lowers Tb by exerting a coherent action on POA warm- and cold-sensitive neurons. Since these effects were antagonized by naloxone, the action of morphine on warm- and cold-sensitivie cells seems to be mediated by an opiate receptor.

1848 SIMILAR MOTOR EFFECTS OF 5HT AND TRH IN CHRONIC SPINAL RATS AND MONKEYS. Hugues Barbeau* and Paul Bédard*, (SPON: Louis Larochelle), Dept. Anatomie, Laboratoires de neurobiologie, Université Laval, Pav. Notre-Dame, 2075 ave de Vitré, Québec, Qué. G1J 5B3.

We have previously established (Bédard, P., Barbeau, H. et al., Brain Research, Vol. 169-2, June 1979) that 5 days after transsection of the spinal cord in rats, DL-5hydroxytryptophan, 100 mg/kg i.p. induces a powerful muscle contraction in extensor muscles of the tigh. This effect of 5-HTP progressively increases until the twentieth day after transsection. It is possible to quantify the response to 5-HTP and various agonists of serotonin by measuring the integrated EMG of extensor muscles of the tigh. This effect is not mimicked by drugs acting on other systems (Noradrenaline, Dopamine, Acetylcholine and GABA).

Thyrotropin-releasing hormone (TRH) is a tripeptide which has, besides it's hypophysiotropic action, been shown to be an active substance in the central nervous system of several species. The mechanisms of this action however remains unclear.TRH given at a dose of 5 to 10 mg/kg i.p. to rats spinalised at least 15 days previously elicits a strong motor response in both extensor and flexor muscles which is identical to that produced by 5-HTP and 5HT agonists. The effect of TRH last between 10 to 60 minutes and can be markedly inhibited by cyproheptadine 10 mg/kg. Glutamic acid 10 mg/kg i.p., one of three amino-acids which constitute TRH, did not reproduce the effect of TRH. A similar effect was seen in a monkey rendered paraplegic one month previously.

This finding suggest that TRH directly stimulates 5HT receptors located on neurons situated in the lumbar spinal cord in both rodents and primates.

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1849 BEHAVIORAL SUPERSENSITIVITY TO APOMORPHINE WITHOUT REGIONAL CHANGES IN cAMP IN RAT BRAIN. Vernice E. Bates, Robert H. Lenox, G. Jean Kant, and James L. Meyerhoff. Dept. of Medical Neurosciences, Walter Reed Army Inst. of Research, Washington, DC, and Dept. of Psychiatry, University of Vermont, Burlington, VT.

Stereotypy has been related to stimulation of striatal dopamine (DA) receptors. Supersensitivity of the striatal DA receptor has been inferred from an increased stereotypic response to DA agonists, in: 1) a hyperthyroid model in guinea pigs; 2) guinea pigs and rats chronically given amphetamine; and 3) rats chronically given DA antagonists such as haloperidol.

Addition of DA or DA agonists to striatal homogenates results in an increase in cAMP. In models of DA receptor supersensitivity, an increased cAMP response to DA in striatal homogenates has been reported. However, in other studies, no increase in cAMP response was found. We were interested in determining whether the above models of supersensitivity would produce an increased in vivo response of striatal cAMP to apomorphine (APO).

Three models of supersensitivity were studied in the rat. In the first model, animals were administered haloperidol daily for 28 consecutive days; 2.5 mg/Kg initially for 7 days with the dosage increased by 2.5 mg/Kg every 7 days to a final dose of 10 mg/Kg. Seventy-two hours after the last dose, the rats were challenged with APO (0.3 and 10 mg/Kg) and scored for stereotypy by blind raters at 1.5, 3 and 5 min after injection. The rats were sacrificed 7 min after APO challenge by a high power microwave inactivation system developed in our laboratory (Lenox, et. al., J. Cyclic Nuc. Res. 3:367-379, 1977). cAMP and cGMP were measured in corpus striatum, nucleus accumbens, olfactory tubercle, substantia nigra and cerebellum by radioimmunoassay.

In the second model amphetamine 5 mg/Kg was given daily for 14 consecutive days followed by 10 mg/Kg for 7 days. Seventy-two hours after the last dose, APO challenge, stereotypy recording, sacrifice and assay were performed as above. In the third model, thyroxine 250 μg/Kg daily was administered for 28 days. Seventy-two hours after the last dose, the animals were challenged with APO 0.3 mg/Kg. Stereotypy scores, sacrifice and assay were performed as above.

An increased stereotypic response to APO challenge was seen only in the chronic haloperidol model ($p < .05$). Neither cAMP response nor cGMP response to APO was increased following any of the chronic treatments. Significant increases in cGMP were observed in several regions following apomorphine 10 mg/Kg possibly due to increases in motor activity (Meyerhoff et al., Life Sci. 24: 1125-1130, 1979). We conclude that behavioral supersensitivity to APO can occur without demonstrable changes in the in vivo response of cAMP.

1850 EFFECT OF GUANINE NUCLEOTIDES ON AGONIST BINDING TO ANTERIOR PITUITARY DOPAMINE RECEPTORS. Judith E. Beach,*Michael J. Cronin and Richard I. Weiner. Dept. Ob-Gyn. and Reprod. Sci., Univ. of Calif. Med. Sch., San Francisco, Ca. 94143.

Postsynaptic dopamine (DA) receptors in the caudate nucleus appear to be coupled to adenylate cyclase. The binding affinities of agonists, but not antagonists, for these receptors is decreased by the presence of GTP and other guanine nucleotides, as is generally observed for cyclase-linked receptors. We have extended these studies to DA receptors in the anterior pituitary where there is little evidence for a DA-sensitive cyclase. Membrane fractions of the steer anterior pituitary were prepared as previously described (Endocrinology 104:307, 1979). Membranes were incubated with approximately 1 nM of ^{3}H-spiperone (^{3}H-SPIP), a DA antagonist, in a Tris buffer (pH 7.4) at 37 C for 15 min. Bound ^{3}H-SPIP was separated from free at equilibrium by rapid filtration and the trapped radioactivity counted. The ability of various conc. of DA agonists and antagonists to compete for the ^{3}H-SPIP binding was tested in the presence and absence of 0.3 mM GTP, GDP, GMP, Gpp(NH)p, or ATP.

	Ki (nM)		Magnitude
AGENT	CONTROL	+0.3mM GTP	Shift
Agonists:			
DA	703	2162	3
ADTN	32	102	3
apomorphine (APO)	25	70	3
bromergocryptine (CB-154)	16.5	20	1.2
Antagonists:			
spiperone	2.7	2.6	1
d-butaclamol (d-BUTAC)	2.7	3.1	1

GTP caused a significant($p<0.01$)3-fold increase in the inhibition constants (K_i's) for DA agonists with the exception of CB-154. GDP and Gpp(NH)p caused similar effects on agonist binding; however, GMP and ATP had no effect. No change was observed with any of the guanine nucleotides on the affinity of antagonist binding. Hill coefficients of unity were calculated for SPIP,d-BUTAC and CB-154. However, Hill coefficients of approximately 0.5, which were not altered by GTP, were measured for DA, APO and ADTN competitions, indicating either negative cooperativity or multiple binding sites. All of the agonists used in this study including CB-154 are potent inhibitors of prolactin secretion from the anterior pituitary. An interpretation is that ^{3}H-SPIP binds to multiple sites in the anterior pituitary. One of these sites is sensitive to regulation by guanine nucleotides.

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1851 COMPARISON OF MORPHINE ABSTINENCE SYNDROME IN AWAKE AND HIBERNATING GROUND SQUIRRELS. Alexander L. Beckman, Toni L. Stanton, Carmen Eckman*, Martin W. Adler, and Sheryl Glassman*
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The hibernating mammal offers a unique opportunity to expand our understanding of narcotic actions in the CNS because the natural range of brain activity from deep hibernation to the awake state is exceedingly wide. The purpose of these experiments was to describe the morphine abstinence syndrome in the euthermic (i.e., not hibernating) ground squirrel, in which the level of brain activity is comparable to that of other mammals, and during hibernation, when the brain is in a profound state of natural depression.

Euthermic, drug-naive male and female California golden-mantled ground squirrels (Citellus lateralis) were subcutaneously implanted under light ether anesthesia in the interscapular region with two 75 mg morphine pellets during the spring, summer, fall, and winter seasons. Hibernating, drug-naive animals received the same administration of morphine. In all animals, morphine abstinence was either precipitated 72 hours after pellet implantation with naloxone (1 mg/kg, s.c.) or by abrupt withdrawal. The morphine abstinence syndrome was characterized by quantifying the occurrence of signs displayed in the 30-min. period following naloxone injection or 24-hr following removal of the morphine pellets.

Euthermic ground squirrels displayed a pronounced and characteristic morphine abstinence syndrome consisting of exploratory behavior, nesting, grooming, vocalization, shakes, yawning, chewing, chromodacryorrhea, digging, dyspnea, eyetwitch, flattened posture, ptosis, and a forward-extended tail. In addition, there were seasonal and ambient temperature dependent changes in the occurrence of some signs of the naloxone-precipitated abstinence syndrome. Abrupt withdrawal produced only a very mild abstinence syndrome, with few or no signs. Control animals received only naloxone and displayed no abstinence signs.

Experiments on hibernating animals produced strikingly different results. Animals exposed to morphine and tested with naloxone during hibernation displayed no abstinence signs. Similar results were obtained from animals exposed to morphine during hibernation which were deliberately aroused to the euthermic state and immediately tested with naloxone. These results suggest that the state of hibernation alters the development of physical dependence on morphine in some, as yet unknown, manner. (Supported by NIDA Grant DA 02254 and the A.I. duPont Institute).

1852 REPEATED ELECTROCONVULSIVE SHOCK (ECS) AND MORPHINE TOLERANCE: DEMONSTRATION OF CROSS-SENSITIVITY IN THE RAT. Gregory Lucas Belenky and John W. Holaday. Dept. of Medical Neurosciences, Walter Reed Army Institute of Research, Washington, DC.

In a complementary set of studies, we have found repeated ECS sensitizes opiate-naive rats to the acute effects of a single morphine injection, and induction of morphine tolerance sensitizes ECS-naive rats to the acute effects of a single ECS.

In the first experiment, opiate-naive rats were given transauricular ECS (160V, 60Hz, 2 sec) daily for nine days. Twenty-four hours after their last ECS they were tested for sensitivity to morphine using single subcutaneous doses of 4 or 15 mg/kg of morphine sulfate. Following morphine, the repeated-ECS groups were more cataleptic ($F = 5.26$; df 1,21; $p = .032$), more analgetic [F(tail flick latency) = 4.73; df 1,21; $p = .041$] and hypothermic ($F = 8.19$; df 1,21; $p = .009$) when compared to sham-ECS control groups.

In the second experiment, ECS-naive rats were made tolerant to morphine by the implantation of morphine sulfate pellets (75 mg) 72 hours and again 24 hours prior to testing. On the day of testing the morphine-pellet rats were tolerant to morphine as indicated by the lack of significant differences in tail flick latencies, respiratory rates, and body temperatures when compared to a placebo-pellet implanted control group. Following these pre-measures, both groups of rats were given a single trans-auricular ECS (160V, 60Hz, 2 sec). Post-ECS the morphine tolerant rats were more cataleptic ($t = 2.55$, df = 7, $p = .038$), more analgetic [t(tail flick latency) = 3.50, df = 7, $p = .011$], respiring less ($t = 6.41$, df = 7, $p = .0004$) and hyperthermic ($t = 2.86$, df = 7, $p = .024$) when compared to the placebo-pellet implanted control group.

These experiments demonstrate, on the one hand, repeated ECS sensitizes to the effects of morphine, and, on the other hand, morphine tolerance potentiates the effects of ECS. This cross sensitivity suggests the physiological changes following repeated ECS and the induction of morphine tolerance share common neurobiological mechanisms.

1853 THE DIFFERENTIAL EFFECTS OF DORSAL HORN OR VENTRAL HORN INTRASPINAL MICROINJECTIONS OF NOREPINEPHRINE (NE) AND 5-HYDROXYTRYPTAMINE (5HT) ON NOCICEPTIVE C-FIBER REFLEXES (CFR) IN THE ACUTE DECEREBRATE SPINAL CAT. J. A. Bell* and T. Matsumiya* (Spon: W. R. Martin), NIDA Addiction Research Center, Lexington, KY. 40583

CFR's recorded from an L_7 ventral root were evoked by stimulation of the ipsilateral superficial peroneal nerve with a stimulus intensity (15 v 2 msec duration) sufficient to activate C-Fibers. All microinjections (vol 0.5 µl) were performed via glass micropipettes (tip diameter 50 µm) placed either 1 mm (dorsal horn) or 3 mm (ventral horn) below the surface of the spinal cord in the L_7 segment. Control dorsal horn or ventral horn microinjections of NaCl (2 M, pH 3) had no effect on the CFR. Dorsal horn microinjections of 5HT rapidly depressed the CFR to 59% of control at 15 min. Ventral horn microinjections of 5HT facilitated the CFR to 280% of control at 15 min. Dorsal horn microinjections of NE depressed the CFR to 50% of the control at 15 min, whereas ventral horn microinjections of NE facilitated the CFR to 374% of control at 15 min. Pretreatment of the preparation with intravenous administration of naltrexone (0.5 mg/kg) antagonized the depressant effects of the dorsal horn microinjections of NE (101% of control at 15 min), whereas the depressant effects of the dorsal horn microinjections of 5HT were not antagonized by naltrexone (62% of control at 15 min). These data support a hypothesis that NE acts in the dorsal horn of the spinal cord to depress the segmental nociceptive CFR by activating an intermediary system which is sensitive to an opiate antagonist (naltrexone) blockade, whereas 5HT may act directly in the dorsal horn to depress the CFR.

1854 LITHIUM(LI)AND TRYPTOPHAN(TP)SYNERGISM IN CENTRAL SEROTONERGIC PATHWAYS IN THE MURICIDAL RAT, Sr. P. Broderick, I. Sanghvi*, V. deP. Lynch* and P. Cervoni*+. St John's Univ., Coll. of Pharm., Jamaica, NY, 11439 and USV Pharm. Corp., Tuckahoe, NY 10707 (+Lederle Lab, Pearl River, NY 10965).

Therapeutically, the use of Li or TP as thymoleptic remains controversial. The effects of Li on TP on central serotonergic pathways were studied in rat muricidal(mouse killing)behavior(MB), a model pragmatically employed to screen antidepressant drugs.

We have previously shown(Pharmacol., 1978)Muricidal Behaviour Antagonism(MBA)by Li and TP, both acutely and chronically, in isolated, male Long Evans rats, with no significant neurotoxic impairment on rotarod performance. Li and TP acted synergistically on MBA; the synergism was also reflected in Li and TP plasma levels.

In present study, forebrain and hindbrain steady state 5-HT and 5-HIAA levels were determined in these muricidal aberrant rats. The steady state levels of 5-HT and 5-HIAA were 0.12 ug/gm and 0.28 ug/gm respectively. Both acute and 7 day treatment with Li, 0.5 meq/kg, i.p. and TP, 100 mg/kg, i.p. alone and in combination, significantly increased($p<0.001$)steady state 5-HT and 5-HIAA levels. 5-HT turnover studies revealed a 5-HT turnover rate of 0.26-0.29 ug/gm/hr, and a turn over time of 204-208 min. for muricidal controls. Li and TP combination significantly($p<0.001$)restored neuronal activity to a more usual range,(0.64-0.72 ug/gm/hr and 84-96 min. respectively). In order of activity, the effects were Li+TP>TP>Li. Hindbrain 5-HT turnover was significantly higher($p<0.001$)over forebrain 5-HT turnover in the chronic Li+TP combination group. However, within the forebrain a significant difference ($p<0.01$)in 5-HT steady state values was found between right and left hemispheres in 83% of the animals following treatment with Li, 0.5 meq/kg/day, i.p. for 7 days.

Our data show that Li acts, at least, in part, through serotonergic pathway and the MB can be modified by serotonergic influences. Results also show a decreased plasma TP level, consistent with decreased 5-HT 5-HIAA and 5-HT turnover in muricidal rats, and suggest that Li and TP act synergistically on 5-HT pathways, possibly via increased TP uptake into brain. This synergism has important therapeutic implications.

1855 EFFECTS OF CHRONIC OPIATE RECEPTOR ANTAGONISM BY NALTREXONE ON ASPECTS OF MATERNAL BEHAVIOR IN MICE. David Brown* and George R. Peterson. Pharmacol. Dept., Wright St. U. Sch. Med., Dayton, OH 45435.

Panksepp and collaborators (Pharmacol. Biochem. Behav. 9: 213, 1978; Neurosci. Abs. 3:303, 1977) reported that the narcotic antagonist naloxone disrupted certain mother-infant interactions in various species, thus suggesting that endogenous opiate-like substances may play a role in the mediation of intraspecies social bonding. By use of a long-term delivery system consisting of 1.5 mm beads containing 2 mg of naltrexone base in a 90/10 polylactic/glycolylic acid copolymer (Dynatech R/D Comp., Cambridge, MA; beads supplied by NIDA), the consequences of chronic blockade of opiate receptors on maternal behavior could be explored.

As soon as feasible after delivery, mothers were implanted subcutaneously with either two naltrexone beads, two sham beads or were left untreated. The naltrexone was 100% effective as judged by its ability to block a > ED_{99} dose of morphine sulfate (i.p., 20 mg/kg) for approximately 30 days. The behavior of sham-implanted and untreated mice were never significantly different from one another. However, pup-retrieval time of naltrexone-treated mothers was disrupted and significantly prolonged for at least the first 10 days. The pups of untreated mothers gained weight at a significantly slower rate than controls for the first 5 days, after which time treated and controls did not differ. The fact that treated animals eventually showed weights that were not different from controls may have been attributable to a significantly higher mortality rate (30% vs. 7%) among treated animals, thus removing the most slowly growing pups from the population pool. The naltrexone-effect on retrieval times was reversible: removal of the beads restored normal behavior. In addition, animals manifesting normal behavior first would show the disturbed behavior upon naltrexone implantation. Placing treated mothers with litters from untreated parents indicated that the behavior of the mothers rather than the pups had been altered. Attempts to reverse this naltrexone-mediated behavior with clonidine (10-250 μg/kg, i.p.), a specific α-agonist that reverses opiate-withdrawal signs, were unsuccessful. These observations support the hypothesis of a role for endorphins in mediating social bonding and do not support one for a noradrenergic component in these behaviors. (Supported by Biomed. Res. Support Grant Program, N.I.H. 1-507-RR07155-01 and 1-R01 DA02004-01 [N.I.D.A.]).

1856 EFFECT OF ALCOHOL INGESTION ON PHYSIOLOGICAL ACTION TREMOR. Scott A. Burgstahler*, Robert S. Pozos, Roger W. Petry*, Paul Iaizzo*. Department of Physiology, University of Minnesota, Duluth, School of Medicine.

During voluntary motion of the hand there is a concomitant high frequency involuntary oscillation called Physiological Action Tremor (PAT). Since it has been reported that alcohol can decrease the amplitude of Pathological Action Tremors, the present study was undertaken to see if alcohol would also have a similar influence on PAT. If this occurs, insight into the relationship between Physiological and Pathological action tremors would be obtained.

PAT was recorded using an AVR-250 accelerometer taped on the dorsal side of a styrofoam sandwich which enclosed the hand. EMGs of the extensors and flexors of the wrist were recorded using Beckman biopotential surface electrodes. Accelerometer and EMG signals were recorded onto a Hewlett Packard tape recorder and later analyzed on a PDP-12 computer. Subjects were allowed to drink the alcoholic beverage of their choice until they felt intoxicated. An intoximeter (CMI) was used to quantitate the blood alcohol content of the subjects.

In non-intoxicated subjects, data analysis showed that the maximum displacement values (2500 μ) from acceleration records of PAT of the hand occurred at 8-12 hz. However, there was another significant frequency band of 2-4 hz in most subjects. After intoxication (0.1 mg % blood alcohol content), the amplitude of PAT was significantly reduced, but the frequency range remained constant.

Ingested alcohol has been reported to decrease sympathetic neuronal activity and reduce the amplitude of tremor in Pathological situations. Arterial injections of alcohol in the periphery appear to have no effect on the amplitude or frequency of the tremor and therefore the effects of alcohol are though to occur at the spinal cord or higher levels. Based on our observations, PAT might have a significant neural component. Also since alcohol decreases the amplitude of PAT and is reported to do the same with Clinical Essential Tremors, the same mechanism controlling PAT might also be the substrate for these clinically observable tremors.

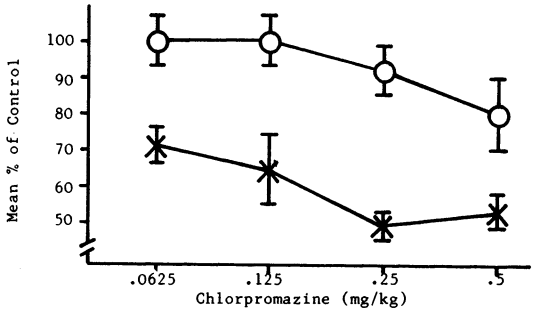
Research was supported in part by funds from Sea Grant(DOC-NA-519A-B-00025. Authors gratefully acknowledge the use of the intoximeter lent by Dr. J. Schaefer, Director of A.O.D.A.P., University of Minnesota.

1857 CORRELATION BETWEEN THE EFFECTS OF CHLORPROMAZINE ON SPINAL CORD ACTIVITY AND ALPHA-ADRENERGIC BLOCKADE. J.S. Carp*, P. Brigham* and R.J. Anderson (SPON: A. Raines). Dept. of Pharmacol., Geo. Washington Univ., Washington, D.C. 20037.

The effects of chlorpromazine (CPZ) on monosynaptic spinal cord transmission and on adrenergic, cholinergic, and histaminergic receptors were compared in alpha-chloralose anesthetized cats. Monosynaptic transmission between dorsal and ventral roots (L6, L7, or S1) in intact cats was significantly depressed ($P < .05$) by doses of .0313 to .5 mg/kg of CPZ i.v. This response plateaued at approxmately 50% of the control response.

In spinal, vagotomized cats, the cardiovascular responses to a series of agonists (norepinephrine, acetylcholine, epinephrine, histamine and isoproterenol) were recorded before and after cumulative doses of CPZ ranging from .0156 to .5 mg/kg. Only the response to epinephrine was depressed by CPZ in this dose range. Larger doses (from 2 to 10 mg/kg) depressed the response to norepinephrine. Since the isoproterenol response was unaffected, the effect of CPZ on norepinephrine and epinephrine was probably due only to alpha-adrenergic blockade.

The slopes of the dose-response curves obtained from the adrenergic blockade and the spinal cord depression were parallel (see below), suggesting that CPZ is affecting spinal cord responsiveness through an alpha-adrenergic mechanism. The descending noradrenergic bulbospinal pathway (Anden et al., Eur. J. Pharm. 2:59, 1967) is the most likely site of action, since previous work has shown that CPZ has no depressant effect on spinal cord activity in spinal animals (Preston, JPET, 118: 110, 1956).



1858 COMPARING THE EFFECTS OF NALOXONE AND PICROTOXIN ON SCHEDULE-CONTROLLED RESPONDING IN THE PIGEON: POSSIBLE GABA-ANTAGONISTIC EFFECTS OF NALOXONE. Richard B. Carter and J. David Leander*, Depts. of Psychology and Pharmacology, Univ. of North Carolina, Chapel Hill, N.C. 27514.

The narcotic antagonist naloxone has recently been demonstrated to possess GABA-antagonistic properties (Dingledine, et al., Eur. J. Pharmacol. 47:19-28, 1978). For this reason we decided to compare its effects to a known indirect antagonist of GABA, picrotoxin. The effects of naloxone (10.0-80.0 mg/kg, i.m.) and picrotoxin (0.1-0.56 mg/kg, i.m.) were studied alone and in combination with doses of pentobarbital (3.0 and 10.0 mg/kg, i.m.), diazepam (1.0-10.0 mg/kg, p.o.), clonazepam (0.3-3.0 mg/kg, p.o.) and muscimol (0.25 and 0.50 mg/kg, i.v.) on the responding of pigeons maintained under a multiple FR 30, FI 5-min schedule of food presentation. Naloxone and picrotoxin alone both produced dose-related decreases in responding without appreciably affecting the FI patterning. Pentobarbital alone produced dose-related increases in responding. Diazepam alone produced an inverted U-shaped dose-effect function, increasing rates at intermediate doses and decreasing them at the highest dose. Clonazepam and muscimol alone both produced dose-related decreases in responding. When administered in combination with either naloxone or picrotoxin, pentobarbital shifted each curve to the right by a factor of 2 or more. The 3.0 mg/kg dose of pentobarbital did not affect FI patterning either alone or in combination with naloxone or picrotoxin whereas the 10.0 mg/kg dose did. In contrast, 3.0 mg/kg of diazepam attenuated the effects of a 0.56 mg/kg dose of picrotoxin, but not of an 80.0 mg/kg dose of naloxone. Diazepam alone or in combination always influenced FI patterning. Similarly, 1.0 mg/kg of clonazepam also attenuated the effects of 0.56 mg/kg of picrotoxin, but not of 80.0 mg/kg of naloxone. Clonazepam alone or in combination always influenced FI patterning. Muscimol, a direct GABA receptor agonist, did not attenuate the effects of either 0.56 mg/kg of picrotoxin or of 80.0 mg/kg of naloxone at any dose. Thus, naloxone would appear to share some similarities with picrotoxin when their effects are compared on schedule-controlled responding in the pigeon. Supported by USPHS grants DA-01711 and ES-01104 and a research grant-in-aide from Sigma Xi.

1859 ETHANOL ORAL SELF-ADMINISTRATION IN NAIVE AND TOLERANT DROSOPHILA MELANOGASTER. S. S. Chawla*[1], J. M. Perron*[2] and C. Radouco-Thomas[1]. Unité de Recherche sur l'Abus des Drogues et de l'Alcool, Hôp. St-François d'Assise, Dépt. Pharmacol., Faculté de Médecine[1] and Dépt. Biol., Faculté des Sciences[2], Université Laval, Québec, Canada.

A method to measure the microvolumes of ethanol solutions self-administered orally by Drosophila melanogaster Meigen is described. The apparatus consists in a battery of eight experimental units, each one composed of a plastic chamber and an injection circuit. The net amount of ethanol taken has been examined in various experimental conditions: ethanol alone, ethanol-sucrose solutions at various concentrations and ethanol-sucrose cube. The net values for each day were obtained by computing the difference between gross values and evaporation per day figures. The data obtained in naïve drosophila show that the amount of self-administered ethanol is directly related to the concentration of ethanol; the consumption of sucrose solutions is inversely proportional to the concentration of sucrose. Observations on 14 days showed that the lethality increased with the raise in the concentration of ethanol and decreased with the raise in the concentration of sucrose (sucrose-water solutions 0,5%-3%).

Experiments have been carried out also on adult drosophila which have been exposed to ethanol during their entire life cycle. The preliminary results suggest that this new self-administration method could be envisaged for the study of the experimental dependence on ethanol and other addictive psychotropic drugs.

1860 DIFFERENTIAL EFFECTS OF CAFFEINE ON REGIONAL BRAIN BIOGENIC AMINES IN RATS. Dorothy T. Chou, Holly Cuzzone*, Jean Springstead*, Rida Ali* and Kenneth Hirsh. General Foods Corporation, Technical Center, Tarrytown, New York 10591.

Caffeine is well known to increase alertness and decrease drowsiness and fatigue. However, the site and mechanism of its effects on the central nervous system (CNS) are not completely understood. We report here the results of a study of the possible involvement in caffeine's CNS effects of three biogenic amines, serotonin (5-HT), norepinephrine (NE) and dopamine (DA). In order to identify the site of action, the endogenous levels of the biogenic amines in regional brain structures were studied before and after caffeine administration. Ninety male Sprague Dawley rats weighing 175-200 gm were used. The caffeine doses studied were 0, 2.5, 12.5, 25 and 50 mg/kg given by mouth. The animals were decapitated 1 hour after intubation with caffeine solution and the cerebral cortex, corpus striatum, hypothalamus, hippocampal formation, pons-medulla, cerebellum and midbrain raphe areas were dissected out over ice. 5-HT, NE and DA were extracted simultaneously from each of the brain areas using the butanol extraction method and assayed fluorometrically. A nonparametric statistical method was used to analyze the data. Our results show that the low dose of caffeine, 2.5 mg/kg P.O., selectively increased the 5-HT level in midbrain raphe ($p < 0.025$). Doses of 12.5, 25 and 50 mg/kg P.O. produced a dose-related increase in 5-HT level in midbrain raphe, and also in cerebral cortex and cerebellum with the response to 50 mg/kg being significantly greater than water control. The 5-HT concentrations in the remaining 4 brain areas were unchanged by caffeine at the doses studied. Caffeine, 50mg/kg P.O., selectively increased DA level in corpus striatum. NE levels were unchanged in all brain areas at the caffeine doses studied. Our conclusion that caffeine modified the disposition of 5-HT in cerebral cortex, cerebellum and particularly in midbrain raphe suggests that caffeine's action on CNS may be, at least in part, mediated through a serotonergic pathway. It has been postulated that brain serotonergic systems, especially the midbrain raphe serotonergic pathway, participate in sedation and sleep mechanisms. In addition, caffeine induced DA increases in corpus striatum may indicate the possible involvement of the dopaminergic system in caffeine's action on mood elevation and motor activity stimulation since the dopaminergic pathway has been suggested to participate in control of both motor function and mood. Furthermore, our results show that caffeine changes the endogenous levels of these 3 biogenic amines in a non-uniform manner in the 7 brain areas studied. These observations imply that caffeine exerts differential effects at different brain structures and on the different neurotransmitter systems.

1861 THIOHEXITAL ENANTIOMERS: PHARMACOLOGICAL ACTIVITY. H. DIX Christensen, W.C. Goad*, Philip Abraham* and F.I. Carroll*. Dept. Pharm., OUHSC, Okla. City, OK 73190 and RTI, Research Triangle Park, N.C. 27709.

Quantitative pharmacological differences exist between the optical antipodes of barbiturates. Racemic thiohexital in humans has a fast metabolism rate, 25% per hour, but possesses undesirable side effects of hiccoughs and twitching of extremities (Mark et al., Anes. 29:1159 (1968)). The relative anesthetic potency, toxicity, and incidence of side effects as a function of the configuration of thiohexital were investigated in Charles River, CF-1 male mice. The AD_{50} (loss of righting reflex), and LD_{50} values with 95% confidence limits after intraperitoneal administration are as follows:

Stereoisomer	(mg/kg)	Slope	Potency Ratio
AD50			
Racemic	25.8 (24.5-27.2)	1.14 (1.06-1.22)	-
(+)	18.8 (17.4-20.3)	1.25 (1.06-1.48)	1.37
(-)	27.3 (25.9-28.8)	1.16 (1.09-1.24)	1.06
LD50			
Racemic	63.5 (58.0-69.5)	1.23 (1.02-1.49)	-
(+)	59.8 (56.0-63.9)	1.14 (1.05-1.23)	1.06
(-)	66.1 (59.9-72.9)	1.21 (1.06-1.40)	1.04

There were no significant differences in onset, 3.68 ± 0.40 min., and duration of anesthesia, 12.11 ± 1.92 min., when the compounds were administered at the respective AD_{50} value to sets of 40 mice. Although the incidence of tremor on recovery was less for the (+) stereoisomer than (-) or racemic mixture, it is still significant compared to other alkyl-substituted barbiturates. When administered intravenously, the onset and potency are dependent upon the rate of injection, because of the high lipid solubility.

This study in addition to others with barbiturate enantiomers suggests that binding to receptor(s) is determined by the absolute configuration.

1862 p-CHLOROPHENYLETHYLAMINE - A UNIQUE SEROTONIN AGONIST. Eunyong Chung Hwang and Melvin H. Van Woert*. Depts. of Neurol. and Pharmacol., Mount Sinai Sch. of Med., New York, N.Y. 10029.

p-chlorophenylethylamine (p-CPEA) is structurally similar to p-chloroamphetamine (p-CA) and both compounds are potent releasers of synaptosomal serotonin (5HT). Like p-CA, we found that the release of synaptosomal 5HT by p-CPEA *in vitro* can be completely counteracted with 10^{-6}M fluoxetine (a selective 5HT uptake inhibitor) suggesting a similar mechanism of action for both drugs. However, 4 hours after p-CPEA (50 mg/kg i.p.) mouse whole brain 5HT and 5-hydroxyindoleacetic acid (5HIAA) levels were increased to 156% and 180% of control respectively, while p-CA (20 mg/kg i.p.) has been reported to decrease brain 5HT and 5HIAA by 53% and 25% respectively (Fuller, R. et al., Neuropharmacol. 14, 739, 1975). Fluoxetine pretreatment (10 mg/kg, 0.5 hour before p-CPEA) prevented the 5HT reduction at 0.5 hr (due to release); however, it did not block the elevation of brain 5HT and 5HIAA at 2 and 4 hours after p-CPEA (50 mg/kg). Each i.p. injection of p-CPEA (50 mg/kg) produced the "serotonin behavioral syndrome" without development of tolerance. Repeated injection of p-CA produced tolerance to its behavioral effects.

In order to test for neurotoxicity, mice were injected with p-CPEA (50 mg/kg) and sacrificed 1 and 2 weeks later; brain 5HT and 5HIAA were normal at these times. In contrast, p-CA produces a prolonged reduction in brain 5HT and 5HIAA levels.

p-CPEA is taken up at nerve terminals by the serotonergic neuronal transport process and releases endogenous serotonin. Unlike p-CA, p-CPEA is not a neurotoxin and may increase tryptophan hydroxylase activity.

1863 PENTOBARBITAL-INDUCED ALTERATIONS OF ADENOSINE 3':5' CYCLIC MONOPHOSPHATE (cAMP) METABOLISM IN RAT BRAIN. M.L. Cohn and M. Cohn, Dept. Anes., Magee-Womens Hosp., Univ. Pittsburgh, Sch. Med., Pittsburgh, PA 15213.

Our previous findings that cAMP dose-relatedly regulates duration of narcosis led us to investigate whether brain cAMP concentrations are altered by anesthetics. However, cAMP measurements in either whole or specific brain structures failed to establish a relationship between cAMP brain content and state of narcosis. Here, we investigated in-vivo and in-vitro whether cAMP synthesis or degradation is altered by barbiturates. Male Sprague-Dawley rats were anesthetized with intraperitoneal sodium pentobarbital (50mg/kg); controls were untreated. Following sacrifice by either microwave radiation (MR) (5 sec. exposure) or decapitation (D), brains were immediately removed, homogenized in .4N perchloric acid (1ml/100mg of wet brain), centrifuged, neutralized and filtered. After D, brain cortex slices were incubated under aerobic conditions in Krebs-Ringer bicarbonate buffer pH 7.4 (30°C) with or without pentobarbital (10^{-6}M). Sequential aliquots of incubation mixture and brain extract samples were analyzed with our newly devised high performance liquid chromatographic method (Cohn et al, Pittsburgh Conference on Analytic Chemistry and Applied Spectroscopy, p. 620, 1979). Contrary to previous reports that in mammalian brain major metabolic products of ATP and cAMP are adenosine and adenine, our findings in untreated rats sacrificed by MR showed smaller concentrations of the two metabolites and much higher accumulations of deamination products inosine and hypoxanthine. Under hypoxic conditions (D) we found less inosine and hypoxanthine but a significant accumulation of adenosine. The shift in cAMP metabolism produced by pentobarbital, while similar, was more pronounced than that induced by D. In-vitro incubation studies revealed that pentobarbital reversibly inhibits deamination of 5'AMP, leading to adenosine accumulation with no concomitant rise of cAMP. Our in-vivo and in-vitro chromatographic evidence that barbiturates inhibit deamination in cAMP metabolic pathways provides a molecular basis for the 50-year old observation that anesthetics decrease brain ammonium concentrations. That pentobarbital increases adenosine, which allegedly regulates cerebral blood flow, may partially explain the barbiturates' therapeutic usefulness against ischemia and stroke. Recently, it was proposed that inosine and hypoxanthine are endogenous ligands of benzodiazepine receptors. Our earlier finding that, like barbiturates, diazepam-induced narcosis is dose-relatedly shortened by cAMP suggests that diazepam may also duplicate barbiturates' specific inhibition of deamination and subsequent lowering of the two metabolites' concentrations. Future investigations will verify our hypothesis.

Supported by DA00605.

1864 A SPECIFIC EFFECT OF MORPHINE IN THE HIPPOCAMPUS IN VITRO. William A. Corrigall and Mary Ann Linseman. Dept. Neurobiology, Addiction Research Foundation, Toronto, Ontario, M5S 2S1.

The acute effects of morphine sulphate on field potentials evoked in the isolated transverse hippocampal slice preparation from the rat were studied over a bath concentration range of 0.5 to 50 uM. Field potentials were elicited in the stratum pyramidale of CA1 by stimulation in the stratum radiatum. The field potential recorded in control medium generally showed a single population spike representing synchronous activation of the pyramidal cells. Morphine had a consistently stimulant effect on the response, manifest as a dose-related appearance/increase of a second, and occasionally subsequent, population spikes. This action of morphine appears to be stereospecific since it was consistently antagonized by naloxone (0.1 - 10 uM), and it could be duplicated by comparable doses of levorphanol but not of dextrorphan.

The size of the morphine effect was dependent on the input/output (I/O) function of the pyramidale field potential; that is, the effect was maximal at the top of the I/O curve, but it was generally small or absent near threshold.

Several experiments suggested that the effect was not produced at the level of the excitatory synaptic input to the pyramidal cells. Recordings in the stratum radiatum (the site of the synapses on the pyramidal neurons which are activated by radiatum stimulation) simultaneous with those in pyramidale showed that an increase in the population EPSP did not cause the increase in CA1 discharge. The effect was not dependent on the specific input pathway as it was also observed when the field potential was evoked via synapses on the basal dendritic tree by stimulation in stratum oriens. Synaptic input itself was not essential since the effect occurred when the pyramidal cells were driven antidromically by stimulation of the alveus.

The additional population spikes could result however from a decrease in recurrent inhibition upon the CA1 neurons, a presumed GABA-mediated effect. The morphine effect was indeed reversed by the addition of GABA (1 mM) to the bath. Picrotoxin (2.5 uM), a GABA antagonist, mimicked the effect of morphine.

These data are evidence for a specific, primary effect of morphine in the hippocampus, which at the cellular level may consist of a selective depression of the interneurons normally mediating recurrent inhibition to the CA1 pyramidal cells, resulting in net excitation of the latter.

1865 LESIONS OF THE SUBSTANTIA NIGRA REVEAL BENZODIAZEPINE RECEPTORS WITH AND WITHOUT γ-AMINOBUTYRIC ACID RECEPTOR LINKAGE. Tommaso Costa*, Agu Pert and Candace B. Pert. Biological Psychiatry Br., NIMH, Bethesda, MD 20205 (SPONSOR: L. K.Y. Ng)

Four weeks following the destruction of cell bodies in the substantia nigra by microinjection of 1 μg of kainic acid, specific [^{3}H]diazepam binding was reduced 60% in the lesioned side compared to the unlesioned control side. The ability of γ-aminobutyric acid (GABA) (10 μM) to enhance [^{3}H]diazepam binding was significantly reduced on the lesioned side. A parallel, but smaller (40%) decrease on the lesioned side was observed also for [^{3}H]-muscimol binding while the restricted conformation analog of GABA, 4,5,6,7-tetrahydroisoxazolo(4,5-c)pyridin-3-ol (THIP) was more potent in displacing [^{3}H]muscimol bound on the intact side than on the lesioned one. These data markedly contrast with the pattern of changes observed when the striato-nigral input is lesioned (Shibuya et al., Neuroscience Abst., this vol.). In this case, a dramatic increase in sensitivity of [^{3}H]diazepam binding to GABA receptors is accompanied by increased GABA receptors and decreased benzodiazepine (BZ) receptors.

Are the BZ receptors on cell bodies within the nigra closely and permanently coupled to GABA receptors while BZ receptors on terminals which project to the nigra are not associated with GABA receptors? This is unlikely since THIP, which labels the uncoupled form of the GABA receptor (Karobath et al., Nature, 278, 1979) is more potent in displacing GABA receptor ([^{3}H]muscimol) binding on the intact side.

An explanation consistent with all of our data is that the quantity of GABA/BZ-coupled receptors is a function of the ratio between free GABA receptors and free BZ receptors. This ratio is different for membranes obtained from various brain regions, and changes during altered states of sensitivity.

GABA's differential ability to enhance [^{3}H]diazepam binding (50-150%) depending on which brain region is examined (spinal cord > cerebellum > cortex) may be an approximate measure of the GABA-coupled BZ receptor.

1866 ON THE MULTIPLICITY OF BENZODIAZEPINE RECEPTORS AND THEIR DISTRIBUTION IN THE RAT BRAIN. Joseph Coupet and Vera A. Szucs-Myers*, American Cyanamid Co., Medical Research Division, Pearl River, NY 10965.

High affinity benzodiazepine binding sites in the mammalian brain have been described by many investigators. A high degree of correlation has been found between the binding affinities of a series of benzodiazepines and their potencies as anxiolytics, muscle relaxants and anticonvulsants. These findings strongly suggest that these sites may function as pharmacological receptors for the benzodiazepines. Is it possible that the diverse therapeutic effects of the benzodiazepines are mediated by the same receptor sites? Scatchard analyses of the binding of labelled benzodiazepines in various brain regions revealed a single population of binding sites. However, when we examined the dissociation curves ("off rates") for ^{3}H-diazepam from its binding sites, we uncovered biphasic lines of decline in the rat cerebral cortex and the hippocampus. The apparent half time (t-1/2) of the fast component was 1.5 - 2.0 minutes, and that of the slow component 8.0 minutes. In other brain regions ^{3}H-diazepam dissociated monophasically with a t-1/2 of approximately 5.0 minutes.

In an attempt to further characterize the "benzodiazepine receptors", we displaced ^{3}H-diazepam with an excess of unlabelled CL 218,872, a triazolopyridazine anxiolytic lacking sedative properties, and compared the results with those obtained with displacement by unlabelled diazepam or flunitrazepam. While the t-1/2 of the fast component was unaffected by CL 218,872, the t-1/2 of the slow component increased. These findings, taken together with other pharmacological data of CL 218,872 indicate: 1) that there exist two or more types of "benzodiazepine receptors in the rat brain; 2) that the "slow receptor type" may be associated with ataxia or sedation; and, 3) that pharmacologically active benzodiazepines have almost identical affinities for all receptor types, but have different rates of dissociation from each type of receptor.

1867 DEVELOPMENT OF TOLERANCE TO OPIATES AND OPIOID PEPTIDES IN CULTURES OF MOUSE SPINAL CORD WITH ATTACHED DORSAL ROOT GANGLIA. Stanley M. Crain, Bea Crain*, Tara Finnigan* and Eric J. Simon*. Depts. of Neuroscience, Physiol. and Neurology, Albert Einstein College of Medicine, Bronx, N.Y. 10461; (E.J.S.) Dept. of Medicine, New York University College of Medicine, N.Y. 10016.

Acute opiate-depressant effects on sensory-evoked dorsal-horn network responses of organotypic explants of fetal mouse spinal cord with attached dorsal root ganglia (Crain et al.,Br. Res. 133,'77; 157,'78) disappeared after chronic exposure to analgesic levels of morphine (1 μM) for > 2-3 days in culture at 35°C. (Drug exposures were begun about 1-3 weeks after explantation.) Characteristic dorsal cord responses could then be evoked by dorsal root ganglion (DRG) stimuli in the presence of morphine -- even after acute increases in concentration (up to 100-fold). Tolerance also developed after chronic exposure of cord-DRG explants to low concentrations (ca. 0.01 μM) of the enkephalin analog, [d-ala^2,MePhe^4,Met-(O)^5-ol]-enkephalin (Sandoz 33-824). The latter cultures showed cross-tolerance to met-enkephalin and opiates; dorsal cord responses could still be evoked even after acute exposure to high levels of morphine. Morphine-tolerant cultures also showed cross-tolerance to met-enkephalin and to the Sandoz opioid peptide (33-824).

The tolerant state did not develop if the cultures were incubated at lower temperature, 20°C, during exposure to 1 μM morphine for as long as 7 days. (Control 2-week-old cultures incubated in regular media at 20°C for 1 week showed no significant electrophysiologic deficits.) Furthermore, development of tolerance was partly antagonized by raising the Ca^{++} concentration of the medium to 10 mM during chronic exposure to 1 μM morphine. The latter results are consonant with our acute experiments showing that the depressant effect of opiates and opioid peptides on dorsal cord responses were often markedly reduced in the presence of high Ca^{++} in the culture medium (Crain et al., Br. Res. 157,'78).

The sensory-evoked dorsal-horn population responses in tolerant cultures often developed unusually large amplitudes during maintenance in 1 μM morphine or after acute introduction of naloxone. Explants chronically exposed to morphine continued to show evidence of tolerance for about 2 days after transfer to drug-free culture medium, but characteristic sensitivity to opiates returned during the subsequent few days. Organotypic cord-ganglion cultures provide, therefore, a valuable model system for analyses of cellular mechanisms underlying development of opiate tolerance and dependence in mammalian CNS tissues under flexible, controlled conditions in vitro.
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1868 EFFECTS OF d-AMPHETAMINE ON DOPAMINERGIC NEURONS IN THE VENTRAL TEGMENTAL AREA OF THE RAT. M. Dalsass, D.C. German, R.S. Kiser, and S. Speciale. Depts. Psychiat. and Physiol., Univ. Tex. Health Sci. Ctr., Dallas, TX 75235.

Systemic d-amphetamine (d-A) can release dopamine (DA) from nerve terminals in the caudate-putamen and block the reuptake of this amine. This effect is accompanied by a decrease in the spontaneous firing rates of the substantia nigra (A9) DA neurons projecting to the caudate-putamen. Biochemical and electrophysiological data indicate that this decreased firing rate is mediated by a descending GABA-ergic projection from the caudate-putamen to the A9. For example, Bunney & Aghajanian (Arch. Pharmacol., 304, 255-261, 1978) showed electrophysiologically that picrotoxin, a GABA antagonist, reversed the d-A-induced depression in A9 impulse flow. Furthermore, after kainic acid lesions of the caudate nucleus, increased doses of d-A were needed to decrease A9 impulse flow, and picrotoxin failed to reverse the d-A effect. The present study sought to examine whether the medially adjacent DA neurons in the ventral tegmental area (A10), which project to the ventral portions of the caudate-putamen and nucleus accumbens, responded to d-A by a similar feedback mechanism.

Single unit activity was recorded from the A10 region in chloral hydrate anesthetized rats (400 mg/kg). DA neurons in this region are characterized by their slow (1-9 Hz) spontaneous firing rates, and long (>2msec.) action potential durations. Intravenous administration of d-A (1-1.5 mg/kg) produced a greater than 50% decrease in the firing rate of these neurons. Subsequent administration of picrotoxin (2-3.4 mg/kg) failed to reverse the effects of d-A while the DA antagonist, haloperidol (1-2 mg/kg) did. The same procedures were repeated with rats that received kainic acid (2 μg/μl) lesions of the ventral caudate/nucleus accumbens area. Histological examination of these regions showed massive cellular destruction which presumably affected descending pathways projecting to the A10 region. However, the A10 neurons responded to the same doses of d-A and haloperidol as in the normal rat. These findings suggest that the effects of d-A on A10 neurons are not mediated by a ventral caudate/nucleus accumbens feedback loop but perhaps by a direct action on A10 DA neurons. (Research supported by USPHS grants MH-27574, MH-30546, & MH-05831).

1869 α_2-ADRENERGIC RECEPTOR DESENSITIZATION: RECEPTOR UNCOUPLING FROM Ca^{++} CHANNEL IN RAT PAROTID CELLS. James N. Davis, Wendy Maury*, and Robert McDaniel*. VA Medical Center and Duke University Medical Center, Durham, N. C.

Parotid acinar cells are a particularly useful model for the study of α_2-adrenergic receptors since they have easily measured membrane receptor binding and a well-defined, Ca^{++} dependent response, the release of K^+. We previously described a type of α-adrenergic receptor desensitization that was mediated by a rapid alteration in the membrane receptor (Strittmatter et al., J. Biol. Chem. 252: 5478, 1977). This type of desensitization took 15 minutes to become complete, was voltage-dependent, occurred in mildly acidic (pH 6.5-7.0) conditions, and did not interfere with the muscarinic cholinergic response of these cells, also a Ca^{++} dependent K^+ release.

We now report a second type of α-adrenergic desensitization that occurs at physiological pH (7.4). Cells exposed to 100 μM epinephrine and then washed lose both their α-adrenergic and muscarinic cholinergic responses. This second type of desensitization is more rapid than the previously described receptor changes, being complete in 2-3 minutes and spontaneously reversing in 10 minutes. α-Adrenergic stimulation is required for the desensitization since epinephrine in the presence of excess phentolamine does not produce it. However when Ca^{++} is introduced into desensitized cells by the ionophore A23187, they release K^+ as well as washed controls showing that desensitization must prevent Ca^{++} entry. Desensitization can be elicited even in the absence of Ca^{++} (with EGTA) and is voltage-dependent. Although no cholinergic response is present, [^{3}H]-QNB binding to desensitized cells is the same as washed controls.

Our data demonstrate that α-adrenergic stimulation produces a heterologous desensitization of the adrenergic and cholinergic responses in parotid acinar cells. These data support experiments (Putney et al. J. Physiol. 268: 139, 1977) suggesting that these two receptors share the same Ca^{++} channel. We suggest that persistent α_2 stimulation leads to an uncoupling of the channel from both its adrenergic and cholinergic receptors. Since many nerve terminals have both α_2-adrenergic and muscarinic cholinergic receptors, this type of desensitization may play an important role in synaptic communication.

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1870 EFFECTS OF β-ADRENERGIC DRUGS ON APOMORPHINE-INDUCED MODIFICATION OF BEHAVIOR IN MONKEYS AND CHICKENS. B. Delbarre and M. Goustard*,(SPON: R.W. Keller, Jr.), Laboratoire Ch. Exp. Fac. Medicine 37032, Tours, France and Laboratoire d'Ethologie Comparee, 91530, Saint-Cheron, France.

Drugs with β-adrenergic actions are known to affect mood and behavior. For example, propranolol, a β-adrenergic blocker, has been used as a therapeutic agent in the control of psychotic symptoms. Recently, we showed that clenbuterol, a β_2 adrenoreceptor stimulant, has antidepressant activity (Delbarre et al., Mol. Biol. and Pharmacol. of Cyclic Nucleotides 255-258, 1978). In the present investigation, we studied the effects of clenbuterol and DL propranolol on the behavior of monkeys (Macaca Fascicularis) treated intramuscularly (IM) with apomorphine (HCL) 0.4 mg kg^{-1}. Five to thirty minutes after administration, apomorphine produces hyperactivity, increased fearful reactions to observers and stereotyped behavior such as chewing, biting, licking, self-picking, self-grooming and vocalizations. Clenbuterol 5 mg kg^{-1} or DL propranolol 5 mg kg^{-1} administered IM ten minutes before apomorphine significantly attenuated or suppressed the apomorphine-induced behavioral modifications. In chickens IM apomorphine 0.1 mg kg^{-1} induced hypermotility and pecking. At the same dose-range, clenbuterol and DL propranolol antagonized the actions of apomorphine. Similarly, we have shown that neuroleptic drugs blocked apomorphine-induced behavioral alterations in monkeys and chickens (Delbarre et al., Collegium Internat. Neuro-Psychopharmacol., 10th Congress, 1976; Goustard et al., J. Pharmacol. (Paris) 8: 4, 1977). Our evidence suggests that the psychotropic activity of β-adrenergic drugs may be easily and reliably evaluated by testing the ability of these agents to block apomorphine-induced behavioral alterations in monkeys and chickens.

1871 ELECTROPHYSIOLOGICAL CORRELATES OF NORMAL AND HALOPERIDOL-INDUCED IMMOBILITY IN RATS. Marc De Ryck* and Philip Teitelbaum. Psychol. Dept., Univ. of Illinois, Champaign, ILL 61820.

Wakeful immobility in rats (n=10), chronically implanted with bipolar electrodes in somatosensory cortex (SSC) and hippocampus (H), was accompanied by low voltage fast activity (desynchronization, DESYNC) in SSC and large amplitude irregular activity in H. However, between periods of wakeful immobility and prior to the onset of slow wave sleep (SWS--i.e., large amplitude slow waves in SSC and H), intermittent hypersynchrony mixed with DESYNC appeared in SSC. This activity consisted of spindle-shaped bursts of biphasic spike or spike-wave discharges with frequencies of 7-10 spikes/sec and durations of 1-30 sec. Spike amplitudes (.3-2.2 mV), which always exceeded wave amplitudes (.2-.7 mV), were 2.5-13 times larger than the background DESYNC. SSC spiking spindles occurred in apparently awake but immobile rats and were associated with head bobbing in phase with the spike discharges together with fine whisker tremor. SSC spiking spindles in rats may represent an EEG correlate of a distinct immobility state occurring between wakeful immobility and SWS. Similar "twilight states" have been reported in cats and monkeys by Sterman, Rougeul and their associates.

.5-5 mg/kg haloperidol (HAL) produced catalepsy/akinesia accompanied by a dose-dependent increase in occurrence of SSC and H large amplitude slow waves similar to those observed in normal SWS. Thus, at these dosages, HAL predominantly produces somnolent immobility. At higher dosages (10-15 mg/kg), this SWS pattern was replaced, often abruptly, by almost continuous SSC spiking spindles (e.g., during 65-97% of a 30 min. period). Cholinergic agents (.5 mg/kg physostigmine, 8 mg/kg arecoline) completely abolished HAL-induced SSC spiking spindles and produced continuous DESYNC. Likewise, anticholinergic drugs (10 mg/kg atropine, .5 mg/kg scopolamine) abolished HAL-induced SSC spindles, but replaced them by continuous large amplitude slow waves (synchronization, SYNC). When anticholinergic and cholinergic agents were sequentially injected in rats with HAL-induced SSC spindles, the shift from cortical SYNC to DESYNC passed through a stage in which spiking spindles temporarily reappeared. In contrast to their effect on HAL-induced SSC spiking spindles, neither cholinergic nor anticholinergic agents suppressed petit mal or grand mal seizures associated with very high dosages of HAL (20-45 mg/kg). Thus, to the extent that SSC spiking spindles represent a dopaminergic-cholinergic imbalance, their reversal by both cholinergic and anticholinergic drugs suggests that this EEG correlate of normal immobility and HAL-induced akinesia depends on a narrow range of dopaminergic-cholinergic interactions. Supported by NIH Grant RO1 NS 11671.

1872 REGULATION OF β-ADRENERGIC RECEPTORS AND cAMP ACCUMULATION IN VITRO IN SLICES OF RAT CEREBRAL CORTEX. Mark D. Dibner and Perry B. Molinoff. Dept. of Pharmacology, Univ. of Colorado Med. Ctr., Denver, CO 80262.

The effects of incubating slices of rat cerebral cortex with the β-adrenergic agonist isoproterenol (ISO) on β-adrenergic receptor density and on catecholamine-stimulated cAMP accumulation were investigated. Exposure of slices to ISO led to a 40% decrease in receptor density (as measured by Scatchard analysis of IHYP binding). The effect of ISO was complete by 3-5 min and had an EC50 of 10 μM. In contrast, ISO-stimulated cAMP accumulation decreased by approximately 75% as measured by a protein binding assay. The effect of ISO on cAMP accumulation was more rapid (complete by 0.5 min) and had an EC50 of approximately 0.01 μM. Thus, incubation with ISO under appropriate conditions led to decreases in β-adrenergic receptor mediated cAMP accumulation without changing receptor density. The effects of ISO on receptor density and on ISO-stimulated cAMP accumulation were blocked by coincubation with the β-adrenergic receptor antagonist sotalol. The cAMP effects appeared to be receptor specific since PGE_1 stimulation of cAMP was not decreased following incubation with ISO nor was IHYP binding decreased following incubation of slices with PGE_1. Incubation with ISO did not significantly alter the IC50 for displacement of IHYP binding by either agonists or antagonists. Lastly, the observed decreases in β-adrenergic receptor numbers could be slowly reversed by reincubation of slices in the absence of ISO or rapidly reversed by incubation of homogenates with guanine nucleotides before binding.
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1873 LEAD-INDUCED HYPERACTIVITY IN MICE AND BLOOD-BRAIN BARRIER FUNCTION. Floyd R. Domer and Carlos Wolf*, Dept. of Pharmacology, Tulane Univ. School of Medicine, New Orleans, La. 70112.

The offspring from CD-1 mice were exposed to 0.5% sodium or lead acetate in the drinking water from time of birth. After weaning, locomotor activity was evaluated. Individual mice were placed in a box which had the bottom marked off into two-inch squares. The numbers of squares crossed during a 10-minute period of observation was recorded. These observations were made three times at weekly intervals. The lead-exposed mice were hyperactive and smaller than the sodium-exposed mice. Amphetamine and caffeine caused a decrease in locomotor activity of the lead-exposed mice whereas methylphenidate and pemoline caused an increase in locomotor activity of the lead-exposed mice. The permeability of the blood-brain barrier (BBB) was evaluated by the intravenous injection of either radioactive technetium ($^{99m}TcO_4^-$) or radioiodinated human serum albumin (I^{131} RISA) sixty minutes prior to sacrifice. A blood sample was obtained by cardiac puncture and the brain was dissected out. The content of radioactivity in the brain relative to that of the blood was the criterion for judgment of the permeability of the BBB. When technetium was used to assess BBB function permeability was decreased by amphetamine, caffeine and pemoline and increased by ephedrine and methylphenidate in the lead-exposed animals relative to sodium-exposed animals. When RISA was used to assess BBB function permeability was increased by amphetamine (1 mg/kg) and decreased by amphetamine (3 mg/kg), methylphenidate (20 mg/kg) and ephedrine (40 mg/kg) in the lead-exposed mice relative to the sodium-exposed mice. It was concluded that lead-induced hyperactivity is not a valid model for evaluating drugs for use in children with hyperactivity or minimal brain dysfunction. In addition, changes in permeability of the BBB to technetium and RISA caused by the drugs are opposite. This suggests that different mechanisms are responsible for the BBB for the two probes.

1874 EFFECTS OF LSD AND 2-BROMO-LSD ON VISUALLY EVOKED ACTIVITY OF SINGLE CORTICAL NEURONS IN THE CAT. A. Dray and G.G. Somjen Dept. Physiology, Duke Univ. Med. Center, Durham, N.C. 27710.

Systemic or local administration of LSD causes complex changes in the discharge pattern of visual cortical neurons when these are activated by physiological optical stimulation (Rose & Horn, 1977, Expt. Brain Res. 27, 71; Hilmy et al. 1977, Neurosci. Abst. 3 562; Fox & Dray, 1979, Brain Res. 161, 107). To determine whether these changes are related to the hallucinogenic properties of LSD the actions of this drug have been compared with the supposed non-hallucinogenic analogue 2-bromo-LSD(BOL). Data were collected from single neurons in the striate cortex of anesthetized, immobilized cats. Drugs were administered intravenously or locally by microelectrophoresis. Visual stimulation usually consisted of an optimally orientated illuminated light bar moving back and forth across the receptive field. Systemic LSD either enhanced or depressed visually evoked responses. These effects were clearly dose related, enhancement being observed with low doses (0.1-10 ug/kg) and depression more frequently at higher doses (10-25 ug/kg). Accompanying this were changes in neuronal receptive field properties, directional selectivity and unstimulated background discharges. Such effects were observed on cells with both simple and complex receptive fields. Electrophoretic LSD produced similar dose dependent effects suggesting a direct action of LSD on the visual cortex. Unexpectedly systemic BOL (10-25 ug/kg) also produced similar effects to LSD. However comparisons on the same neurones suggested that it was some 20-100 times less active in producing enhancement of visually evoked activity but equipotent in producing depression. Electrophoretic BOL rarely produced changes in evoked activity, possibly due to inadequate expulsion from micropipettes. These results suggest that enhancement of visually evoked activity is an important action of LSD related to its hallucinogenic properties and that BOL in higher doses should produce visual disturbances in the cat (Fanchamps, 1978, Handb. Exp. Pharm. 49, 567).
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1875 SLOW POTENTIATION OF CA1 HIPPOCAMPAL SLICE FIELD POTENTIALS AND ACUTE EFFECTS OF LOW-DOSE ETHANOL. Dominique Durand and Peter Carlen. Neurobiology Lab of the Addiction Research Foundation Clinical Institute, Playfair Neuroscience Unit, Toronto Western Hospital, University of Toronto, Toronto, Canada.

Field potentials were recorded from CA1 hippocampal neurons in the in vitro transverse hippocampal slice preparation. Orthodromic stimulation of the stratum radiatum at low frequency (.1 to .2 Hz) induced a slow but steady increase in the size of the field potential (2 to 10 fold in 30 to 60 min.). Input/Output (I/O) curves of the population spike recorded at 10 min. intervals over a 30 to 60 min. period showed a definite increase in amplitude and a shift to the left. This decrease in the threshold of the response cannot be attributed to an increased afferent volley since the size of the presynaptic spike remained constant.

Because of this slow potentiation, effects of low-dose ethanol (50 mM to 100 mM) were difficult to assess. However, after stabilization of the response, ethanol was added to the bath. Ethanol at 100 mM consistently produced a reversible depression of the field potential of 25%. 50 mM had different effects: in a total of 13 slices, 7 showed a depression (5 to 20%) of the population spike (5 of which were accompanied by acute tolerance ie. return to baseline during ethanol perfusion); 4 slices showed an increase (50 to 10%) of the response, 3 of which were accompanied again by acute tolerance; 2 slices did not show any effects. The time course of the peak ethanol response was between 15 to 30 min. The response returned to baseline during the ethanol infusion after another 15 to 30 min. period.

These depressing effects of ethanol were reversible within 30 min. by switching back to the control solution and were accompanied by an increase threshold as shown by a shift to the right of the input/output curves recorded during the ethanol perfusion period. Because the ethanol-induced increase in the size of the population spike was correlated with shorter periods of stimulation before stabilization, we feel that this response could also be attributed to further potentiation of the field potential, the later "tolerance" to this increase being then the depressing effects of ethanol.

These initial results suggest that clinically relevant doses of ethanol (50 to 100 mM) are effective in vitro but further experiments are necessary to understand these acute physiological changes.

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1876 **SPECIFIC PHOSPHOPROTEINS IN THE FUNCTION OF OPIATE RECEPTORS.** **Yigal H. Ehrlich, Leonard G. Davis and Peter Keen*. Univ. of Mo.-Columbia, Sch. of Med., Mo. Inst. of Psychiatry, 5400 Arsenal St., St. Louis, MO. 63139**

We have reported that preparations containing synaptic membranes from the neostriata of morphine-dependent rats demonstrate a 50% decrease in endogenous phosphorylative activity compared to preparations from placebo treated controls. Examination by SDS-gel electrophoresis revealed that this decrease was directed specifically towards membrane phosphoproteins designated F and H (M.W. 47K and 15-20K, respectively; Life Sci. 23: 137, 1978). Subsequent studies in our laboratory have demonstrated that high concentrations of alkaloid opiates (0.5-5mM) and low concentrations of enkephalin and β-endorphin (10^{-8}-10^{-4}M) selectively inhibit in-vitro the phosphorylation of the same proteins (F and H) affected by long-term morphine in-vivo (Davis and Ehrlich, Adv. Exp. Med. Biol., in-press). We have hypothesized then that the membrane-bound enzymatic machinery for the phosphorylation of proteins F and/or H may be linked functionally, and perhaps also physically, to opiate receptors. Therefore, we have extracted our membranes with the non-ionic detergent Brij-36T. This treatment was reported by Simon, et al (Science, 190: 389, 1975) to extract stereospecific opiate-binding-sites from synaptic membranes. When synaptic membranes were first incubated with γ-^{32}P-ATP and then extracted with Brij-36T, close to 100% of the phosphorylated protein F and some of the H co-extracted with the opiate receptor. Moreover, the Brij extract and the membrane-residue retained phosphorylative activity. Endogenous phosphorylation of protein F was in evidence only in the extract. Chromatography of the Brij extract by gel filtration (Biogel-A1.5m) yielded macromolecular protein complexes that contained protein-bound [^{3}H]etorphine and the endogenous phosphorylation systems. Furthermore, concentrations of Brij-36T which were shown by Simon et al (1975) to distort the opiate binding site, caused selective inhibition of the endogenous phosphorylation of F and H, without affecting the endogenous phosphorylation of other proteins in synaptic membranes. Finally, opiate-inhibited phosphorylation of F and H was accompanied by altered activity of adenylate cyclase in the same preparations. Preliminary experiments indicate possible cause-effect relationships between these systems. The results suggest that the function of opiate receptors may involve complex interactions between opioid-peptides, their binding sites, and membrane-bound systems that phosphorylate specific phosphoproteins. Supported in part by a grant from the Epilepsy Foundation of America to Y.H.E., and by intramural funds from the MIP.

1877 EFFECTS OF CHRONIC INSTILLATION OF DOPAMINERGIC DRUGS INTO SPECIFIC SITES. Everett H. Ellinwood, Jr., George Dougherty, Ron E. Pruitt and M. Marlyne Kilbey, Dept. Psychiatry, Behav. Neuropharmacology Sect., Duke Univ. Med. Ctr., Durham, NC 27710

Several hypothesis relating Dopamine system mechanisms to the effects of dopamine agonists and antagonists have been proposed for experimental models of clinical disease ranging from dyskinesia to psychosis. Most frequently chronic drug induced behavioral changes, e.g., stimulants and neuroleptics are considered mainly in terms of post-synaptic receptor changes rather than the interaction of neuronal with synaptic mechanisms. This report will review data from several studies in which dopamine system sites, e.g., either unilateral substantia nigra and caudate are chronically treated with dopamine agonists or antagonists instilled via cannulae connected to Alza osmotic minipumps, then tested for post-treatment responsivity to apomorphine and amphetamine. For example, preliminary data analysis indicates that chronic amphetamine into the caudate leaves a residual state where animals rotate contralateral following i.p. amphetamine, yet show no rotational preference to apomorphine. This implies a pre-synaptic mechanism. On the other hand, chronic L-Dopa instillation into the substantia nigra renders animals that rotate ipsilateral to apomorphine which is in keeping with a subsensitive neuronal soma dopamine receptor mediating an inhibition of firing rate. In general, our results are consistent with a significant pre-synaptic role for residual chronic drug effects.

1878 CORRELATION BETWEEN STEREOTYPED BEHAVIOR AND DOPAMINE RECEPTOR BINDING IN AN ANIMAL MODEL OF TARDIVE DYSKINESIA. Jeremy Z. Fields, Chinwuba R. Okafor*, Bruce I. Diamond, & Richard L. Borison. Dept. Pharmacol., Chicago Med. Sch. & Mt. Sinai Hosp., Chicago IL 60612

Anticholinergic agents such as Cogentin(COG) and Artane(ART) are often coadministered with antipsychotic drugs to reduce extrapyramidal side-effects. These side-effects are thought to result from a shift in the functional balance between acetylcholine (ACh) and dopamine(DA) in the striatum(ACh> DA). It has recently been suggested that these anticholinergics may, in addition, promote the future development of neuroleptic-induced tardive dyskinesia(TD)(DA> ACh). To understand how this might occur, we tested the effects of these drugs in an animal model of TD. Chronic haloperidol(HAL)(i.p.,0.5mg/kg,14 days) produced a "sensitization" of striatal DA activity: stereotyped behavior(StB), induced by a challenge dose of apomorphine(APO)(0.25 mg/kg) 4 days after HAL withdrawal, was increased 5 to 6 fold while the number of DA receptors(DAr)(3H-spiroperidol binding) was increased 1.3 to 1.5 fold. Muscarinic cholinergic receptors(AChr) (3H-QNB binding) did not change in any of these studies(it is interesting that a DA antagonist increased the number of DAr but the cholinergic antagonists did not increase the number of AChr).

If either COG or ART(0.5 mg/kg) are coadministered with HAL (but not if they are given alone), there is a further small but significant increase in StB. No significant parallel increase in DAr could be detected. Amantadine(AMAN)(25 mg/kg), another anti-parkinsonian agent(believed to act as an indirect DA-mimetic) in combination with HAL, reduces the StB and decreases DAr binding. When APO(0.5 mg/kg) was coadministered chronically with HAL, the StB is significantly reduced while the HAL-induced increase in DAr is completely blocked. Chronic APO alone, in this paradigm, produced little change in either StB or in DAr.

Our data indicate that although there is not a 1 to 1 correspondence between StB and DAr, qualitatively, both parameteres change in parallel fashion. It has been shown clinically that the administration of DA-mimetics antagonizes the abnormal movements in TD. We have now shown experimentally that the conjoint treatment of a neuroleptic with a DA-mimetic produces not only an antagonism to the neuroleptic-induced behavioral hypersensitivity, but also reverses neuroleptic-induced increases in DAr.

Thus a more clinically rational treatment choice for neuroleptic induced extrapyramidal side-effects would be the DA-mimetic agent AMAN, rather than the anticholinergics COG or ART.

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1879 ASSESSMENT OF ANTICONVULSIVE EFFECT OF DIAZEPAM IN PENTOBARBITAL DEPENDENT MICE. B. A. Flint and I. K. Ho, Dept. Pharmacol. & Toxicol., Univ. Miss. Med. Ctr., Jackson, Miss. 39216.

The effect of diazepam administration on the threshold for pentylenetetrazol (PTZ) or audiogenic induced convulsions in pentobarbital dependent mice was assessed. Male ICR mice were rendered dependent on pentobarbital by the subcutaneous implantation of a 75-mg pentobarbital pellet (acid form).Control animals received placebo pellets. After three days of pellet implantation the pellets were removed and animals were administered saline or diazepam, 1 mg/kg, i.p. Six hours following pellet removal CNS hyperexcitability was evaluated by two different methods. At least three different doses of PTZ were administered s.c. to separate groups of animals. The percent of mice having a clonic convulsion within five minutes was recorded. A second method used for assessing CNS hyperexcitability was by administering at least three different subconvulsive doses of PTZ s.c. and after five minutes challenging each group with an audiogenic stimulus. Mice were administered the audiogenic stimulus by placing them in an audiogenic seizure box equipped with a two inch in diameter bell. The percent of mice having a tonic convulsion within thirty seconds after the initiation of the audiogenic stimulus was recorded. Diazepam was observed to increase significantly the ED50 for PTZ and audiogenic induced convulsions. The involvement of the GABA system related to the observed increase in ED50 for PTZ in diazepam administered animals was evaluated. The GABA levels in pentobarbital dependent animals was significantly lower than that of the placebo control mice. A further decrease in GABA was also noticed to occur in dependent mice that convulsed after the administration of PTZ as compared to those that failed to convulse. The GABA level in diazepam treated pentobarbital dependent mice was significantly elevated as compared to non-treated pentobarbital dependent animals. Also, the activity of L-glutamate-1-decarboxylase measured in dependent mice that convulsed was significantly lower than those of non-convulsed dependent mice. Thus, it was shown that diazepam is effective in increasing the ED50 for PTZ and audiogenic induced convulsions in pentobarbital dependent animals. Also, an observed decrease in GABA levels seemed to be associated with the PTZ and audiogenic induced convulsive response increase in pentobarbital dependent mice. Therefore, it is possible that the GABA system is involved in the increased hyperexcitability observed at six hours after pellet removal in pentobarbital dependent mice (Supported by Grant DA-01403 from the National Institute on Drug Abuse).

1880 MORPHINE ENHANCES AND DIAZEPAM SUPPRESSES THE NEUROTOXICITY OF SYSTEMICALLY ADMINISTERED KAINIC ACID. Terry A. Fuller and John W. Olney. Washington Univ. School of Med., St. Louis, MO 63110.

Systemically administered kainic acid (KA) induces wet-dog shakes (WDS), convulsions and acute neuronal necrosis in various brain regions, particularly the hippocampus and olfactory cortex. Since WDS is a behavioral phenomenon observed in rats undergoing naloxone-precipitated withdrawal from opiates, we explored in a previous study, the effects of naloxone pre-treatment on KA-induced WDS and found that naloxone conferred a mild protective action in relation primarily to WDS but also to the convulsions and brain damage induced by KA. Ben-Ari et al., (Br. Res., 165, 362-365, 1979) found that the anticonvulsant, diazepam suppressed convulsions and blocks the "distant" hippocampal degeneration associated with local injections of KA into the amygdala. Here we report that morphine augments and diazepam suppresses both the convulsions and brain damaging effects of systemically administered KA.

When adult male Sprague-Dawley rats were given 7 mg/kg KA subcutaneously (sc), only 1 of 14 (7%) exhibited convulsions and sustained acute damage to the hippocampus and olfactory cortex. When the same dose of KA was preceded by injection of morphine, 10 mg/kg sc, 10 of 10 animals (100%) convulsed and sustained hippocampal and olfactory cortical damage. When 29 rats were injected with 12 mg/kg KA sc, 27 (93%) exhibited convulsions and the typical pattern of brain damage, but when this dose of KA was preceded by 20 mg/kg diazepam sc, the pattern of brain damage was characteristically modified with pathological changes being mild and only detectable in one brain region (CA3 hippocampus). The severe convulsions typically observed in rats treated with 12 mg/kg KA were eliminated but the incidence of WDS was not reduced by diazepam pre-treatment.

Our findings suggest that KA-induced convulsions and brain damage are closely associated phenomena; morphine augments both effects and diazepam suppresses both. The suppressant action of diazepam differs from that of naloxone in that WDS were influenced by naloxone but not diazepam. Additional research will be required to adequately clarify the mechanisms and receptors involved in the modification of KA neurotoxicity by these agents. Support by NIH grants NS-09156, DA-00259, ES-07066, a Huntington's Chorea Fdn. grant and RSD Award MH-38894 (JWO).

1881 METABOLIC CORRELATES OF TOLERANCE TO ETHANOL IN TWO SPECIES OF DROSOPHILA. F. Garcin[1], S. Radouco-Thomas[1], S. S. Chawla*[1], J. M. Perron*[2] and C. Radouco-Thomas[1]. Unité de Recherche sur l'Abus des Drogues et de l'Alcool, Hôp. St-François d'Assise, Dépt. Pharmacol., Faculté de Médecine[1] and Dépt. Biol., Faculté des Sciences[2], Université Laval, Québec, Canada.

Two sibling species of drosophila were used: Drosophila Melanogaster (Colmar) and Drosophila Simulans (Villeurbane). In a first set of experiments, adult individuals of both species were submitted to ethanol selection pressures according to the method of David et al (1974). For each generation, the 50% lethal ethanol concentration (LC_{50}) was determined. Concentrations of ethanol ranging from 6 to 20% (Drosophila Melanogaster) and from 1 to 10% (Drosophila Simulans) were used.

In a second set of experiments, the animals were exposed to ethanol (LC_5) during the developmental stages and submitted in adulthood to the same selection pressures as mentioned above.

In the two sets of experiments the specific activity of alcoholdehydrogenase (ADH) was determined spectrophotometrically in the whole body and the separated head (brain) of adults. The enzymatic polymorphism was revealed by polyacrylamide gel electrophoresis (Borner 1974).

In the flies submitted to selection in adulthood without prior ethanol exposure, the ethanol tolerance (LC_{50}) increased from generation to generation. The tolerance increase seemed to be parallel in both species although the initial tolerance was lower in Drosophila Simulans than in Drosophila Melanogaster.

In flies exposed to ethanol during all the developmental stages the selection pressure seemed to be more efficient. The concomitant changes in ADH electrophoretic pattern and specific activity are discussed for both experiments.

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1882 PHYSICAL DEPENDENCE TO FK-33,824 [Tyr-dAla-Gly-MePhe-Met-(O)-ol], A SYNTHETIC METHIONINE ENKEPHALIN ANALOGUE, IN THE CHRONIC SPINAL DOG. P. E. Gilbert* and D. R. Jasinski. NIDA Addiction Research Center, Lexington, KY 40583.

FK-33,824 is a pharmacologically active peptide with some pharmacologic actions in the chronic spinal dog that resemble those of morphine (Gilbert and Jasinski, Fed. Proc., 1979). To confirm that FK-33,824 is a morphine-like drug, a direct addiction study was done in the chronic spinal dog. FK-33,824 was infused through an indwelling jugular catheter every 4 hr in a dose of 0.35 mg/kg/infusion in five chronic spinal dogs. The animals were stabilized at this dose of FK-33,824 for 2 weeks prior to the beginning of precipitation and suppression experiments. Throughout the study, 1 to 5 min after each drug infusion the dogs vomited. (This action of FK-33,824 was also observed in other dogs that received the drug only one time in acute studies.) Tolerance did not develop to this effect of FK-33,824, nor could the vomiting be blocked by the prior administration of 1.0 mg/kg of naltrexone in nondependent dogs. Naltrexone precipitated an abstinence syndrome in animals receiving FK-33,824 chronically. This abstinence syndrome resembled the naltrexone precipitated abstinence syndrome in morphine-dependent dogs. The most prominent signs of precipitated abstinence were stepping, salivation, mydriasis, tachycardia and tachypnea. Naltrexone was only about 1/8 as potent in precipitating an abstinence syndrome in FK-33,824-dependent dogs as it was in morphine-dependent animals. The dogs began to show signs of withdrawal abstinence approximately 4 hr after their last infusion of FK-33,824. Withdrawal from FK-33,824 had a more rapid onset and earlier peak (10-12 hr) than withdrawal from morphine. Both FK-33,824 and morphine suppressed withdrawal abstinence in 12-hr abstinent FK-33,824-dependent dogs. In conclusion, the chronic administration of FK-33,824 produces physical dependence in the dog. Additionally, the emetic effects of FK-33,824 are probably due to interactions at a non-opioid receptor since this action of FK-33,824 is not antagonized by doses of naltrexone, which precipitates an abstinence syndrome in dogs receiving FK-33,824 chronically and antagonizes the miotic and flexor reflex depression produced by single doses of FK-33,824.

1883 DEVELOPMENT OF OPIATE MECHANISMS IN THE GUINEA PIG SMALL INTESTINE IN RELATION TO THE ONTOGENY OF NEURONS KNOWN TO BE COMPONENTS OF THE ENTERIC NERVOUS SYSTEM. Alan R. Gintzler*, Taube P. Rothman and Michael D. Gershon. (Spon. K. Pfenninger.) Dept. of Anatomy, Columbia Univ., College of P&S, New York, New York 10032.

The development of opiate mechanisms in the guinea pig small intestine has been studied with reference to the development of transmitter mechanisms of neurons known to be components of the enteric nervous system. At 25 days' gestation, neurons and a primitive neuropil could be found in the enteric mesenchyme. At this time markers for two enteric neurotransmitters could be demonstrated, i.e., synthesis of ^{3}H-acetylcholine (^{3}H-ACh) from ^{3}H-choline, and specific axonal uptake of ^{3}H-5-hydroxytryptamine (^{3}H-5-HT). This early gut also showed high affinity, stereospecific binding of ^{3}H-diprenorphine that was antagonized by levallorphan but not dextrophan. Thus, opiate receptors are detectable in the gut as soon as neurons can be identified. Neuronal precursors, however, are probably present prior to 25 days. Putative serotonergic precursors were recognized by their uptake of ^{3}H-5-HT into cell bodies as early as day 20. Adrenergic innervation appeared gradually between days 32 and 48. Functional innervation of the longitudinal layer of smooth muscle was established much later in ontogeny. Spontaneous tone and a tetrodotoxin-sensitive, 5-HT-induced relaxation of the muscle were detected at day 42 (neither norepinephrine nor ATP mediate this effect). Contraction in response to ACh appeared at day 48 and responses to electrical stimulation were elicited at days 50-56. Acute and chronic (tolerance-dependence) effects of opiates were also apparent by day 56. The early appearance of opiate receptors is consistent with their being associated with either cholinergic or serotonergic neurons or both. Moreover these early opiate receptors suggest that opiates or endogenous substances that act on opiate receptors might have effects not revealed by standard indices of opiate actions. Supported by NIMH Grant #DA10772.

1884 CHOLINERGIC INTERACTIONS WITH STIMULANT-INDUCED MOTILITY. Larry P. Gonzalez and Everett H. Ellinwood, Jr., Dept. Psychiat., Behav. Neuropharm. Sect., Duke Univ. Med. Ctr., Durham, NC 27710

Recent evidence has implicated the involvement of striatal cholinergic (Ch) interneurons in a feedback regulation of activity in the nigrostriatal dopamine (DA) pathway. Since psychomotor stimulants are believed to act primarily through their effects on central DA activity, Ch modulation of DA neurons might play a role in mediating the effects of stimulant drugs. The present study examined the effects of altered Ch activity on behavioral stereotypy resulting from the administration of psychomotor stimulants. To determine the involvement of regulatory feedback mechanisms, a comparison was made between the effects of altered Ch activity on stereotypy resulting from treatment with the direct receptor stimulant apomorphine and effects resulting from agents which alter pre-synaptic DA release (amphetamine and methylphenidate). Altered feedback regulation of presynaptic DA activity would be expected to interfere with the effects of those stimulants acting pre-synaptically to a greater extent than with the effects of a direct receptor stimulant. The subjects were 258 male, Sprague-Dawley rats, 200-250 g. The measurement of stereotyped movements was performed in a Stoelting activity monitor, modified to permit quantification of restricted repetitive behaviors. This apparatus produced an analog signal with a frequency of oscillation equal to the frequency of occurrence of movements within the activity monitor. Twenty min. after an i.p. injection of either saline, physostigmine, neostigmine, scopolamine, or methylscopolamine, subjects received a second i.p. injection of either saline, amphetamine, methylphenidate, or apomorphine. Amphetamine, methylphenidate, and apomorphine produced dose-related increases in movement frequencies between 1 and 15 Hz, with a significantly greater increase in 8 Hz movements. We have previously demonstrated a significant correlation between such movements and the occurrence of stereotyped sniffing. Physostigmine significantly reduced the effects of all three stimulants. The effects of low doses of the stimulants were facilitated by scopolamine, but higher doses were not. Neostigmine and methylscopolamine did not alter stimulant-induced motility. These results indicate an involvement of central Ch neurons in the mechanisms by which stimulants alter behavior. The finding that apomorphine-induced motility is also affected by alterations in Ch activity suggests that these effects may not involve feedback regulating mechanisms, but rather suggests a more direct involvement of Ch neurons in the mediation of stimulant-induced behavioral effects.

1885 EFFECTS OF PICROTOXIN AND PHYSOSTIGMINE ON ETHANOL-INDUCED INHIBITION OF HIPPOCAMPAL UNIT ACTIVITY. Larry A. Grupp. Department of Pharmacology, University of Toronto and Addiction Research Foundation, Toronto, Canada.

We have previously demonstrated that high doses of ethanol inhibit spontaneous cell firing in the dorsal hippocampus of the awake rat. This report deals with the interaction of ethanol with each of two agents which increase the responsivity of hippocampal neurons by different mechanisms. Picrotoxin, a gaba antagonist may, in part, produce an increase in neuronal excitability by disinhibition, while physostigmine, an acetylcholinesterase inhibitor, may increase the excitatory cholinergic tone by maintaining high levels of acetylcholine.

Rats chronically prepared with hippocampal microelectrodes, fronto-cortical EEG electrodes and a jugular catheter, were pretreated intravenously either with picrotoxin (0.5 or 1.0 mg/kg) or physostigmine (0.15 mg/kg) 15 min prior to the infusion of ethanol (800 mg/kg). Recordings were taken for the entire pretreatment and ethanol periods (15 min each) and for a 15 min baseline period preceding the drug infusions.

Picrotoxin pretreatment increased unit firing rate above baseline levels, produced a reduction in heart rate and induced epileptiform (spike and wave) activity in the frontal cortex. The subsequent administration of ethanol produced an inhibition of firing which did not differ in degree from that obtained without pretreatment, although the final firing level achieved was considerably higher than that in the absence of any pretreatment. Cortical EEG was briefly normalized to low amplitude fast activity before reverting back to spike and wave activity, and heart rate reduction appeared to be reversed.

Physostigmine pretreatment produced decreases and increases in firing rate, yielded an essentially activated EEG, but did not decrease heart rate appreciably. The administration of ethanol for those units enhanced by the pretreatment brought about a quantitatively similar inhibition in rate to that obtained in the absence of pretreatment although final firing levels were again higher than in the absence of pretreatment. Those units inhibited by the pretreatment did not appear to show any further rate reduction following ethanol. Cortical EEG following ethanol showed little change from the activated mode, while heart rate also remained unaffected. Control experiments with neostigmine ruled out changes secondary to either cardiovascular or peripherally mediated effects.

These data indicate that the manipulation of hippocampal responsivity either by interfering with gaba-mediated inhibition or by augmenting cholinergically-mediated excitation does not alter the degree of firing depression produced by ethanol. (Supported by the Addiction Research Foundation of Ontario).

1886 VINYL GABA ALTERS AMPHETAMINE STEREOTYPY IN RATS. John Hammerstad, Lisa Gronke, John Nutt*, and Daniel Casey*. Depts. of Neurol. and Psych., U. Oregon Health Sci. Cent., Portland, OR 97201.

Initial studies of GABA-dopamine interactions in basal ganglia suggested that a striatonigral GABA system is inhibitory to the nigrostriatal dopamine pathway. However, direct injections into substantia nigra or systemic administration of GABA agonists have produced facilitation rather than inhibition of motor behaviors thought to be mediated by the nigrostriatal dopamine system. As part of a study of the role of GABA in an animal model of tardive dyskinesia, we made some further observations on this unexpected phenomenon by examining the effect of vinyl GABA, an irreversible catalytic inhibitor of GABA transaminase, on amphetamine stereotypy in the rat.

Eighteen hours after administration of vinyl GABA (1,000 mg/kg i.p.), control and treated animals (300 gm, male S-D rats) were given saline or dextroamphetamine (2 mg/kg). After two hours of behavioral observation, the animals were sacrificed and brains quickly removed and frozen for biochemical studies. In comparison with control animals, the vinyl GABA treated animals demonstrated an increase in the sniffing, gnawing stereotypy thought to be mediated through the neostriatal dopamine system while locomotor activity mediated by mesolimbic striatum was totally abolished. Striatal homogenates showed a 2-3 fold increase in GABA, a 42% inhibition of GABA transaminase activity and a 27% reduction in succinic semialdehyde activity. This dual action of elevated GABA on amphetamine stereotypy suggests differing interactions between GABA and dopamine systems in neostriatum and mesolimbic striatum. The results also provide additional evidence that treatments intended to increase GABA activity augment motor behavior mediated by neostriatum. Additional data, including results of GABA and dopamine binding in animals treated with neuroleptics, will be presented.

1887 ACTION OF CLONIDINE ON DOPAMINOCEPTIVE NEURONS OF THE SNAIL BRAIN
John C. Hancock, Department of Pharmacology, East Tennessee State University, College of Medicine, Johnson City, Tennessee 37601.

The action of clonidine (10^{-6} to 10^{-3}M) was studied on identified dopaminoceptive neurons in the abdominal ganglion of the snail, Helix aspersia. Standard microelectrode techniques were used to establish presynaptic, postsynaptic and local anesthetic effects of clonidine. On cell RPal (Judge, S.E. et.al. Comp. Biochem. Physiol., 61: 475-481, 1978), clonidine caused a hyperpolarization of the cell membrane, a decrease in input resistance and an inhibition of spontaneous firing. The membrane response to clonidine was less than that caused by dopamine (10^{-7} to 10^{-3} M) or methoxamine (10^{-7} to 10^{-3}M) but greater than that caused by isoproterenol (10^{-6} to 10^{-3}M). Disruption of synaptic transmission by elevating the extracellular Mg^{++} concentration did not alter the responses to these drugs. Dihydroergotamine (DHE: 10^{-6} to 10^{-3}M) but not chlorpromazine (10^{-3}M) or haloperidol (10^{-3}M) blocked the responses to clonidine, dopamine, methoxamine and isoproterenol on the post synaptic membrane. Stimulating the left pallial nerve evoked an inhibitory potential of long duration preceeded by an EPSP (ILD-E) in cell RPal. Stimulating the anal nerve evoked an antidromic potential followed by multiple firing. DHE but not chlorpromazine blocked all synaptically evoked potentials without affecting the antidromic potential. Clonidine (10^{-6} to 10^{-3}M) produced a progressive decrease in the synaptically evoked potentials without affecting the antidromic potential. For threshold stimulation, complete block of the ILD required 15 ± 1.7 min while blockade of the ILD-associated EPSP required 31 ± 1.8 min. For supra maximal stimuli, blockade of the ILD required 31 ± 1.7 min while blockade of the ILD-associated EPSP required 67 ± 1.5 min. Blockade of multiple firing accompanying stimulation of the anal nerve required 61 ± 1.9 min. Following complete blockade of the synaptic potentials by clonidine, the cell membrane was normally responsive to dopamine (10^{-7} to 10^{-3}M).

The results in elevated Mg^{++} demonstrate that clonidine has a direct post synaptic action on dopaminoceptive neurons to produce a dopamine-like response. The blockade of the responses to clonidine, dopamine, methoxamine and isoproterenol by DHE suggests that all of the drugs act on a dopamine receptor mediating inhibition. The failure to affect the antidromic potential eliminates the possibility of a local anesthetic action. The effect of clonidine to block synaptic potentials without affecting the cell response to dopamine suggests that clonidine has a presynaptic action to inhibit dopamine release.

1888 Dexamphetamine Increases Striatal Single Unit Activity in Freely Moving Rats.
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In awake freely moving rats, dexamphetamine (5 mg/kg, i.p.) significantly increased ($p < .001$) the firing rate of a group of individual striatal neurons (n = 13) by 100% during periods of drug-induced locomotion and stereotyped behavior. Individual cells increased their firing rate for periods ranging from 1 to 4 hours. However, the group as a whole had significantly elevated firing rates over the entire four hour time when contrasted with the preinjection rate. A group of saline (i.p. injection) control cells (n = 5) manifested no change in rate over a similar comparison period. This result agrees with a previous multi-unit recording study in freely moving rats which found that dexamphetamine (1-10 mg/kg, i.p.) caused small populations of striatal neurons to increase their average rate of discharge. In contrast, dexamphetamine has been reported to cause increases, decreases, or bi- and tri-phasic changes in firing of individual striatal neurons in immobilized, artificially respired rats. The discrepancy between data derived from freely moving and immobilized animal emphasizes the hazards of inferring neuronal correlates of dexamphetamine-induced behavior from experiments on immobilized non-behaving animals.

1889 BENZODIAZEPINE RECEPTORS IN PRIMARY CULTURES OF CEREBRAL CORTEX.
T.K. Harden and K.D. McCarthy*, Dept. of Pharmacology, Univ. of North Carolina, School of Medicine, Chapel Hill, N.C. 27514.

Benzodiazepine receptors were studied in primary cultures of rat cerebral cortex using ^{3}H-flunitrazepam (^{3}H-FNT) as a radioligand. Cultures containing mixed populations of neurons and glia were prepared from 18 day gestation rats and grown for 4 days. Scatchard analysis of ^{3}H-FNT binding to membrane fractions obtained from these cultures revealed a single high affinity binding site with a Kd (≈ 1.4 nM) identical to that observed in membrane preparations of adult cerebral cortex. Clonazepam, diazepam, oxazepam, and chlordiazepoxide inhibited the binding of ^{3}H-FNT with apparent Ki values (0.3, 8, 20, and 800 nM, respectively) that were similar to those determined for adult cerebral cortex. A variety of other non-benzodiazepine psychoactive drugs had little or no effect on binding at concentrations of competing drug up to 100 μM. Cultures grown for 4 days possessed the greatest amount of ^{3}H-FNT binding (101 ± 6 fmol/dish) and the highest specific activity of receptors (230 fmol/mg protein). Increasing the duration of the culture period resulted in a decrease in the number of binding sites per dish (day 8 = 44 ± 4 fmol; day 11 = 27 ± 4 fmol; day 15 = 13 ± 2 fmol). The decrease in ^{3}H-FNT binding sites coincided with a similar diminution in the number of neuronal elements as determined by (1) phase contrast microscopy, (2) transmission and scanning electron microscopy, and (3) veratridine-stimulated Na^+ uptake. In contrast, the number of glial cells and amount of protein per culture increased markedly over the same time period. When neuron-containing cell clusters in 3 day cultures were separated from the glial cell bed by physical agitation, greater than 90% of the ^{3}H-FNT binding sites were recovered in the cell cluster fraction. Growth of cultures in the presence of antimitotics resulted in virtual elimination of glial cells from the cultures with a much smaller reduction in the number of neurons. Under these conditions the number of ^{3}H-FNT binding sites was 60-70% of that observed in control cultures. Specific binding of ^{3}H-FNT was not detectable in pure astroglial cultures or in mixed glial cultures prepared from newborn rat cerebral cortex. The data indicate that primary cultures of rat cerebral cortex may serve as a useful model system for the study of benzodiazepine receptors. Preliminary results suggest that specific ^{3}H-FNT binding sites in these cultures may reside primarily on neuronal elements.

1890 EFFECTS OF ETHANOL ON SYNAPTOSOMAL CALCIUM TRANSPORT.
R. Adron Harris and William F. Hood. V.A. Med. Center and Dept. Pharmacol. U. Missouri, Columbia, MO 65212.

Effects of ethanol and related drugs on synaptosomal calcium transport were investigated using the K^+ depolarization-stimulated uptake of ^{45}Ca by intact synaptosomes (Blaustein, J. Physiol 247:617,1975) and the ATP-dependent sequestration of ^{45}Ca by intra synaptosomal organelles (Blaustein et al.,J. Gen. Physiol. 72: 15,1978) isolated from whole brain. In vitro addition of ethanol (EtOH), acetaldehyde (Acet)or pentobarbital (PB)inhibited calcium transport by both of these processes in a dose-dependent fashion:

Drug	$-K^+$	$+K^+$	ΔK^+	-ATP	+ATP	ΔATP
	nmol Ca/mg protein/1 min.			nmol Ca/mg protein/ 5 min.		
Control	3.0	8.0	5.0	0.3	1.3	1.0
Ethanol						
50 mM	3.0	7.1	4.1	0.3	1.3	1.0
200 mM	2.9	6.4	3.5*	0.3	1.2	0.9
800 mM	2.9	4.9*	2.0*	0.2	0.9*	0.7*
Acetaldehyde						
100 mM	1.9	4.4*	2.5*	0.2	0.6*	0.4*
Pentobarbital						
0.5 mM	2.6	4.8*	2.2*	0.4	1.2	0.8*

*Significantly different from control $p<0.01$

Results are from rats; similar effects were obtained in mice. The effects of these drugs were reversed when the membranes were centrifuged and resuspended in drug-free buffer. The effects were apparent as soon as 1 min. after drug addition and persisted at least 60 min. Ethanol inhibited the depolarization-dependent uptake equally over a range of assay temperatures (0°to 37°) but inhibited the ATP-dependent uptake only at higher temperatures.

Mice ingesting an ethanol-containing (7%v/v)liquid diet for seven days displayed altered synaptosomal calcium transport as compared to pair-fed controls. Depolarization-dependent and ATP-dependent transport were reduced by about 25% and 15%,respectively, by chronic alcohol exposure and the inhibitory effects of ethanol and pentobarbital added in vitro were reduced by chronic alcohol ingestion. Data from several strains of mice suggest that this effect is correlated with the development of tolerance to the behavioral effects of ethanol. In summary, the depolarization-dependent uptake was more responsive to alcohol than the ATP-dependent storage of calcium. These processes may be related,respectively,to the inhibition of depolarization-stimulated neurotransmitter release observed with moderate concentrations of ethanol and the increased resting release of neurotransmitter produced by high concentrations of ethanol. Both processes reflect the mechanisms responsible for ethanol tolerance and should prove useful for the study of these mechanisms. Supported by the National Council on Alcoholism and the Veterans Administration.

1891 THE RESPIRATORY AND CARDIOVASCULAR EFFECTS OF SINGLE AND MULTIPLE INJECTIONS OF MORPHINE IN THE DECEREBRATE RABBIT. A.H. Hassen and E.W. Riehl.* Div. of Allied Health and Life Sciences, University of Texas at San Antonio, San Antonio, Texas 78285.

Seventeen New Zealand rabbits (male, wgt. 2.3-3.7 kg) were used in a study of the respiratory and cardiovascular effects of morphine. During ether anesthesia, animals were decerebrated at the level of the superior colliculus and the trachea, femoral artery and femoral vein were cannulated. Upon completion of surgery, ether was removed and a minimum of one hour was allowed to elapse prior to the administration of morphine. Respiratory frequency and blood pressure were monitored. Heart rate (HR) and mean arterial pressure (MAP) were derived from direct measurements of blood pressure. Maximum changes occurred within 15 minutes of the intravenous administration of morphine. Results were stable over 30 minutes. In one group of animals, morphine was administered as a single, 6 mg/kg, injection. A second group received three, 2 mg/kg, injections, administered 30 minutes apart. Measurements before and thirty minutes after the single, 6 mg/kg, injections are shown in Table 1. Measurements before and thirty minutes after each of three, 2 mg/kg, injections are shown in Table 2. All values are MEAN±S.E.

TABLE 1.

	Control	6 mg/kg
Resp. Freq. (Breaths/Min)	46.5±5 N=5	23±6
H.R. (Beats/Min)	251±18.5 N=5	229±11
MAP (mmHg)	85±6.5 N=5	77±6

TABLE 2.

	Control	2 mg/kg	2 mg/kg	2 mg/kg
Resp. Freq. (Breaths/Min)	45±2.5	28±2.5 N=12	21±2	21±3
H.R. (Beats/Min)	281±11	253±14 N=10	241±15	243±14
MAP (mmHg)	82±5	73±4 N=10	70±4	72±6

Control injections of 1 ml phosphate buffered saline elicited small, transient, changes in blood pressure. Naloxone terminated the morphine response. These data demonstrate that respiratory frequency in the decerebrate rabbit is depressed to a greater degree than either heart rate or blood pressure. A single, 6 mg/kg, administration of morphine is found to be less effective than three, 2 mg/kg, injections. It is noted that the second and third injections are less effective than the first; the third injection producing little or no change. (Supported by NIDA Grant DA 01753-01).

1892 CYCLIC AMP AND OTHER ADENINE NUCLEOTIDES INHIBIT CA++-DEPENDENT POTENTIALS IN SYMPATHETIC POSTGANGLIONIC NEURONS. B.K. Henon and D.A. McAfee, Division of Neurosciences, City of Hope National Medical Center, Duarte, CA 91010, USA.

Previous studies in this laboratory have demonstrated that α-adrenergic agonists inhibit the inward Ca^{++} current in postganglionic sympathetic neurons of the rat. This results in depression of three Ca^{++}-dependent potentials: the shoulder on the repolarizing phase of the action potential, the hyperpolarizing afterpotential (HAP), and the Ca^{++} spike. Cyclic AMP has been implicated in both the mediation of α-adrenergic responses and in the control of Ca^{++} metabolism. Thus, we have examined the effect of several adenine nucleotides on Ca^{++}-dependent potentials recorded intracellularly from the rat superior cervical ganglion (in vitro).

Cyclic AMP depressed the HAP by 14% (n=4) at 0.01 mM and by 25% (n=9) at 1.0 mM. Dibutyryl and 8-Bromo cyclic AMP (n=3) also decreased the HAP about 25% at 1 mM. A number of other parameters were measured in the presence of 1 mM cyclic AMP. The duration of the HAP was reduced from 400 msec to about 250 msec. The action potential amplitude was only slightly reduced (1-3%), but its shoulder was significantly decremented. The input resistance was not affected. The Ca^{++} spike elicited in 1 μM TTX and 10 mM TEA was depressed by cyclic AMP, and this effect was not blocked by the α-antagonist, phentolamine, indicating that the effect of cyclic AMP was not due to release of endogenous catecholamines. Cyclic AMP hyperpolarized the postganglionic neurons (9 of 21 trials), but the effect was inconsistent and not dose dependent.

The phosphodiesterase (PDE) inhibitor Ro 20-1724 mimicked but did not potentiate the cyclic AMP effect on the HAP. In contrast, theophylline, a structurally dissimilar PDE inhibitor, caused a large increase in the magnitude and duration of the HAP. It is possible that theophylline has effects on Ca^{++} mechanisms unrelated to its PDE activity.

Adenosine (1 mM) reduced the HAP amplitude by 22% (n=4) and had a similar effect to cyclic AMP on the action potential amplitude and shoulder. 5'AMP also reduced the HAP (24%, n=4).

While cyclic AMP mimicked the effects of α-agonists on the Ca^{++}-dependent potentials, adenosine and 5'AMP also showed some of these effects. Thus, it is possible that cyclic AMP acts extracellularly on adenosine receptors or that adenine nucleotides act nonspecifically to antagonize Ca^{++}-dependent processes. These experiments, then, do not critically test the hypothesis that α-adrenergic actions are mediated by cyclic AMP. They do demonstrate that cyclic AMP has electrogenic actions. (Supported by NS-05820 and BRSG to C.O.H.)

1893 DIFFERENTIAL SENSITIVITY OF TWO TESTS OF DOPAMINERGIC FUNCTION TO ANTICHOLINERGIC ACTIVITY. W. F. Herblin. Central Research & Development Department, E. I. du Pont de Nemours & Company, Wilmington, DE 19898.

Evaluation of the interaction of scopolamine HBr with four neuroleptics on apomorphine-induced rearing in mice has revealed a consistent effect. Scopolamine HBr at about 3 mg/kg p.o. will double the dose of neuroleptic required to produce 50% inhibition of the rearing. This indicates that the apomorphine-induced rearing is 5-10 times less sensitive to anticholinergic activity than is amphetamine-induced turning in rats with unilateral lesions of the substantia nigra. In the latter test, the ED_{50} of neuroleptics is doubled by about .3-.5 mg/kg p.o. scopolamine HBr. It is thus possible that amphetamine-induced rotation is mediated predominantly by the striatum in which a strong cholinergic-dopaminergic balance is known to exist, while the apomorphine-induced rearing is mediated by the mesolimbic system in which the cholinergic function has been found to be considerably weaker.

1894 IN VIVO DETERMINATION OF ENDOGENOUS BIOGENIC AMINES IN RAT BRAIN USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY AND PUSH-PULL CANNULAE. J.N. Hingtgen*, C.C. Loullis, P.A. Shea and M.H. Aprison. Institute of Psychiatric Research and Depts. of Psychiatry and Biochemistry, Indiana U. School of Medicine, Indianapolis, IN 46223

In previous studies from these laboratories we have demonstrated that increases in total 5-hydroxytryptamine (5-HT) levels in specific brain areas (as well as increases in 5-HT levels in nerve ending fractions) were correlated with the disruption of food-reinforced behavior in pigeons and rats following the administration of L-tryptophan or D, L-5-hydroxytryptophan (5-HTP). One limitation in these experiments is that the trained animal must be sacrificed to permit the neurochemical determinations. Combining the methods of push-pull cannulation with those of high performance liquid chromatography (HPLC), we have measured a number of biogenic amines in the perfusate of freely moving rats. In an initial study, the hypothalamus was chronically implanted with a push-pull cannula and was perfused with 0.9% NaCl. Fifteen minute samples were collected through the push-pull cannula (flow rate: 25 μl/min) and 100 μl were injected on the HPLC without any extraction or prepurification procedure. Simultaneous determination of the levels of norepinephrine, dopamine, 5-HTP, 5-HT, and 5-hydroxyindole acetic acid (5-HIAA) in the perfusate was accomplished by means of HPLC with electrochemical detection. The HPLC system utilized a C-18 reverse phase column coupled with a glassy carbon detector and employed a modified version of the methods of Shea and Jackson (TRANS. AMER. SOC. NEUROCHEM. 10: 183, 1979). Results indicate that this combination of push-pull perfusions and HPLC assay methods can provide a simple, rapid, and sensitive technique for the in vivo simultaneous determination of the compounds released from discrete brain areas. In preliminary studies, in which 50 mg/kg D, L-5-HTP was injected (s.c.) into rats implanted with push-pull cannulae, significant increases in 5-HTP, 5-HT and 5-HIAA were measured over time using these methods. This technique could provide a useful tool in the assessment of neurochemical changes in brain during ongoing steady-state behaviors or during the disruption of behavior following administration of drugs, precursors, or other perturbations. (Supported in part by Research Grant MH-03225-20 from NIMH; Postdoctoral Grant PHS-TO1- MH 10695-13 from NIMH; and Indiana Dept. of Mental Health Grant 178-679-005 and Indiana Attorney General's Fund.

1895 Brain Serotonin and Spontaneous Locomotor Activity Changes Following Oral Tryptophan. K. Hirsh, L. DiMarco*, R. Pomerantz*, H. Cuzzone*, J. Springstead*, R. Ali* and D. Chou. General Foods Corporation Technical Center, Tarrytown, New York 10591.

It has been shown (1) that L-tryptophan (L-T) 0.03, 0.1, 0.3, 1.0 and 3.0g/Kg, p.o. in a 10% acacia vehicle produced a dose dependent trend toward depression of spontaneous locomotor activity (SLA) in mice. The effect was statistically significant at the two highest doses. In an effort to help define the mechanism of this action we studied the possible involvement of the brain serotonergic system. We determined the endogenous brain serotonin (5-HT) levels one hour after oral administration of the above doses of L-T, D-tryptophan (D-T) and the two combined, DL-tryptophan (DL-T) in 180 mice. The whole brain and brain stem, excluding the cerebellum, was dissected out over ice, after cervical dislocation. 5-HT was extracted using the butanol method and assayed fluorometrically. The results showed that brain 5-HT increases in a dose dependent manner as shown in the following table after oral L-T administration. It can be seen that brain 5-HT level reached a plateau at 0.3 g/Kg. At this dose L-T did not significantly reduce the SLA. At higher doses although brain 5-HT did not rise further, the SLA was more and more depressed. It is possible, based on this evidence alone that a non-serotonergic mechanism is involved in L-T effect on SLA. Further support for this contention comes from our results with D-T and DL-T. D-T by itself changed neither the brain 5-HT level nor the SLA. However, when combined with L-T it competitively inhibited depression of SLA (1) while brain 5-HT levels were raised in a manner identical to that observed after L-T alone. In summary we have demonstrated, in mice, an uncoupling of the rise in brain 5-HT level from SLA depression after L-T both in terms of the dose required to evoke these changes and in terms of the production of one response (increased 5-HT level) without the other (depressed SLA) through the use of a competitive inhibitor (D-T).

(1) McCardle, K. and Hirsh, K: Fed. Proc. 35 268, 1976.

Dose g/kg	Brain 5-HT ug/g	Dose g/kg	Brain 5-HT ug/g	Dose g/kg	Brain 5-HT ug/g
water	.683 ± .022				
acacia	.672 ± .042				
L-T 0.03	.743 ± .025	D-T 0.03	.712 ± .027	DL-T 0.06	.728 ± .032
L-T 0.10	.840 ± .040*	D-T 0.10	.737 ± .052	DL-T 0.20	.806 ± .043*
L-T 0.30	.903 ± .054*	D-T 0.30	.741 ± .037	DL-T 0.60	.864 ± .047*
L-T 1.00	.891 ± .047*	D-T 1.00	.720 ± .052	DL-T 2.00	.865 ± .041*
L-T 3.00	.891 ± .030*	D-T 3.00	.771 ± .043	DL-T 6.00	.861 ± .046*

*$P \leq 0.05$ values compared to acacia vehicle

1896 INTERACTION OF DIBUTYRYL CYCLIC AMP WITH THE EFFECT OF MORPHINE AND METHIONINE-ENKEPHALIN ON SPONTANEOUS AND EVOKED NEURONAL FIRING IN RAT MESENCEPHALIC RETICULAR FORMATION (David A. Hosford and Henry J. Haigler, Department of Pharmacology, Emory Univ., Atlanta, GA 30322)

Morphine sulphate and methionine-enkephalin (met-enkephalin), administered microiontophoretically (MI) into the rat mesencephalic reticular formation (MRF), produced different effects on spontaneous neuronal firing and on firing evoked by a nociceptive stimulus (NS: foot pinch) (Hosford and Haigler, Fed. Proc. 38: 740, 1979). The effects of the drugs on spontaneous firing did not correlate with their effects on firing evoked by a NS (evoked firing). Since cyclic AMP(cAMP), administered i.v., antagonized morphine-induced analgesia in mice (Ho et al, J. Pharmacol. Exp. Ther. 185: 347, 1973), we administered dibutyryl cAMP (dBcAMP), a phosphodiesterase resistant analog of cAMP, MI to determine if it antagonized the effects of MI morphine and MI met-enkephalin on neuronal firing in the rat MRF.

After male Sprague-Dawley rats (220 to 370 grams; n=65) were anesthetized with chloral hydrate (400 mg/kg i.p.), a scalp incision was made, and a hole was drilled in the skull. A five-barrel micropipette, containing 10 mM met-enkephalin, 50 mM morphine sulphate and 0.5 m dBcAMP in three of its four side barrels, was lowered into the MRF. The central barrel of the micropipette was used for recording neuronal firing and the fourth side barrel was used as a current "balance" barrel. All neurons tested (n=48) responded to the NS with a significant ($p<.01$: 1 way ANOVA) increase in firing. As described previously (Hosford and Haigler, 1979) there were three classes of neurons: a). MI morphine blocked evoked firing, MI met-enkephalin did not (n=4); b). MI met-enkephalin blocked evoked firing, MI morphine did not (n=2); c). both drugs blocked evoked firing (n=1). In all of these neurons (n=7) MI dBcAMP antagonized the blockade of evoked firing induced by both morphine and met-enkephalin. Morphine and met-enkephalin may block the increase in neuronal firing evoked by a NS via the common mechanism of inhibiting intraneuronal production of cAMP. The effect of dBcAMP on spontaneous firing was excitatory ($p<.01$: 1 way ANOVA) in some neurons (n=17), but not in others (n=19), and it was not correlated with the dBcAMP antagonism of the morphine or enkephalin blockade of evoked firing. This may reflect differing actions of cAMP in pre- and post-synaptic neuronal elements. (Supported in part by Helen Miller endowment fund 5729, NIDA Grant 1-R01 DA-01344-03 and a grant from the Women's Auxiliary of the Veterans of Foreign Wars.)

1897 LATERALIZED PERIORAL SENSORIMOTOR FIELD ACTIVATED BY INTRANIGRAL GABAergic DRUGS AND BY SYSTEMIC APOMORPHINE AFTER 6-OHDA NIGRECTOMY. J.P. Huston, B. Nef*, G. Papadopoulos* and H. Welzl*. Institute of Psychology III, University of Düsseldorf, Düsseldorf, FRG.

Unilateral injection of the GABA agonist muscimol (20 ng in 0.1 µg saline) into the substantia nigra of rats caused contralateral turning as well as an asymmetry in responsiveness to tactile stimulation. I.e. contralateral to the side of the injection touching of the lip, cheek or vibrissae elicited a reflex that consisted of a withdrawal of the lip, followed by a quick orientation towards and biting of the probe.

Unilateral injection of the GABA antagonist picrotoxin (200 or 300 ng in 0.1 µl saline) induced rotation in the direction ipsilateral to the injected hemisphere, and higher ipsilateral responsiveness.

We observed that systemic injection of apomorphine clearly primes the biting reflex to tactile stimulation of the perioral area in intact rats and guinea pigs. The apomorphine-primed perioral biting reflex is blocked on the side ipsilateral to the substantia nigra lesioned by 8 ug of 6-OHDA. I.e. apomorphine primed the perioral biting reflex contralateral to the 6-OHDA lesioned substantia nigra during periods of contralateral turning. Injection of d-amphetamine in these animals induced ipsilateral turning and increased responsiveness to tactile stimulation on the side ipsilateral to the lesioned substantia nigra, but did not prime the perioral biting reflex.

These experments demonstrate: (a) transient reversible asymmetries in sensorimotor responsiveness correlated with direction of pharmacologically induced turning, and (b) a neuropharmacological basis of the perioral biting reflex, probably involving substantia nigra mediated dopaminergic-GABAergic systems.

1898 N-DIPROPYLACETATE INDUCES A PREFERENTIAL INCREASE IN NERVE TERMINAL GABA IN VIVO. M.J. Iadarola, A. Raines and K. Gale*. Dept. of Pharmacology Georgetown Univ. Schools of Medicine and Dentistry, Washington D.C. 20007.

N-Dipropylacetate (DPA) and amino-oxyacetic acid (AOAA) produced dose related elevations of brain GABA as well as protection against maximal electroshock seizures (MES 150 mA, 0.2 sec, 60 hz) in rats. The ED_{50} for DPA in MES (200 mg/kg) caused a 15% increase in whole brain GABA. In contrast, AOAA, 20 mg/kg, increased whole brain GABA by 60% but only produced a 10% protection in MES (at ED_{50} for MES, AOAA, 60 mg/kg, elevated GABA over 3 fold). In view of the modest effect of DPA on brain GABA levels we were interested in determining whether the GABA increase produced by this drug in vivo is associated with nerve terminals. For this purpose we developed a surgical transection technique to unilaterally sever all connections between substantia nigra (SN) and the forebrain. This resulted in the complete destruction of GABAergic fibers afferent to substantia nigra (SN) leaving glial cells and neuronal perikarya intact. One week postoperatively, GABA concentration in the SN of the transected hemisphere was 10-20% of control. In the SN largely devoid of GABAergic nerve terminals, AOAA, 30 mg/kg, produced a marked (2-fold) increase in GABA content. In contrast DPA, 300 mg/kg, did not change the GABA content in the GABA-denervated SN. Since DPA and AOAA had similar effects in the intact SN (25% increase in GABA), it appears that the elevation of GABA produced by DPA is dependent upon the presence of GABAergic nerve terminals, whereas AOAA primarily elevates GABA in non-nerve terminal components.

As we have previously reported (Iadarola et al., Soc. for Neurosci. 444, 1978 and J. Neurochem. in press) an an analysis of GABA increases in several brain regions revealed that areas which were most affected by DPA were among those least affected by AOAA.

Taken together, our experiments suggest that the antiseizur efficacy of these drugs correlates with their ability to raise GABA in nerve terminals in specific brain regions and not with increases in GABA concentrations in whole brain. We have obtain ed additional support for this conclusion by examining the effects of ℵ-vinyl GABA (a specific and irreversible GABA transaminase inhibitor) on MES as well as on GABA concentrations in various brain regions and in the GABA denervated SN.

1899 OPIOID HYPERMOTILITY IN RATS: DIFFERENTIAL EFFECTS OF PUTATIVE μ, κ AND σ RECEPTOR AGONISTS. Edgar T. Iwamoto. Department of Pharmacology, University of Kentucky, Lexington, KY 40536.

Sprague-Dawley male rats were acclimated to a lucite observation cage; body movements were recorded observationally, and horizontal activity was recorded using a motility meter (Optovarimex). In agreement with previous data (Martin and Sloan, 1977, Handb. Exp. Pharmacol, Vol. 45/I, Springer-Verlag, p. 49), 0.25, 0.5, 1.0 and 2.0 mg/kg s.c. of the putative agonist, morphine sulfate, induced grooming-chewing stereotypies, and increased locomotor and rearing behavior; periods of motionlessness separated the morphine-induced hyperactivities. Saline-injected animals exhibited infrequent exploration or slept. Forward locomotion and grooming were also induced by 62.5 μg/kg levorphanol or 5 mg/kg l-pentazocine, but not after corresponding doses of dextrorphan or d-pentazocine. Ketazocine (2.5, 5, 10 mg/kg s.c.), the prototype κ agonist, depressed locomotion and produced antinociception, sedation, decreased respiratory rate, loss of corneal reflex and Straub tail; grooming behavior and catalepsy were not elicited. Naloxone, 0.1 mg/kg i.p., antagonized the hyperactivity syndromes induced by morphine and levorphanol, and the sedation induced by ketazocine. SKF-10,047, the prototype σ receptor agonist, induced continuous sniffing and locomotion (2.5, 5, and 10 mg/kg s.c.) that was antagonized only by large doses (>20 mg/kg s.c.) of naloxone. Both morphine and SKF-10,047 induced ipsilateral turning in the 6-hydroxydopamine circling behavior model suggesting possible indirect activation of the nigrostriatal pathways. Thus, μ, κ and σ prototypes induce separate locomotor syndromes in the rat with differential sensitivity to naloxone antagonism; with respect to locomotor activity in rats, additional support for the concept of multiple opioid receptors is provided (Martin et al. JPET 197:517, 1976).

The research described in this report conforms with the 'Guide for the Care and Use of Laboratory Animals' prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, DHEW Publication No. (NIH)78-23. Supported in part by BRSG RR 05374, Division of Research Facilities and Resources, NIH.

1900 BEHAVIORAL HYPERSENSITIVITY WITH APOMORPHINE: PREVENTION BY L-DOPA. Vernice Jackson, Carl Miller*, ALN Prasad*, Stuart Snider*, and Stanley Fahn. Dept. Neurology, College of P & S, Columbia University, New York, NY 10032.

Intermittent stimulation of dopamine receptors by dopamine agonists such as apomorphine may cause behavioral hypersensitivity. To test this possibility we administered the following drugs: 1a) unoperated rats received apomorphine, 1 mg/kg i.p. once weekly for four weeks; 1b) some also received daily doses of L-dopa 250 mg/kg + carbidopa 25 mg/kg p.o.; 2a) substantia nigra-lesioned rats received apomorphine i.p. once weekly; 2b) some substantia nigra lesioned rats received daily doses of L-dopa + carbidopa.

The unoperated and the lesioned groups developed behavioral hypersensitivity manifested by a gradual increase in motor activity and stereotyped behavior during the four weeks. This occurred more rapidly in lesioned rats. The unoperated and lesioned rats receiving L-dopa exhibited significantly less motor activity and stereotyped behavior than those not receiving L-dopa.

We believe that the more continuous stimulation of dopamine receptors in rats treated with L-dopa prevented the development of behavioral hypersensitivity during intermittent apomorphine injection.

1901 EFFECTS OF HYDERGINE® ON PERIPHERAL BLOOD ALCOHOL LEVELS. Francine Joiner*, David Fenimore* (SPON: T. Samorajski). Dept. Biol., Texas Woman's Univ., Houston, TX 77030.

Female C57BL/6J mice with a mean age of 9 months in groups of 6-8 animals were used in these experiments. In the first experiment, mice were given a single dose of ethanol (2 g/kg) or a combined dose of ethanol (2 g/kg) and dihydroergotoxine mesylate (DHET: active substance of Hydergine) (2.0, 4.0, or 8.0 mg/kg). In experiment No. 2, mice were maintained on daily doses of ethanol only (2 g/kg) or ethanol plus DHET (2, 4, or 8 mg/kg) for a period of 21 days. Blood samples were collected from each animal on the 21st day of the experiment following the last dose of ethanol or ethanol plus DHET. In the third experiment, mice received 2 mg/kg ethanol, 15 minutes apart. One group received DHET first and the other, ethanol. Blood samples (100 μl) from a cut of the tail were collected at 5, 15, 30, 60, 120, and 180 minutes after ethanol only or DHET plus ethanol treatment. Samples were analyzed by headspace gas chromatography. Results indicated: (a) Acute treatment with combined oral doses of ethanol and DHET decreased blood alcohol levels in a dose-dependent manner. (b) Chronic administration of ethanol plus DHET reduced blood alcohol levels. (c) Blood alcohol levels were lower when ethanol was given 15 minutes before DHET than when DHET was given 15 minutes before ethanol.

1902 EEG AND POWER SPECTRAL EFFECTS OF NARCOTIC AGONISTS IN THE RAT CONTRASTED WITH THOSE PRODUCED BY MIXED AGONIST-ANTAGONISTS AND PURE ANTAGONISTS. Sarala Kareti*, J. Edward Moreton and Naim Khazan. Dept. Pharmacol. and Toxicol., Univ. of Maryland, Sch. of Pharmacy, Baltimore, MD 21201.

Rats were prepared with chronic cortical and temporalis muscle electrodes. Continuous recording of the electroencephalogram (EEG) and electromyogram (EMG) was made for two days before and continued for two days after acute intravenous administration of physiological saline or moderate doses of the narcotic agonist morphine (10 mg/kg), the agonist-antagonists nalorphine (5 mg/kg), cyclazocine (2 mg/kg), and buprenorphine (1 mg/kg) and the pure antagonists naloxone and naltrexone (5 mg/kg each).

During the quiet awake state of the control period, the characteristic low-voltage, high-frequency EEG yielded a power spectrum with minimal amount of power mainly in the 0-10 Hz band. The narcotic agonist morphine first produced a behavioral stupor associated with intermittent high-voltage, slow-wave EEG bursts. This stage was followed by a more intense behavioral stupor and continuous EEG slow-wave activity. The agonist-antagonists nalorphine, cyclazocine, and buprenorphine produced a morphine-like behavioral profile and EEG synchrony. However, the pure antagonists naloxone and naltrexone exerted no discernable effect on EEG or behavior.

In an attempt to further delineate similarities and differences between the above EEG effects, these EEGs were subjected to power spectral analysis using the Nicolet MED-80. It was found that during behavioral stupor associated with continuous EEG high-voltage slow activity, a distinction between the pure agonist and the mixed agonist-antagonists could be made using the EEG power spectra. Morphine produced EEG synchrony whose power spectrum occupied mainly the 0-7 Hz frequency band. However, synchronous EEG produced by nalorphine and cyclazocine demonstrated a more condensed lower frequency activity which peaked at 1-3 Hz. On the other hand, the pure antagonists naloxone and naltrexone exerted no effect on either direct EEG or the EEG power spectra.

Furthermore, the agonist-antagonist buprenorphine produced behavioral stupor and continuous EEG high-voltage slow activity at the 1 mg/kg dose level, and a power spectrum similar to that produced by morphine. Earlier studies have shown that at the 1 mg/kg dose, buprenorphine displays an agonistic activity.

These findings suggest that narcotic agonists, agonist-antagonists, and pure antagonists exhibit characteristic EEG power spectra which may correlate with their different pharmacologic profiles. (Supported by NIDA Grant DA 01050.)

1903 CROSS-TOLERANCE BETWEEN ETHANOL AND MORPHINE. J.M. Khanna*, H. Kalant*, A.D. Le* and J. Mayer* (SPON: Y. Israel). Dept. Pharmacol., Univ. of Toronto, and Addiction Research Foundation, Toronto, Canada M5S 1A8.

Adult male Wistar rats were fed chronically a liquid diet providing 35% of the calories as ethanol (12-14 g/kg ethanol daily), while pair-fed controls received the corresponding diet with alcohol replaced by an equicaloric concentration of sucrose. Rectal temperatures, after test doses of ethanol or morphine, were measured in several groups of rats at various times during chronic ethanol treatment. The fall in rectal temperature after a challenge dose of ethanol (3.0 g/kg) was significantly lower in the alcohol group than in controls, indicating tolerance to ethanol-induced hypothermia as a result of chronic ethanol treatment. They also developed cross-tolerance to the hypothermic effect of morphine (15 and 30 mg/kg), whereas no cross-tolerance to the hyperthermic effect of morphine (5 mg/kg) was seen. Administration of morphine (30 mg/kg i.p.) for 3 days resulted in tolerance to morphine hypothermia and also cross-tolerance to ethanol-induced hypothermia.

In other experiments, guinea pigs were treated by subcutaneous implantation of morphine (4 x 75 mg) or placebo pellets for 3 days. Longitudinal muscle/myenteric plexus preparations obtained from the morphinized animals showed tolerance to the inhibitory effect of morphine on the electrically evoked contraction, as measured by parallel shifts in the log dose response curves. The extent of shift was approximately 1.8 log units. The same preparations were cross-tolerant to ethanol, showing a shift of about 0.8 log units in the LDR curves. Similar results were found in preparations, obtained from naive guinea pigs, preincubated with morphine in vitro (40 x 10^{-6} M for 18 hrs) before testing.

These studies fit with our hypothesis that tolerance and cross-tolerance among drugs develop to drug effects rather than to drug per se. Therefore drugs sharing a common effect, even by different mechanisms, might show cross-tolerance for that effect.

1904 DIFFERENTIAL EFFECTS OF "DOPAMINERGIC AGONISTS" ON MEASURES OF DOPAMINERGIC FUNCTION. C.D. Kilts*, D.A. Smith*, M.G. Ondrusek, R.B. Mailman, R.A. Mueller and G.R. Breese. Departments of Psychiatry, Pharmacology and Anesthesiology, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27514.

Apomorphine (APO) and the ergot derivatives lergotrile (LERG) and bromocriptine (BROM) increase locomotor activity of rats, an effect presumed to be related to a stimulation of dopaminergic activity. However, these drugs differ in how they affect locomotor activity in terms of potency (APO > LERG > BROM); latency to onset (LERG > BROM > APO); and duration (LERG = BROM > APO). Studies conducted utilizing 6-hydroxydopamine or α-methyltyrosine-pretreated animals indicate that inherent differences exist in the mechanisms underlying the locomotor increases evoked by APO, LERG and BROM. In addition to these behavioral assessments, the present study investigated several biochemical measures that have been hypothesized to reflect dopaminergic activity. The relative potency of these drugs in competing for ^{3}H-spiroperidol binding sites in a total particulate fraction of rat striata was BROM > APO = LERG. BROM was also more potent than LERG in displacing ^{3}H-spiroperidol (0.5-0.7 nM) from rat striatal membranes when the agonists were injected (i.p.) 2 hr before sacrifice. Micromolar concentrations of APO, but not BROM or LERG, stimulated basal adenylate cyclase activity in homogenates of rat striata. The in vitro stimulation of striatal adenylate cyclase activity by dopamine (5 μM) was antagonized by these drugs (LERG > APO > BROM) suggesting that these compounds are partial antagonists of this enzyme. In addition, it has been demonstrated that none of these drugs altered striatal cyclic AMP content in vivo. Therefore, these data are inconsistent with the postulate that cyclic AMP mediates the locomotor increases caused by APO, LERG and BROM. (Supported by USPHS Grants HD-10570, HD-03110, ES-01104 and MH-00013.)

1905 THE EFFECTS OF METOPRINE ON HISTAMINE-INDUCED HYPOTHERMIA. Michael C. Klein* and Sheldon B. Gertner* (Spon:A.G.Gona). Dept. Pharmacol. N.J. Med Sch. (CMDNJ) Newark, N.J. 07103.

Considerable data have accumulated to implicate histamine as a neurotransmitter within the central nervous system (CNS). However its precise physiologic function is still obscure. Central injections of histamine into the ventricular system or the anterior hypothalmus have resulted in a dose dependent fall in body temperature in rats which could be antagonized by histamine receptor antagonists (Brezenoff, H.E., and Lomax,P., Experientia, 26: 51,1970; Lomax,P., and Green, M.D., Prog. Brain Res., 42: 252,1975). Furthermore, systemic injections of histidine, the amino acid precursor of histamine or central injections of specific histamine receptor agonists have also induced significant hypothermic effects (Green, M.D., et al., Life Sci., 16: 1293, 1975; Cox, B., et al., J. Thermal Biol., 1:205, 1976).

Such data may implicate a functional role for histamine involving central thermoregulation. To further investigate this possibility male Sprague-Dawley rats were sterotaxically implanted with stainless steel canulae in the lateral ventricles (i.c.v.). One week later conscious animals were placed in restraining cages and injected (i.c.v.) with metoprine [2-4-diamino-5-(3,4 -dichlorophenyl)-6-methylpyrimidine] (10^{-5} molar). This drug has been demonstrated to be one of the most potent in vivo inhibitors of histamine N-methyl transferase (Duch, D.S., et al., Biochem.Pharmacol., 27: 1507,1978), the major and probably only significant metabolizing enzyme of histamine within the C.N.S. One hour later animals were injected (i.c.v.) with varying concentrations of histamine HCl (1-5ug). All experiments were conducted in a walk-in environmental chamber maintained at 20-22°C. Basal temperature was recorded by a thermistor probe inserted approximately 6 cm. into the rectum and gently taped to the tail.

Metoprine, in the doses used, significantly attenuated the histamine induced fall in body temperature (1-3°C) as well as reduced its duration of action (which was usually 2 to 3 hours). Central injections of metoprine alone, in the same doses, had no significant effects on body temperature. These paradoxical findings suggest that although metoprine may be a potent histamine N-methyl transferase inhibitor, it may also posess histamine receptor blocking activity. We are currently investigating the mechanism of this action. (Supported by grant #170000 GSBS,NJMS).

1906 Erythrosin B (Food, Drug, and Cosmetic Red No. 3) Inhibits Dopamine Uptake In Rat Caudate Synaptosomes: A Kinetic Study. J. Lafferman*, E. Silbergeld, NINCDS, NIH, Bethesda MD 20014 (SPON: R. Irwin)

Artificial food colors have been hypothesized to cause hyperkinetic behavioral disorders in certain children (Am. J. Nurs. 75, 797 (1975); Why Your Child Is Hyperactive (Random House, 1975)). A neurochemical study was undertaken to determine if erythrosin B has any properties of a central excitatory agent; we report here on its effects on synaptosomal dopamine metabolism. Synaptosmes were prepared as the P2 pellet from rat caudate and erythrosin B was added in vitro. Erythrosin B inhibited ^{3}H-dopamine uptake by synaptosomes with an IC_{50} equal to 45uM. The Michaelis-Menten kinetic constant (Km) decreased from 33.6nM to 13.5 nM and Vmax was decreased from 310 to 90 pmol/gram wet weight/5 min. From a Lineweaver-Burk plot of uptake kinetics, the type of inhibition observed is characteristic of uncompetitive or coupling inhibition. Uncompetitive inhibition of dopamine uptake by erythrosin B in vitro suggests that in the presence of dye, dopamine has a higher affinity for the transport carrier and the carrier-dopamine complex has a decreased efficacy of transporting dopamine across the synaptosomal membrane. One possible mechanism for this would be through an action by erythrosin B on sodium interactions with the carrier moiety.This is consistent with the lipophilic (Proc. Natl. Acad. Sci. 744, 2914 (1977)) and anionic nature of erythrosin B,to dissolve into the lipid membrane and to attract cations. In studies of synaptosomal ^{22}Na influx, it was found that erythrosin B increases sodium influx at concentrations around 0.5 uM. The results suggest that erythrosin B may be neuroactive if it acts similarly in vivo. An increase of dopaminergic transmission by the inhibition of dopamine removal from the synaptic cleft is consistent with hyperkinetic behavior which has been hypothetically associated with artificial food colors.

1907 AN ENDOGENOUS BARBITURATE/PICROTOXININ-LIKE SUBSTANCE IN MAMMALIAN BRAIN? Fredrik Leeb-Lundberg, *Christian Napias, *and Richard W. Olsen. University of California, Riverside, CA 92521.

α-Dihydropicrotoxinin (DHP) binding to mammalian brain membranes was inhibited by small amounts of the 100,000 x g supernatant fraction obtained from the same homogenate, and also by some purines and pyrimidines. Furthermore, DHP binding was inhibited by pharmacological concentrations of benzodiazepines, both depressant and excitatory. [^{3}H] DHP binding to fresh rat cerebral cortex membranes was measured by a centrifugation assay, giving a K_d of 2-3 μM and a B_{max} of 4 pmol/mg protein. Binding was highly enriched in the synaptosomal and light microsomal fractions; it was inhibited by biologically active picrotoxinin analogs, barbiturates (both depressant and excitatory), cage convulsants (Ticku et al. Mol. Pharm. 14, 381 [1978]; Life Sci. 22, 1643 [1978]; Neuropharm. 18, 315 [1979]), and by pyrethroid insecticides (Leeb-Lundberg, Napias and Olsen, in preparation). All of these drugs appear to have physiological effects involving Gamma-amino butyric acid (GABA) receptor-regulated Cl^- channels. The high affinity binding sites for these drugs (some K_d values in nM range) suggest the possibility of an endogenous ligand in brain. DHP binding was inhibited 50% by 50 μg of supernatant protein from rat cortex homogenate, assayed 10 min at 0° without preincubation, using 1 mg/ml membrane protein, [^{3}H] DHP at 10 nM, 29 Ci/mmol, in 0.1M NaCl, 20 mM HEPES, 1 mM CaCl2, pH 7.0. 50% inhibition of DHP binding was also obtained with 100 μM hypoxanthine and cytosine (but not several other bases nor nicotinamide at 1 mM, and by 1 μM benzodiazepines, including diazepam, flunitrazepam, nitrazepam and the convulsant analog RO5-3663. These results suggest that picrotoxinin/barbiturate binding sites might be receptor sites for endogenous ligands such as purines and pyrimidines (which also interact with high affinity binding sites for benzodiazepines) and that these substances may share some pharmacological actions at the level of Cl channels in GABA-mediated inhibitory synapses. Supported by NSF Grant BNS 77-24414 and NIH Grant NS-00224.

1908 KAINIC ACID-INDUCED NEUROLOGICAL SYNDROME: PARTIAL REVERSAL BY BACLOFEN AND OTHER PUTATIVE GABA-MIMETICS. Jeffrey Liebman, Patrick Bernard*, Richard Sobiski*, Gary Pastor* and Karen Dawson*. Research Dept., Pharmaceutical Div., CIBA-GEIGY Corp., Summit, NJ 07901

Kainic acid (KA) selectively destroys certain types of neurons when injected systemically or in various rat brain regions. This action is hypothesized to occur through a synergistic interaction with endogenously released glutamic acid, although not all reports are consistent with this proposal (Nadler, Life Sci. 24: 289, 1979). Because baclofen (BF) has been reported to reduce the release of glutamic acid in vitro (Potashner, Canad. J. Physiol. Pharmacol. 56: 150, 1978) it was of interest to evaluate the possible interaction of BF with KA. The effects of gamma-butyrolactone (GBL) and naloxone (NX) were also examined.

In the present experiments, KA (10 mg/kg i.v.) was administered to rats, which were then observed for 90 min afterwards by "blind", experienced observers. Wet dog shakes became apparent approximately 20-25 min after injection. At 30-35 min, hypersalivation was noted and was usually followed several min later by clonic seizures usually involving the jaw and forelimbs. KA also elicited a unique behavior, persistent scratching of the face with the hind legs. The ability of test drugs (i.p.) to antagonize its effects was evaluated by pretreating rats 30 min prior to i.v. KA.

BF (10-20 mg/kg) reduced the incidence of KA-induced clonic seizures. This effect contrasted strikingly with its inability to prevent metrazol- or electroshock-induced seizures. Similarly GBL (30, 100 or 300 mg/kg) selectively antagonized KA-induced seizures. Hypersalivation was reduced by the highest doses of BF (20 mg/kg) and GBL (300 mg/kg) tested. Hindleg scratching was antagonized by low doses of BF (3 mg/kg and up) and by high doses of GBL (300 mg/kg). None of these treatments altered the incidence of wet dog shakes. NX (3 and 30 mg/kg) failed to alter any of these KA-induced signs. Intrastriatally administered KA in rats reliably reduces striatal choline acetyltransferase levels, presumably by destroying striatal cholinergic neurons. BF (20 mg/kg i.p. at 1 hr before and again 2, 5 and 8 hr after intrastriatal KA, 0.5 μg, 1 μl) totally failed to antagonize this neurochemical effect of KA.

These results indicate that BF and GBL, which are not effective in traditional models of anticonvulsant activity, ameliorate the convulsant action of KA, as well as other induced neurological effects. The inability of BF and GBL to reverse KA-induced wet dog shakes and striatal choline acetyltransferase depletion suggests that the effects of KA may be mediated by multiple neurochemical substrates having differential susceptibility to various antagonists.

1909 A CONTROLLED RELEASE IMPLANT FOR CHRONIC ADMINISTRATION OF COCAINE. D.R. Liston*, C.D. Ebert*, S.W. Kim*, and J.W. Gibb (SPON: L.W. JARCHO). Department of Biochemical Pharmacology and Toxicology and Department of Pharmaceutics, University of Utah, Salt Lake City, Utah 84112.

The effects of chronic drug exposure can differ substantially from those of acute administration. Depending on the properties of the drug, either tolerance or sensitization to drug effects may result from chronic administration. Chronic studies on the effects of rapidly metabolized drugs, such as cocaine, present several technical problems, e.g., repeated injection requires either high doses or short dose intervals, the former causing wide variations in plasma drug concentrations while the latter can be technically inconvenient. Utilization of constant i.v. infusion requires some constraint of the animal. We are evaluating a sustained release drug delivery system composed of hydrogel polymers for the chronic administration of cocaine HCl. Advantages of the hydrogel system include suitable release kinetics for hydrophilic drugs and a high degree of tissue compatibility. In vitro release kinetics were determined for the polymeric, monolithic device emersed in twice-distilled water at 22°C. For gels equivalent to those implanted into test animals, typical first-order release kinetics were observed with a release rate of 8 mg/hr at 2 hours and 0.6 mg/hr at 36 hours. Subcutaneous placement in rats of gels containing a total dose of approximately 500 mg/kg cocaine HCl produced an increase in locomotor activity, followed by a progression to moderate stereotypic behavior within 6 hours after implant. Stereotypy continued for at least 36 hours. Preliminary biochemical experiments have indicated that tyrosine hydroxylase activity in the neostriatum is not altered 36-40 hours after implant. Current in vitro experiments with a combined polymeric device composed of a hydroxyethylene methylacrylate/cocaine HCl core encapsulated in a methoxyethyl methacrylate outer layer indicate that zero-order release kinetics can be achieved with cocaine for over ten days. This delivery system appears applicable to a wide range of drugs whose chronic delivery may be interesting and otherwise technically difficult. (Supported by USPHS grants GM 07579 and DA 00869.)

1910 BIPHASIC INTERACTION OF DIRECT-BUT NOT INDIRECT- ACTING GABA AGONISTS AND ANTAGONISTS WITH DOPAMINE-MEDIATED EVENTS. K. G. Lloyd and P. Worms *, Dept. of Neuropharmacology, Synthélabo-LERS, 31, Ave P. V. Couturier, F 92220 Bagneux, France

The control by GABA neurons of nigrostriatal dopamine (DA) -utilizing neurons has been demonstrated using both biochemical and behavioural methods. Thus, at anticonvulsant doses GABA-mimetics decrease striatal DA release, block apomorphine stereotyped behaviour and potentiate neuroleptic (eg haloperidol)-induced catalepsy. GABA-receptor antagonists have the opposite effects. However, in view of recent observations that DA agonists have biphasic actions in these test systems, purportedly due to dose-dependent pre-versus post-synaptic actions, we have investigated the interaction of a wide dose-range of GABA agonists and antagonists on DA-mediated events. For these studies catalepsy was measured utilizing the 4-cork test in male albino rats after an injection of 0.6 mg/kg i.p. of haloperidol. Stereotypies were rated in male albino mice after 0.5 mg/kg, s.c. of apomorphine. Direct acting GABA mimetics (SL 76 002, muscimol) showed a clear-cut biphasic action : haloperidol-induced catalepsy was antagonized and apomorphine stereotypies enhanced at low doses (12.5 mg/kg i.p. for SL 76 002 ; 0.25 mg/kg i.p. for muscimol, $p < 0.01$ vs reference compound alone in both cases) and markedly potentiated catalepsy and diminished stereotypies at high doses (100 mg/kg, i.p. for SL 76 002 ; 2.0 mg/kg i.p. for muscimol, $p < 0.01$ vs haloperidol alone). Accordingly, bicuculline (a direct GABA receptor antagonist) increased haloperidol-induced catalepsy at low (0.03 mg/kg, i.p.) doses and antagonized catalepsy at higher (0.3 mg/kg, i.p.) but still nonconvulsant doses. In contrast, agents (AOAA, gamma-acetylenicgaba) which indirectly (by means of inhibition of GABA-transaminase) increase GABA synaptic activity only potentiated catalepsy. Also inhibition of GABA synthesis by allylglycine only demonstrated an antagonism of haloperidol catalepsy. These results demonstrate that changes of GABA receptors has a biphasic curve whereas alteration of GABA levels has only a monophasic dose-response. This might be due either to the differential morphological arrangement of pre-versus post-synaptic GABA receptors and/or the existence of extra-synaptic GABA receptors which respond to injected GABA agonists or antagonists but which are relatively insensitive to alterations in the synaptic concentrations of this neurotransmitter.

1911 BEHAVIORAL EFFECTS OF CENTRALLY ADMINISTERED CYCLIC GMP. Irwin N. Lourie*, Michael M. Krieger* and Nagendran S. Thampi. Res. Dept., Norristown State Hospital, Norristown, PA 19401.

Intracerebroventricular (ICV) cannula were implanted in 92 day old male NSH Sprague-Dawley derived rats. Following a two week recovery period subjects were conditioned to a novel environment for one hour daily until behavior stabilized (after eight days). On subsequent days and 30 minutes after the ICV administration of either saline or dibutyryl cyclic 3',5' guanosine monophosphate (dbcGMP) animals were observed for one hour. Behavioral observations reported consisted of five consecutive one minute samples taken at the beginning of the assay. Behavior observed consisted of two types. Normal exploratory measures were made in which the following showed dbcGMP related effects: dynamic activity (forward motion) (DA), vertical exploratory activity (VE), total exploratory time (TE), and habituation rate (HR). Atypical behavior could be described by catatonia (C), wet dog shakes (WDS), convulsions (CON), and characteristic cholinergic effects. Dose range was 5 to 100 µg with all doses administered in 10 µl of saline with an n of 5 to 7 subjects per dose.

There are four observable dose responsive effects of dbcGMP on exploratory activity. At doses between 5 and 10 µg there is a 40% decrease in DA without any decrease in TE, indicating a specific effect on motor activity sparing the motivational component. Between 10 and 100 µg there is a biphasic dose response on these same measures ranging from depression at 10 µg, excitation at 50 µg, and subsequent depression at 100 µg. Concomitant with the depressed phase is an effect seen with neuroleptics and certain amygdaloid lesions: a two-fold increase in exploratory habituation rates. A fourth effect is a linear log dose response decrease in VE activity with complete suppression at 100 µg. Appearance of atypical behavior begins to occur at 100 µg or with repeated dosing. WDS and C are reversible but CON behavior is invariably irreversible leading to extensive brain damage and death. The multiplicity of effects of dbcGMP and its effectiveness at low doses seems to suggest a complex modulatory role for this substance in neural mechanisms.

1912 ^{3}H-SPIROPERIDOL BINDING SITES IN HUMAN CAUDATE AND PREFRONTAL CORTEX, M.E. Maguire, A.C. Andorn*, and L.E. Weber*. Departments of Pharmacology and Psychiatry, Case Western Reserve University, School of Medicine, Cleveland, OH 44106.

The examination of ^{3}H-spiroperidol (^{3}HSP) binding in rat striatum has shown the existence of two binding sites with different pharmacologies (Andorn and Maguire, Soc. for Neurosci. IV:504, 1978). The high affinity site (K_D=20pM) exhibits a pharmacology that is classically dopaminergic, while the second site (K_D=2nM) appears pharmacologically to be an α-adrenergic receptor even though dopamine is presumably the transmitter. Our data to date suggest that properties of ^{3}HSP binding and the associated pharmacology are similar in postmortem human caudate. Examination of human prefrontal cortex suggests only a small amount (if any) of a high affinity site, while the density of a lower affinity binding site is comparable to that seen in the caudate. However, the pharmacology of this lower affinity site differs markedly from the pharmacology of either of the caudate sites. Although neuroleptic potency was similar, and apomorphine and serotonin were potent competitors (apomorphine > serotonin), dopamine and norepinephrine competed only at mM concentrations. Our prefrontal cortical data is similar to data recorded by others in frontal cortex and not only suggests that the prefrontal cortical site is not the same site as those observed in the caudate but that it is primarily serotonergic.

We obtained postmortem tissue samples from a male schizophrenic in whom no tissue or fluid concentrations of psychotropic agents could be detected at autopsy, ^{3}HSP binding studies on prefrontal cortex and caudate show marked changes in affinities for agonists and antagonists. The differences in binding make it difficult to determine if a change in density also exists in prefrontal cortex although there are no immediately apparent differences between normal or unmedicated schizophrenic caudate. It is premature to speculate on the clinical relevance of these observations. (NSF PCM 77-24693 and NIH GRS RR05140A2)

1913 A PHYLETIC STUDY OF BICUCULLINE-SENSITIVE GABA RECEPTOR BINDING. E. Mann* and S.J. Enna (SPON: I. Butler). Depts. Pharmacol. and of Neurobiol. and Anat., Univ. Texas Med. Sch., Houston, Tx. 77025

γ-Aminobutyric acid (GABA) appears to be an inhibitory neurotransmitter in both vertebrates and invertebrates. In addition, recent studies have indicated that there may be present, at or near this receptor site, a modulator(s) of GABA binding, which can be removed by the detergent Triton X-100, and which may play a role in the mechanism of action of the benzodiazepines. The present study was undertaken to describe the pharmacological, biochemical and kinetic properties of the GABA receptor in a variety of species in an attempt to better define these characteristics. For the study, ^{3}H-GABA receptor binding was studied in membrane fractions prepared from vertebrate whole brain or invertebrate cephalic ganglia using a previously published proedure (Enna and Snyder, Mol. Pharm. 13:442, 1977). Specific GABA receptor binding was defined as the amount of ^{3}H-GABA displaced by unlabeled bicuculline (0.1 mM). In tissue not treated with Triton, a significant amount of bicuculline-displaceable ^{3}H-GABA binding was detected in the brains of all 9 vertebrate species studied, with the hagfish, the oldest vertebrate, binding over twice as much ^{3}H-GABA as the spiny dogfish, the net oldest species. All other vertebrates bound similar amounts of ^{3}H-GABA, being one-third to one-fourth that observed in the hagfish. In contrast, after Triton-treatment, the hagfish displayed the least amount of bicuculline-sensitive ^{3}H-GABA binding and, under these conditions, the amount of binding observed increased in an evolutionary fashion. Kinetic analysis indicated that Triton treatment enhanced binding in all vertebrates, except the hagfish, by increasing the affinity of the receptor for GABA. No measurable bicuculline-sensitive GABA receptor binding was noted in any invertebrate studied, suggesting that the receptors in these species are relatively insensitive to this antagonist.

These results suggest that bicuculline-sensitive GABA receptors are present in the brains of all vertebrates and that during the course of evolution there developed a Triton-sensitive substance (s) whose presence modifies the kinetic properties of this receptor site. This data, taken together with a previous report (Nielsen et al, Brain Res. 141:342, 1978) indicating a lack of benzodiazepine receptor binding in the hagfish, is further circumstantial evidence that these drugs may interact with a Triton-sensitive modulator(s) at the GABA receptor. (Supported in part by USPHS grants NS-13803 and an RCDA NS-00335 (S.J.E.))

1914 LITHIUM ANTAGONISM TO CLOZAPINE-INDUCED LEUKOPENIA. Philip J. Maple*, Bruce I. Diamond, and Richard L. Borison (SPON: C.M.Combs) Dept. Anesthesia, Mt Sinai Hosp., Chicago, Il 60606

Among neuroleptics, clozapine is unique because it fails to produce extrapyramidal side-effects and it has little demonstrated dopamine blocking activity. The clinical use of this drug in Europe has firmly established its use as a potent antipsychotic. Moreover, clozapine's lack of extrapyramidal system actions precludes its producing tardive dyskinesia. At present, this drug has been taken off the market due to its ability to produce agranulocytosis. We have now conducted studies to test whether lithium can reverse clozapine-induced agranulocytosis. Subjects were white male Swiss mice (25-30 g) which received daily injections of either saline, clozapine (15 mg/kg), lithium chloride (45 mg/kg) or the combination of lithium and clozapine. We found that after three weeks of treatment, that those animals receiving lithium showed a mild leukocytosis to 110% of control values. In contrast, animals receiving clozapine showed a marked leukopenia to 41% of control values, whereas animals receiving lithium-clozapine in combination showed a less drastic reduction of white blood cell count to 55% of control. Moreover, nine weeks after initiation of treatment, there was a recovery of white blood cell count to 64% of baseline values in animals receiving lithium and clozapine. Those animals receiving lithium achieved serum concentrations of 1.2 meq/l. The mechanisms underlying the hematologic actions of lithium remains to be fully elucidated, however lithium has been demonstrated to stimulate granulopoiesis, and it is suggested that it increases production of granulocytes rather than redistributing the granulocytes from the bone marrow to the circulation. In our experiments we have demonstrated both the leukopenic actions of clozapine, and its antagonism by conjoint treatment with lithium. These studies would suggest that combined therapy using lithium and clozpine may prove a clinically safe and rational treatment for schizophrenia.
(Supported by DMHDD grant 910-01)

1915 COMPARISON OF THE PATTERN OF MORPHINE-INDUCED CHANGES IN CORE TEMPERATURE AND MOTOR ACTIVITY IN THE RAT. Gregory E. Martin, Nan L. Papp* and G. Rufus Sessions Merck Institute for Therapeutic Research, West Point, PA 19486 and Walter Reed Army Institute of Research, Washington, D.C. 20012.

Restraining a rat in a plastic cage attenuates morphine-induced increases in core temperature (CT) whether morphine is given i.p. or intracerebrally (i.c.). Perhaps, the blockade of increased locomotor activity (LMA) is the action which results in the attenuated rise in CT. The purpose of the present experiments was to compare the time course of morphine-induced increases in LMA and CT in the rat following the drug's i.p. or i.c. administration.

Two measures of LMA were used. One was in a circular open field (OF) and the other was confinement motor activity (CMA) which records activity when the rat rears on its hind legs. OF counts were totaled at 15-min intervals following the injection of morphine in male rats, whereas CMA counts were totaled at 30-min intervals. CT was measured at 15 or 30 min intervals. Morphine was given i.p. in doses of 5, 15 and 30 mg/kg. It was given i.c. into the pre-optic anterior hypothalamus (POAH) in doses of 10, 20 and 50 µg. There was also a control group given the vehicle solution by each route.

In comparing the pattern of LMA and CT changes following i.p. morphine treatment, the time interval in which motor activity was greatest either preceded (2/6 treatments) or was coincident with the greatest recorded CT (4/6). In the OF test, the peak level of activity was recorded 30-60 min before the peak CT after the 30 and 15 mg/kg doses of morphine, whereas the 5 mg/kg dose and all dose levels in the CMA test elicited peak CT and LMA during the same time interval. Examination of the LMA vs time and the CT vs time curves reveals a marked similarity between the two functions following each dose of morphine given i.p. Although an increase in CT was observed following the i.c. microinjection of morphine, the increase in CT preceded the increase in LMA activity in 5/6 treatment groups. On the other hand, the peak periods for LMA and CT were coincident in 3/6 treatment groups, while the peak LMA followed the peak CT in 2/6 treatments and preceded it in 1/6.

These data indicate that a causal relationship may exist between the increase in LMA and the rise in CT observed following the i.p. administration of morphine. If true, this may partially explain the action of restraint on morphine-induced changes in CT. Since the increase in CT preceded the increases in LMA following the POAH injections of morphine, LMA is probably not crucial to the initiation of this hyperthermic response, but may contribute to its prolongation.

1916 KETAMINE BINDS TO BRAIN OPIATE RECEPTORS. Lawrence H. Matt*, Gopi A. Tejwani* and Joseph R. Bianchine. Dept. of Pharmacology, College of Medicine, The Ohio State University, Columbus, OH 43210.

Ketamine hydrochloride (2-[o-chlorophenyl]-2-[methylamino] cyclohexanone hydrochloride) is a short-acting, anaesthetic analgesic with unique side-effects including ataxia, catalepsy, and epileptiform EEG's. The mechanism by which ketamine causes analgesia and its "dissociative" anaesthesia is unclear. To determine whether an interaction with the endogenous opioid system might be part of the mechanism by which this drug acts, we have performed opiate binding assays to discover if ketamine displaces the stereospecific binding of [^{3}H]-naloxone to the opiate receptor.

The assay system we used is essentially the same as that described by Pert and Snyder (PNAS 70: 2243, 1973). Briefly, after killing a rat by decapitation, we excised the brain and removed the cerebellum from the brain. We then homogenized the brain in 50 mM Tris-HCl, pH 7.4. Two ml aliquots of the brain homogenate were incubated with various concentrations of drug in the presence of [^{3}H]-naloxone (45,000 cpm) for 3 hrs at 0 C. Stereospecific binding was taken to be the binding of [^{3}H]-naloxone in the presence of 1 µM dextrorphan minus its binding in the presence of 1 µM levorphanol. Under these conditions, ketamine was approximately as effective as morphine in displacing [^{3}H]-naloxone from opiate receptors. Preliminary results suggest that the ID_{50} 's of both ketamine and morphine are about 10 nM. (This work was supported by gifts from Mrs. Marion Colwill and Mr. Max Weiss.)

1917 DOES THE LEVEL OF SOUND STIMULATION AFFECT THE NEUROTOXICITY OF KAINIC ACID IN THE GUINEA PIG COCHLEAR NUCLEUS? Douglas E. Mattox*, Robert L. Gulley, and Stephanie Bird. (SPON: M. Vaughan) Dept. Surg. (Div. of ORL) and Anat., University of Texas Health Science Center, San Antonio, TX 78284 and Neuroscience Research Program, Jamaica Plains, MA.

Kainic acid causes a selective degeneration of neurons in the cochlear nucleus which receive primary auditory input. In this study kainic acid, 0.5 g in phosphate buffered saline, was injected in the brain stem adjacent to the cochlear nucleus of adult guinea pigs. At short time periods, this dose of kainic acid causes only a minimal amount of neuronal loss in the rostral anteroventral cochlear nucleus (Bird and Gulley, 1979). Animals injected with kainic acid were placed in a sound-reducing auditory chamber for four hours. Previous studies in this acoustic environment have shown that auditory activity is sufficiently reduced to produce significant morphological changes in the presynaptic terminals after 24 hours (Gulley, Mattox, and Ulrich, personal observation). Control animals received either an injection of phosphate buffered saline followed by four hours of sound reduction or 0.5 g of kainic acid followed by four hours of exposure to ambient noise. Neuronal degeneration in the cochlear nucleus was observed in animals injected with kainic acid after both sound deprivation and exposure to ambient noise. The amount of neuronal degeneration was identical in both groups. No degeneration was observed in sound-deprived animals injected with buffer. Zaczek et al (1978) described attenuation of the effect of kainic acid by various anesthetics which limit neuronal excitation. Our data suggest that, in the cochlear nucleus, decreased sound-evoked activity does not protect postsynaptic neurons from minimally toxic doses of kainate. This observation supports the findings in the cochlear nucleus that presynaptic terminals are not essential to mediate kainate neurotoxicity (Bird and Gulley, '79).

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1918 STUDIES ON PHENCYCLIDINE (PCP) LOCALIZATION IN RAT BRAIN Richard C. Meibach, Stanley D. Glick, and Saul Maayani.* Department of Pharmacology, Mount Sinai School of Medicine, New York, New York 10029.

The deoxyglucose technique as described by Sokoloff, et al. was used in an attempt to define the possible sites of action of PCP by determining its effects upon glucose metabolism. Rats were injected with PCP (5mg/kg) IP, 40 minutes prior to administration of 25 µCi of ^{14}C-deoxy-d-glucose (DDG) (specific activity 40 Ci/mmole). Examination of the autoradiograms revealed a striking effect of PCP upon local cerebral glucose metabolism. Major increases were limited to limbic system structures, particularly those involved in the Papez circuit. These included the hippocampus (molecular layer of the regio superior), subicular cortex, anterior and posterior cingulate cortices, and anterioventral thalamic nucleus. Decreases were noticeable only in auditory structures most notably in the inferior colliculus. In order to quantify the autoradiographic data, experiments were repeated using tritium labeled DDG and counting freshly dissected brain parts in a liquid scintillation counter. The results of these experiments confirmed the autoradiographic data in that the anterior cingulate gyrus had a 26% increase in glucose metabolism while the inferior colliculus reflected a 46% decrease. Other structures which reflected increases in glucose utilization but were not readily apparent in the autoradiograms included the frontal cortex and striatum.

The distribution of PCP within the brain was determined following injection of ^{3}H-PCP. Surprisingly, the distribution closely paralleled that of DDG changes, with the anterior cingulate having the greatest uptake.

These results suggest the possibility that PCP may act primarily on the limbic system and could possibly account for the fact that PCP abuse is often associated with severe emotional disturbances.

1919 EFFECTS OF AMANTADINE ON RAT PLASMA PROLACTIN LEVELS. H.Y. Meltzer and V.S. Fang. Depts. of Psychiatry and Medicine, Univ. of Chicago Pritzker Schl. of Medicine, Chicago, Ill. 60637.

Amantadine (A) is an anti-parkinsonian drug whose mechanism of action is uncertain. There is much evidence against the suggestion that it acts via enhancement of dopamine (DA) release or inhibition of DA uptake. It has also been proposed (Cox and Tha, Eur. J. Pharmacol., 30, 344, 1975) that A may increase receptor sensitivity to serotonin (5-HT). We have investigated these hypotheses by studying the ability of A to influence the increase in rat prolactin (PRL) secretion induced by haloperidol (H) a dopamine antagonist, and 5-hydroxytryptophan (5-HTP), the precursor of 5-HT. We have previously shown that drugs which release DA and/or block its reuptake such as d-amphetamine and methylphenidate antagonize the H- and 5-HTP-induced increases in plasma PRL. A in doses of 50 and 100 mg/kg ip markedly enhanced the increase in plasma PRL produced by 5-HTP 100 mg/kg ip (168± SE 18 ng/ml; 168±36 ng/ml and 78±21 ng/ml respectively). On the other hand, A 50, 100 and 150 mg/kg did not potentiate the increase in PRL produced by H. These results suggest A does enhance 5-HTP activity and does not enhance DA release or block its reuptake. Studies concerned with a specific increase in 5-HT receptor sensitivity following treatment with A will be reported.

1920 SUPERSENSITIVITY AND INCREASED NUMBER OF OPIATE RECEPTORS IN CEREBRAL HEMISPHERES OF RATS WITH HEREDITARY DIABETES INSIPIDUS. Rita B. Messing, Beatriz J. Vasquez, Henk Rigter*, Robert A. Jenser Joe L. Martinez, Jr., J.C. Crabbe, Jr.*, and James L. McGaugh. Dept. of Psychobiol., Univ. of Calif., Irvine, CA 92717.

This study investigated opiate receptors in rats with hereditary hypothalamic diabetes insipidus (Brattleboro strain). These rats have no detectable brain or neurohypophyseal vasopressin. They also have deficiencies in brain endorphins and impaired tolerance development to opiates. Therefore, ^{3}H-dihydromorphine (DHM) binding kinetics were examined in 6 brain areas of rats homozygous for the mutant autosomal gene determining diabetes insipidus (HO-DI rats). DHM binding of these rats was compared to binding in rats heterozygous for diabetes insipidus (HE-DI rats) and normal rats. Washed membranes were pre-incubated at 35°C in 0.05M tris HCl buffer, pH 7.4 with 100 nM of either dextrorphan or levorphanol. ^{3}Samples were then incubated with one of 7 concentrations of ^{3}H-DHM ranging from 0.2 to 13 nM, and stereospecifi binding was determined at each concentration. Apparent dissociation constants (K_D's) and receptor concentrations (B_{max}'s) for each brain region were obtained from Scatchard plots of the data.

BRAIN REGION	APPARENT K_D(nM)		B_{max}(fmol/mg protein)	
	Normal	HO-DI	Normal	HO-DI
Ant. Cortex	3.77	1.54**	80.9	63.9
		6.17+		140.5*
Amygdala	0.94	0.73	20.9	34.0**
Striatum	4.59	2.90**	84.8	127.2
Hypothalamus	6.90	5.16	179.3	123.7
Thalamus	2.84	2.32	50.2	39.6
Midbrain	9.17	7.81	275.2	292.2*

+p<0.02 different from high affinity site.
* and **: p<0.02, p<0.01 respectively, different from normal.

Higher receptor concentrations and affinities for DHM were observed in all assayed areas of the cerebrum (anterior cortex, amygdala and striatum) of the HO-DI as compared to normal rats (see table). In contrast, in assayed areas of the brainstem affinity or number were found between HO-DI and normal rats (see table). In general, ^{3}H-DHM binding kinetics in cerebral hemispheres of HE-DI rats were intermediate to those of HO-DI and normal rats, but HE-DI rats had significantly fewer opiate receptors in the thalamus and midbrain when compared to normal rats.

Our findings suggest that opiate receptor differences between HO-DI and normal rats may be involved in impaired tolerance development of Brattleboro rats. Supported by USPHS AG00538 and MH12526, NSF BNS 76-17370 and the McKnight Foundation (JLMcG).

1921 **HALOTHANE EFFECTS ON SENSORY EVOKED PHOTIC RESPONSES RECORDED SIMULTANEOUSLY FROM RETICULAR FORMATION, THALAMUS AND CORTEX IN FREELY MOVING ANIMALS.** **Luis J. Moreno* (SPON. W.S. Fields) The University of Texas Medical School at Houston, Houston, TX 77025.**

Sensory evoked potentials were recorded simultaneously in response to sensory stimulation from three areas within the central nervous system which have been postulated to be involved in mechanisms of general anesthesia. Permanent semimicroelectrodes (62 μm in diameter) were implanted stereotaxically under pentobarbital (50 mg/μg) anesthesia in the brainstem reticular formation (MRF), ventral posterior nucleus of the thalamus (VPL) and the somatosensory cortex (SC), 5-8 days prior to the experimental day. Experiments were performed on 12 male albino Sprague-Dawley rats (250-350 gr). The present investigation was initiated to establish an electrophysiological measurement to identify the acute effects of several concentrations of halothane (0.25; 0.5; 1.0; 1.5; 2.0 and 2.5%) on sensory evoked field potentials.

The averaged photic evoked responses consist of a positive deflection (P_1), a negative deflection (N_1), followed by a positive (P_2), negative (N_2), and a positive wave (P_3). Only the N_2 components were evaluated in terms of the amplitude and latency because of the consistency within and between animals. The response amplitude of the recording from the SC was the most sensitive to halothane treatment and demonstrated suppression with the lower dose (0.25%). The degree of suppression was dose-related, i.e., with increased dose of halothane the amplitudes were suppressed more and more. The lowest dose of halothane (0.25%) induced excitation of the responses obtained from the MRF and exhibited dose-related suppression with increasing halothane doses (0.5%-2.5%). The VPL responses were affected only from the second dose of halothane (0.5%) and also exhibited dose-related suppression. In all three structures the suppression of the responses was accompanied with shifting the latency response to the right. Differences in the degree of these shifting between the three structures were observed. In conclusion, halothane induced different effects on the three structures investigated in the present study, and were remarkably different from those obtained following pentobarbital treatment (Dafny, 1978, Exper. Neurol., 59:263).

1922 DOPAMINE ANTAGONIST ACTIVITY OF A NOVEL ANTIPSYCHOTIC COMPOUND EN285. Paul R. Myers, Greg R. Christoph*, and Rebecca S. Knight*. Central Research & Development Department, E. I. du Pont de Nemours & Co., Inc., Wilmington, DE 19898.

Neurochemical and electrophysiological methods were used to study the dopamine antagonist properties of EN285, a chemically novel methanodibenzocycloheptapyrrole. EN285 was an effective antagonist of rat striatal dopamine-sensitive adenylate cyclase with an $I_{50} = 6 \times 10^{-7}$ M against 1×10^{-4} M dopamine. Half maximal stimulation of adenylate cyclase activity as measured by cAMP production was observed at 6×10^{-6} M dopamine with maximal stimulation at 1×10^{-5} M dopamine. Trifluoperazine showed 50% inhibition at 1×10^{-7} M. The potency of EN285 is similar to that of chlorpromazine ($I_{50} = 6 \times 10^{-7}$ M; Clement-Cormier et al., J. Neurochem, 25, 1975) in this assay. In electrophysiological experiments, rats were anesthetized with chloral hydrate. Single dopamine-containing neurons in the pars compacta of the substantia nigra were identified on-line by their firing rate (2-7 spikes/sec) and spike duration (> 2 msec) characteristics. EN285 (up to 0.4 mg/kg, i.v.) had little or no effect on spontaneous firing rate. In rats whose nigral cell discharge rate had been reduced 40-60% by d-amphetamine pretreatment, EN285 (0.1-0.4 mg/kg, i.v.) reversed the amphetamine-induced depression in a dose-dependent fashion. The present work demonstrates that EN285 has central dopamine antagonist properties.

1923 ETHANOL SPECIFICALLY POTENTIATES GABAERGIC NEUROTRANSMISSION IN FELINE CEREBRAL CORTEX. Joannis N. Nestoros. Dept. Anaesthesia Research, McGill Univ., 3655 Drummond St., Montreal PQ H3G 1Y6.

Since ethanol is the oldest known "anxiolytic" agent, and since anxiolytic benzodiazepines and barbiturates are known to potentiate GABAergic neurotransmission, the following questions were asked: 1) Does ethanol potentiate the inhibitory effect of GABA on single neurons in the feline cerebral cortex? 2) If there is such a potentiation, is it specifically exerted on inhibitions evoked by GABA or does it involve inhibitions evoked by other postulated neurotransmitters, such as glycine, serotonin and dopamine? The experiments were performed on cats anaesthetized with chloralose, fluothane or methoxyflurane. Control experiments were performed using the "isolated cerebrum" unanaesthetized preparation. The drugs were applied utilizing standard microiontophoretic techniques. The degree of inhibition of single cortical units induced to fire submaximally by Na-L-glutamate was measured from peristimulus histograms generated by the extracellularly recorded spikes.

It was found that ethanol released from micropipettes (0.3 M in 165 mM NaCl) by "electro-osmosis" (by removing the retaining current or by ejecting currents up to 10 nA) or applied intravenously (0.2-2.0 mg/kg) potentiated strongly the inhibition of neuronal firing produced by iontophoretically-applied pulses of GABA, whereas it had no effect on inhibition produced by pulses of glycine, and had an antagonistic effect on the inhibition produced by pulses of serotonin or dopamine. Furthermore, ethanol applied by "electro-osmosis" or intravenously in the same doses potentiated strongly the inhibition of neuronal firing evoked by electrical stimulation of the surface of the cerebral cortex. This electrically-evoked cortical inhibition is believed to be mediated by endogenous GABA, and was found in these experiments to be prolonged in the presence of nipecotic acid, and to be antagonized by bicuculline and picrotoxin, but not strychnine. Ethanol in the aforementioned electro-osmotic and intravenous doses frequently decreased spontaneous firing as well as the firing evoked by Na-L-glutamate. However, this always occurred at doses higher than those affecting the inhibitions under study and precautions were always taken to use doses of ethanol that did not interfere with Na-L-glutamate evoked firing.

When tested on the same neurons, the effects of ethanol on GABA pulses and electrically-evoked cortical inhibition were identical with the effects of flurazepam and chlordiazepoxide, except that the effects of benzodiazepines lasted longer.

The above findings may have implications for the etiology and treatment of alcoholism.

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1924 ETHANOL PROLONGS THE TIME COURSE OF RECURRENT INHIBITION IN THE HIPPOCAMPUS IN RESPONSE TO COMMISSURAL FIBER ACTIVATION. S.A.Newlin, E.D.French*, T.Berger, F.E.Bloom. Alcohol Research Center, The Salk Inst., La Jolla, CA 92037.

Ethanol (3g/kg) was found to increase the period of recurrent inhibition recorded in hippocampal pyramidal neurons consequent to commissural fiber activation. This increase in the length of inhibition may be due to increased excitability of pyramidal neurons in the presence of ethanol.

Single unit responses were measured in CA_1 and CA_3 areas of the dorsal hippocampus using 3M NaCl-filled glass microelectrodes. On-line computer-generated post-stimulus-time histograms provided a measure of the degree of inhibition of spontaneous firing following stimulation. Stimulating current was delivered through a bipolar stainless steel electrode positioned in the contralateral hippocampus. Pyramidal neurons, as identified by firing pattern, responded to commissural fiber stimulation with an initial excitatory response followed by a cessation of activity lasting from 10-300 msec. As previously reported (Spencer and Kandel, Exper.Neurol.,1961) increasing intensities of stimulus voltage produced longer periods of recurrent inhibition. However, after a single i.p. injection of ethanol (3g/kg), the stimulus response curve for the period of recurrent inhibition was shifted to the left and upward, indicating an increase in the time course for inhibition with a given stimulus current. This increase in the time of recurrent inhibition could be due to any of several mechanisms, including: 1) an increased activation of pyramidal neurons which then activate more inhibitory interneurons; 2) an increased activation of inhibitory interneurons or increased transmitter release by inhibitory interneurons in the absence of increased activation of pyramidal neuron activity; 3) a change in pyramidal cell responsiveness to inhibitory synaptic input.

Our findings that iontophoretic application of ethanol (1-3M) on to CA_1 and CA_3 hippocampal neurons increased spontaneous firing rate in the majority (54%) of cells tested would be consistent with an hypothesis of increased pyramidal neuron excitability after ethanol. Moreover, an analysis of field potentials evoked by commissural stimulation revealed that the size of the population EPSP did not change while the size of the population spike was affected in the presence of ethanol.

1925 OPIATE ANALGESIA: EVIDENCE FOR MEDIATION BY A SUBPOPULATION OF OPIATE RECEPTORS. Gavril W. Pasternak, Steven R. Childers and Solomon H. Snyder. Depts. Neurology and Pharmacology, Memorial Sloan-Kettering Cancer Center and Cornell Univ. Med. College, N.Y., N.Y. 10021 and Depts. Pharmacology and Exp. Therapeutics and Psychiatry, Johns Hopkins Med. Sch., Baltimore, Md. 21205.

Animal responses to several different types of opiate analgesics have suggested multiple classes of receptors (Martin et al, JPET 197 517, 1976) and binding studies performed with opiates of high specific activity have demonstrated two distinct binding sites with differing affinities for narcotics (Pasternak and Snyder, Nature 253 563, 1975). In the present study, a novel narcotic antagonist, naloxazone, which appears to block irreversibly high affinity binding sites in vivo has been used to investigate the pharmacological relevance of the two receptor binding sites. Opiate binding in membranes from mice pretreated in vivo 24 hr earlier with naloxazone (200mg/kg) is decreased about 40% despite extensive washing techniques shown to remove reversibly bound ligands. Naloxone-treated controls (200mg/kg) show no decrease. Scatchard analysis of the decreased binding in the naloxazone-pretreated mice demonstrates loss of virtually all high affinity binding with almost no effect on the affinity or number of low affinity binding sites. The rate of return of high affinity binding suggests that in vivo turnover of high affinity receptors takes about 3 days. Sixteen hours after pretreatment with naloxone (200mg/kg) or saline, groups of mice tested for morphine (12mg/kg) analgesia in the tail-flick assay showed an increased latency of 90±4% (n=13) and 91±5% (n=8) respectively of the maximal measurable latency. By contrast, the naloxazone-pretreated group (200mg/kg) whose high affinity binding sites were blocked demonstrated only an 18±6% (n=13) increase in latency, suggesting prevention of analgesia seen in the control groups. When groups of mice similarly pretreated with naloxazone naloxone, or saline were given high (lethal) doses of morphine (500mg/kg), mortality (7/8, 7/8, 8/8 respectively) was the same in all groups. This mortality is opiate specific since the administration of naloxone immediately before the lethal dose of morphine prevents the mortality. Thus, blockade of only high affinity binding sites prevents opiate analgesia while offering no protection from opiate mortality. This mortality is probably a combination of cardiovascular and respiratory effects. These studies suggest that the different classes of receptors mediate different pharmacological functions and suggest the possibility of drugs specific for a single class of receptor.

1926 RAT BRAIN STRIATAL SYNAPTOSOMES BECOME RELATIVELY UNRESPONSIVE AT "OPTIMAL" pH CONDITIONS FOR MEASURING DOPAMINE FORMATION. Robert L. Patrick and Michael T. Rendel*, Neuroscience Sect. Div. Biol. Med. Sci., Brown Univ. Providence, RI 02912.

The rate of dopamine formation in rat brain striatal synaptosomes markedly increases as the pH is lowered from 7.2 to 6.2. Compared to pH 7.2, synthesis was increased 80% at pH 6.6 and 160% at pH 6.2. This increase in synthesis is accompanied by a significant increase in apparent Km for tyrosine as well as in apparent Vmax. Although these kinetic changes are similar to those produced by the depolarizing agent veratridine, it does not appear that synthesis is stimulated at pH 6.2 via synaptosomal depolarization since (1) synthesis stimulation still occurs at pH 6.2 in a calcium-free medium, in contrast to the calcium-dependency of synthesis stimulation observed with veratridine and (2) tyrosine uptake is not inhibited at pH 6.2, in contrast to the inhibition of tyrosine uptake produced by veratridine.

In order to study how the regulatory properties of synaptosomal preparations may vary according to pH, we have examined the effects of various stimulatory and inhibitory agents at pH 7.2, 6.6 and 6.2. The effects of three stimulatory agents (veratridine, amphetamine and phenylethylamine) were significantly diminished at pH 6.2 compared to pH 7.2. Their effects at pH 6.6, however, were quite similar to those at pH 7.2. The effects of two inhibitory agents (dopamine and tyramine) were also significantly antagonized at pH 6.2 compared to 7.2, while their effects at pH 6.6 were similar to those at 7.2. These results suggest that caution must be exercised in choosing an appropriate "optimal" pH for studying synaptosomal synthesis regulation, but it is possible to increase activity by altering pH without necessarily altering the regulatory properties of the system.

1927 A GENERAL METHOD FOR AUTORADIOGRAPHIC VISUALIZATION OF BRAIN RECEPTORS IN VITRO. Candace B. Pert and Miles Herkenham. Biological Psychiatry Branch and Laboratory of Neurophysiology, NIMH, Bethesda, MD 20205

Previous autoradiographic visualization studies have involved either injection of reversible radioactive ligands into animals with subsequent work-ups developed for diffusable substances ("in vivo") or incubations "in vitro" with ligands which form covalent bonds. Since highly specific ligands which form covalent bonds with receptors are not always available, we developed a method for in vitro labeling of receptors which features a fixation procedure of general applicability.

Rats were decapitated, their brains were rapidly removed, frozen on dry ice, cut into 25 micron sections in a cryostat (-12°C), pressed lightly onto slides, and maintained at 4°C for several days. Batches of slides were incubated at 4°C in 200 mls of sodium phosphate buffer (0.05 M, pH 7.4) containing bacitracin (500 µg/ml), aprotinin (1 TIU unit/ml), 100 mM sodium chloride and [^{3}H]diprenorphine (1 x 10^{-8} M) for 10 min. Slides were quickly dipped into five serial washes of 0.2% albumin at 0°C and a final wash of distilled water at 0°C and rapidly frozen with dry ice. After lyophilization overnight, sections were fixed by overnight exposure to concentrated gluteraldehyde vapors in a sealed dish. Finally, the slides were washed in a series of five 10-min rinses in 1 mM glycine (pH adjusted to 7.4) and two washes in distilled water at room temperature. The clean dried slides were dipped in the darkroom into Kodak NTB-2 emulsion (1:1 dilution) and processed by traditional autoradiographic methods.

Slides available at 2 weeks of exposure showed tissue preservation consistently comparable to conventionally fixed tissue, very low background grain counts, comparable low levels in white matter and striking clusters of grains overlying several brain areas previously determined to be enriched in opiate receptors, e.g., striatal patches, amygdaloid nuclei, inferior colliculus and locus coeruleus. Longer exposures will be necessary for low-power visualization of receptor distributions.

We monitored specific binding by processing alternate sections incubated in saturating concentrations of the appropriate non-radioactive drug, cutting out the part of the slide containing the section, and counting it by liquid scintillation spectrophotometry. "Cold" displacer reduced control binding on alternate sections by 80% or more for a number of ligands including [^{3}H]-diazepam and [^{3}H]D-Ala^2-met-enkephalinamide, and this specific binding was maintained throughout all steps of the fixation procedure. The presentation will emphasize how variations of the ligand and incubation conditions on alternate slides can be used for visualization of various receptors and receptor subtypes.

1928 D-AMPHETAMINE REDUCES STRIATAL SUBSTANCE P CONCENTRATION BY PRESYNAPTIC RELEASE OF DOPAMINE. D. J. Pettibone and R. J. Wurtman. Dept. Nutrition and Food Science, M.I.T., Cambridge, MA 02139.

We have previously shown that the acute administration of d-amphetamine, a drug that releases striatal dopamine (Besson et al., Brain Research, 32:403, 1971), reduces substance P immunoreactivity in the rat striatum, 2-4 hrs post-injection (Pettibone, Wurtman and Leeman, Biochem. Pharmacol., 27:839, 1978). We suggested that the reduction might be mediated by activation of dopaminergic receptors. This report further characterizes the effect of amphetamine on striatal substance P and shows its dependence on adequate striatal dopamine concentrations.

The intraperitoneal administration of d-amphetamine sulfate to male, Sprague-Dawley rats (175 g) causes a dose-dependent reduction in substance P immunoreactivity within the striatum; a 10mg/kg dose reduces substance P from 1.37 to 0.97 pmoles/10 mg tissue ($p < 0.01$), 2 hrs post-injection. Substance P levels of substantia nigra or hypothalamus are unaffected. The amphetamine-induced reduction in striatal substance P is blocked in animals pretreated with haloperidol (1,3, or 10mg/kg i.p.). Similarly, when striatal dopamine levels are reduced 70-75%, either by injections of α-methyl tyrosine methyl ester (225mg/kg i.p.) or by lesions of the nigrostriatal tract (induced by intranigral injections of 8µg 6-OHDA), the effect of amphetamine is prevented.

These data indicate that the mechanism by which d-amphetamine reduces striatal substance P content involves the presynaptic release of dopamine from the terminals of nigrostriatal neurons. They raise the possibility that striatal dopamine release may normally participate in the regulation of neuronal substance P concentrations in this brain region. (Supported in part by USPHS grant AM-14228)

1929 MORPHINE-LIKE EFFECTS OF CLONIDINE ON THE EEG AND BEHAVIOR IN THE DOG. Wallace B. Pickworth, Lawrence G. Sharpe and Vaikunth N. Gupta*. National Institute on Drug Abuse, Addiction Research Center, Lexington, Kentucky 40583

Clonidine, an alpha adrenergic agonist, has several effects similar to those of the opiates. We have compared the effects of morphine (0.5, 1.0 and 2.0 mg/kg) and clonidine (11, 33 and 100 µg/kg) on the EEG and behavior of the intact unrestrained beagle dog prepared with chronic electrodes for recording cortical and hippocampal EEG. EEG, heart rate, temperature and respiratory rate were recorded during a 2 hr experiment while the dogs were in a dimly lit sound-attenuated chamber. Their behavior was observed on a video monitor. Drugs were injected intravenously over 10 min from a pump outside the chamber. Morphine and clonidine caused cortical EEG synchrony which was more evident in parietal than occipital recordings. EEG-behavioral dissociation (EEG synchrony during a non-sleeping posture) was evident after all doses of morphine and after 33 and 100 µg/kg of clonidine. Also, both drugs increased total sleep. The increase was dose related after morphine, whereas the sleep after clonidine was maximal after the 33 µg/kg dose.

Spectral analysis of the EEG in clonidine treated dogs indicated that the effect of the drug was maximal at 15 min. The cut off frequency (half-power point) was increased from 6 to 10 Hz in the parietal and occipital EEG by clonidine. The drug increased power in the 4-8 Hz region while it decreased power in the 0.5-4 Hz band. The later effect was especially evident in recordings from the parietal cortex.

Morphine and clonidine caused behavioral sedation, ataxia, staring and vomiting as well as decreases in temperature, heart and respiratory rates. Catalepsy was more evident after morphine than clonidine. Naloxone (30 µg/kg) pretreatment antagonized the EEG and behavioral effects of morphine (1 mg/kg) but not those of clonidine (33 µg/kg). These data are consistent with the proposal that morphine and clonidine act upon different receptors in neural pathways which mediate these EEG and behavioral effects.

1930 POSSIBLE DISRUPTION OF GABAergic NEURAL FUNCTION IN ADULT MICE TREATED AS NEONATES WITH MONOSODIUM GLUTAMATE. William J. Pizzi, James R. Unnerstall* and June E. Barnhart. Department of Psychology, Northeastern Illinois University, Chicago, IL 60625.

Mice, treated as neonates with monosodium L-glutamate(GLU), exhibit a sequalae of somatic and behavioral deficits as adults due to the neurotoxic effects of GLU on the developing nervous system. We have recently shown that neonatal GLU-treated mice are more susceptible to pentylenetetrazol (PTZ) induced convulsions as adults. In order to explore this phenomenon, we have compared the differential response of GLU-treated and control mice to convulsants purported to act via differing pharmacological mechanisms.

Subjects were male HA:ICR mice born in our laboratory and housed with their dams until weaning at 29 days of age. Experimental animals were injected for 10 consecutive days (days 2-11 postpartum) with a gradually increasing dose of monosodium L-glutamate (2.2-4.4 mg/g body weight). Control subjects received equivalent volumes of equimolar NaCl.

All animals were tested between 90-120 days of age. The convulsant potencies of 3-mercaptopropionate (3-MP), an inhibitor of gamma-aminobutyric acid (GABA) synthesis, and bicuculline-HCl (BIC), a GABA receptor blocker, were determined according to the method of Litchfield & Wilcoxon (1949) using the least-squares criterion. A positive response was defined as a generalized clonic convulsion with loss of righting reflex. All agents were administered intraperitoneally using a 500 ul syringe.

GLU-treated mice were significantly more susceptible to 3-MP induced convulsions than controls (GLU: CD_{50}=19.59 mg/kg; NaCL: CD_{50}=31.02 mg/kg; Potency Ratio (PR)=1.58). The response of the GLU-treated animals to 3-MP was greater than the response obtained with PTZ (GLU: CD_{50}=38.7 mg/kg; NaCL: CD_{50}=49.6 mg/kg; PR=1.28). GLU-treated animals were less sensitive to BIC-induced convulsions (GLU: CD_{50}=5.89 mg/kg; NaCL : CD_{50}=4.06 mg/kg; PR=1.45).

The findings that GLU-treated animals are more sensitive to 3-MP and that they show a decreased sensitivity to BIC-induced convulsions suggests a disruption of normal GABAergic transmission in these animals. It is possible that GLU, administered during a critical period of neurochemical development, may permanently interfere in GABA synthesis and metabolism. Decreased GABA production may lead to altered sensitivity or increased numbers of GABA receptors, thus explaining the decreased sensitivity of GLU-treated animals to BIC.

1931 REGIONAL LOCALIZATION OF ^{14}C-METHADONE IN RABBIT BRAIN AFTER INTRACEREBROVENTRICULAR ADMINISTRATION: EFFECT OF NALOXONE PRETREATMENT. Donald A. Powell, Nandkumar S. Shah, Jane D. Yates* and Deborah A. May*. Ensor Fdn. Res. Jab., WM. S. Hall Psychiat. Inst. and V.A. Hosp., Columbia, S.C.

Albino rabbits were anesthetized (Ketamine, 55 mg/kg + chlorpromazine, 5 mg/kg, i.m.) and a cannula was implanted into the lateral ventricle. Seven days after surgery, a 50 µl Merles solution containing 96 µg + 1.47 µCi 2-^{14}C-methadone was introduced into cerebroventricular system. This dose exerted no noticeable behavioral effects. Following decapitation at 0.25, 1 or 2 hr, several brain areas were assayed for ^{14}C-methadone. ^{14}C was unevenly distributed in various regions reaching peak levels within 15 min. The superior colliculus, tegmentum, pons, hypothalamus, caudate, inferior colliculus, septum-nucleus accumbens, and medulla contained from 1.3 to 5.8 µg/g while olfactory tubercle, cortex, cerebellum, hippocampus and superior colliculus contained less than 1.0 µg/g. ^{14}C-methadone considerably declined at 2 hr. With few exceptions, pretreatment with naloxone (10 mg/kg i.p., 15 min) produced no significant effects on methadone levels in several brain regions examined at 15 min after methadone administration; only statistically significant differences were: marked decreases in inferior colliculus, superior colliculus and hypothalamus, a samll decrease in the thalamus, a large increase in the caudate and a small increase in cerebellum. At later time intervals, most noticeable differences in methadone levels in naloxone pretreated rabbits were the decreased levels in hippocampus and the increased levels in medulla. (Supported in part by the Ensor Research Foundation and V.A. Institute Funds Project No. 7155-03).

1932 THE EXCITATORY PORTION OF THE BIPHASIC RESPONSE OF CENTRAL NEURONS TO IBOTENIC ACID. E. Puil, Depts. of Anaesthesia and Pharmacology, University of British Columbia, Vancouver, B.C., Canada. V6T 1W5

At the last meeting of this Society, Puil and Krnjevic reported (Neurosci. Abst. 4:431, 1978) that microiontophoretic applications of ibotenate, a conformationally restricted analogue of glutamic acid, characteristically produced a biphasic effect in cerebral cortical neurons, as in spinal neurons: a strong excitation followed by a powerful, more prolonged inhibition of L-glutamate- and especially of acetylcholine-evoked discharge. As in the case of the potentiation of responses of neurons to L-glutamate by acetylcholine, the potentiation of L-glutamate-evoked firing by subthreshold amounts of ibotenate could be prevented by concurrent, extracellular application of methohexital.

Since the long latency of onset and afterdischarge of the excitatory responses of cortical neurons to ibotenate appeared to be more prominent in cats anaesthetized with diallylbarbiturate than with methoxyflurane, the effects of DL-ibotenate in cerebral cortical neurons were examined both extra- and intracellularly using parallel micropipette assemblies (triple barrel glued to single barrel at intertip distances of ~80µm) in cats with their forebrain isolated by leucotomy during brief anaesthesia with halothane. The most typical response of pre- and post-cruciate cortical neurons to iontophoresis of DL-ibotenate in this unanaesthetized preparation, was a strong excitation with a slow time course and afterdischarge more like the effect of acetylcholine than that of L-glutamate, while the subsequent inhibitory phase appeared to be much less prominent than that found in the previous studies. With intracellular recording, the excitation caused by ibotenate was seen to be related to a depolarizing action and to an ability of the isoxazole to increase the rate of rise of EPSPs evoked by bipolar electrical stimulation of the cortical surface or of the n. ventralis lateralis thalamus/internal capsule region with a tungsten microelectrode. The depolarization produced by ibotenate was in several cases associated with a rise in membrane resistance as measured from the increased amplitude of hyperpolarizing pulses injected through the recording barrel. In spinal motoneurons of cats anaesthetized with diallylbarbiturate, a similar, less pronounced depolarizing action of ibotenate was observed without important changes in membrane resistance. These data are consistent with the possibility that the excitatory portion of the biphasic response of central neurons to DL-ibotenate, which is dependent on the state of anaesthesia, is due at least in part to an action similar to that exhibited by acetylcholine, i.e., through a reduction in membrane permeability to K^+ relative to Na^+.

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1933 CORTICAL SENSORY EVOKED POTENTIALS AND THE ANESTHETIC DOSE OF HALOTHANE IN FREELY BEHAVING RATS. L.S. RABE*, B.M. RIGOR*, L. MORENO*, N. DAFNY. Depts. of Neurobiol. & Anat. and Anesthes., Univer. of Texas Med. Sch. Houston, TX 77025

This study was designed to assess the effect of different doses of halothane on the averaged evoked response (AER) of freely behaving rats since halothane is one of the most commonly used gaseous anesthetics in this country. Stainless steel semimicroelectrodes (60 µm in diameter) were implanted under pentobarbital anesthesia in the visual and auditory cortices of rats several days prior to experimentation, along with a bipolar stimulating electrode surrounding the sural nerve. Four sets of AER's to both acoustic and visual stimuli (32 repetitions) were taken as controls and then following increasing doses of halothane at 0.25%, 0.5%, 1.0%, and 2.0% administered by a vaporizer, (Fluotec 3). At each dose the sural nerve was stimulated to determine onset of anesthesia. The amplitudes of the AER's were analyzed peak to peak and consisted of an initial positive-negative spike (P_1-N_1) followed by a larger positive-negative-positive-negative wave (P_2-N_2-P_3-N_3). The results indicate that the auditory averaged evoked response (AAER) is a more sensitive measure for detecting halothane's effect on cortical areas than the visual averaged evoked response (VAER). In general, the AAER from the auditory cortex was the most affected by the halothane averaged over all doses (59%). The later components (P_2,N_2,P_3,N_3) were more responsive (i.e. had the greatest number of animals with a significant change) than the early components. The effects of halothane on the individual components (P_1,P_2,N_2) showed dose related patterns of responsiveness. The next most responsive set of AER's was the AAER from the visual cortex, (52%), then the VAER from visual cortex, (44%), and the least responsive was the VAER from the auditory cortex, (38%). This last set was the only one not to exhibit increasing responsiveness in the later components. All areas showed dose-related patterns of responsiveness on one or more components. The results from the sural nerve stimulation were that the dose level of 0.5% was the lowest dose at which the rats showed no pain-evoked response to stimulation. In all sets of recordings at this dose (0.5%), the N_2 component showed an increase in amplitude over controls while other components showed mixed responses. These results indicate a relationship between anesthetic dose and cortical AER's and suggest clinical applications for monitoring level of anesthesia.

1934 DIFFERENTIAL DOSE-DEPENDENT EFFECTS OF D-AMPHETAMINE AND APOMORPHINE ON SPONTANEOUS NEURONAL ACTIVITY IN THE RAT NEOSTRIATUM AND NUCLEUS ACCUMBENS. George V. Rebec and Kenneth S. Zimmerman*. Dept. Psychol., Indiana Univ., Bloomington, IN 47405.

Increasing doses of d-amphetamine produce a shift in the firing pattern of neurons in the neostriatum that has been implicated in the dose-dependent transition in behavior from locomotion to focused stereotypy (Rebec and Segal, Brain Res., 1978, 150: 353). To further elucidate the neuronal mechanisms underlying the amphetamine behavioral response, we extended our dose analysis to the nucleus accumbens. We also characterized the dose-dependent changes in firing rate produced by apomorphine. Unit activity, recorded from immobilized, locally anesthetized rats (350-450g), was amplified and displayed by conventional means. All drugs were injected via an in-dwelling intraperitoneal catheter.

Although low doses of d-amphetamine (1.0-2.5 mg/kg) and apomorphine (0.5-1.0 mg/kg) produced a qualitatively similar depression of neuronal activity in the neostriatum and nucleus accumbens, increasing the dose caused dramatic regional differences in firing rate. Thus, whereas neurons in the neostriatum responded to 7.5 mg/kg d-amphetamine with a prolonged increase in unit activity, the same dose produced a mirror-image inhibition in the nucleus accumbens. Increasing the dose of apomorphine (4.0 mg/kg), on the other hand, typically inhibited neuronal activity in the neostriatum but increased firing rate in the nucleus accumbens. The dose-dependent effects of d-amphetamine and apomorphine in both brain sites were reversed by either haloperidol (2.0 mg/kg) or clozapine (20-40 mg/kg).

The results indicate that the amphetamine- and apomorphine-induced behavioral transition from locomotor activity to stereotypy may be mediated, in part, by a differential dose-dependent effect on unit activity in the neostriatum and nucleus accumbens.

This research was supported by Biomedical Research Support Grant 46-314-09 from Indiana University and by USPHS Grant DA-02451 from the National Institute on Drug Abuse.

1935 ENANTIOMERIC INFLUENCES IN NEUROLEPTIC BINDING TO THE DOPAMINE RECEPTOR. Timothy A. Robert, Ernest A. Daigneault and Andrea N. Hagardorn*. Department of Pharmacology, College of Medicine, East Tennessee State University, Johnson City, Tennessee 37601.

The stereoselectivity of the dopamine (DA) receptor has been recognized for some time. Antagonists such as cis-thiothixene, a geometric isomer, and (+) butaclamol, an optical isomer, are more potent both in vivo and in vitro than their corresponding antipodes. The unique properties of enantiomers make them particularly useful for studying receptor interactions. Differences in potency of DA antagonism between optical isomers is exclusively a function of relative affinities determined by a single recognition parameter at the receptive site. A series of optical isomers of phenothiazine derivatives was examined for the ability to displace ^{3}H-DA and ^{3}H-spiroperidol (SP) from crude homogenates of calf caudate nucleus. Levorotatory methotrimeprazine was most effective at displacing both ^{3}H-DA and ^{3}H-SP with IC_{50} values of 2×10^{-6} and 9×10^{-8}M respectively. The corresponding IC_{50}'s for the dextrorotatory isomer were 3×10^{-5} and 9×10^{-6}M. In contrast, the dextrorotatory forms of other phenothiazine derivatives such as promethazine were more effective at displacing ^{3}H-SP. Upon comparing the ratios of *d*- and *l*- IC_{50} values for displacing ^{3}H-DA and ^{3}H-SP by methotrimeprazine, a large difference was observed. The receptor population labeled by ^{3}H-SP exhibits a six-fold higher stereospecificity than that labeled by ^{3}H-DA. These results provide direct evidence suggesting non-equivalence of ^{3}H-DA and ^{3}H-SP receptors in the calf caudate.

Resolved phenothiazines were the generous gifts of Wyeth Laboratories, Inc. and Rhône-Poulenc.

1936 EFFECT OF CHRONIC D-AMPHETAMINE OR β-PHENYLETHYLAMINE ON DOPAMINE BINDING IN RAT STRIATUM AND LIMBIC SYSTEM. Harold A. Robertson, Department of Pharmacology, Dalhousie University, Halifax, N. S., Canada. B3H 4H7.

D-amphetamine, a dopamine (DA)-releasing agent, produces a psychosis in man that is difficult to distinguish from paranoid schizophrenia and which is effectively controlled by neuroleptic drugs. β-Phenylethylamine (PEA), a sympathomimetic amine found in mammalian tissues, is structurally similar to amphetamine, the only difference being that PEA lacks a methyl group on the α-carbon of the side chain. PEA, like amphetamine, produces stereotyped behavior in rats and it has been suggested that PEA may be involved in the aetiology of schizophrenia. It was therefore of interest to know the effects of chronic PEA treatment on DA receptors in the CNS.

Male Wistar rats (200 g) were injected i.p. with saline, d-amphetamine sulphate (5 mg/kg) or β-phenylethylamine-HCL (50 mg/Kg) daily for 22 days. On day 24, animals were killed, tissue pooled from 2 rats and DA receptor binding assays (using 0.2 nM 3H-spiroperidol) were carried out on striatum and the meso-limbic system. The meso-limbic system consisted of pooled nucleus accumbens and olfactory tubercule.

Chronic treatment with either PEA or d-amphetamine produced an increase in specific 3H-spiroperidol binding in both striatum and meso-limbic system.

TREATMENT	SPECIFICALLY-BOUND 3H-SPIROPERIDOL (Fmol/mg protein ± SEM, N=4)	
	Striatum	Mesolimbic System
Saline	82.6 ± 3.3	69.2 ± 2.9
D-amphetamine	102.0 ± 3.5**	87.0 ± 0.6**
β-phenylethylamine	96.6 ± 3.2*	87.2 ± 3.6**

*P < 0.05, **P < 0.01, compared to saline

Recent work has demonstrated increased 3H-spiperone and 3H-haloperidol binding in caudate nucleus and nucleus accumbens of schizophrenic patients (Lee et al, Nature 274: 897, 1978). Rats treated with chronic amphetamine also show enhanced behavioral reactivity to directly acting dopamine agonists (Klawans et al, J. neurol. Sci. 25: 283, 1975). It appears therefore that chronic amphetamine treatment or chronic PEA treatment can produce changes in DA-receptor sensitivity similar to those seen in schizophrenic patients. This finding supports the contention that β-phenylthylamine may be useful as an animal model for schizophrenia. Finally there appears to be no differentiation between extrapyramidal and limbic areas in terms of the changes in DA-receptor binding and this too is in agreement with findings in schizophrenic patients.

Supported by the Canadian MRC.

1937 PRE- AND POSTSYNAPTIC ACTIONS OF RIGID ANALOGS OF DOPAMINE. D.B. Rusterholz, D.B. Goodale, J.R. Flynn*, J.P. Long*, T. Lee* and J.G. Cannon*. Dept. Pharmacology, Coll. Med., Univ. of Iowa, Iowa City, IA 52242.

In order to investigate the structural requirements of dopamine receptors that mediate postsynaptic events as compared to dopamine receptors that mediate presynaptic events, the relative potencies of a series of 18 structural analogs of dopamine were determined in two models. The test series included derivatives of phenethylamine, 2-aminotetralin, benzo[f]quinoline and ergoline.

The agonist potencies of the test compounds at postsynaptic dopaminergic receptors were evaluated using the rotating-rat model of Ungerstedt, et al. (Brain Res. 24, 485, 1970) with the modification that a smaller volume of 6-OH-DA was used in making the unilateral substantia nigra lesions. Contralateral circling behavior was recorded automatically for one hour following s.c. administration of the drug. Potencies were calculated relative to apomorphine using a parallel line bioassay.

Presynaptic receptor agonism was assessed by measuring the drug-induced inhibition of DOPA accumulation in rat striatal samples following administration of gamma-butyrolactone and a DOPA decarboxylase inhibitor. The method of Walters and Roth (N.S. Arch. Pharm. 296, 5 1976) was followed with the modification that NSD1015 was used as the DOPA decarboxylase inhibitor and DOPA was determined using high performance liquid chromatography with electrochemical detection. Potencies relative to apomorphine were calculated using a parallel line bioassay.

Seven compounds were found inactive in one or both preparations, leaving 11 members of the test series with measurable potencies in both models. Although several agents appeared to have some degree of selectivity, a comparison of the rank ordering of the potencies of the test substances by the two models revealed a strong similarity: Spearman's correlation coefficient = .78 ($p<0.01$). These data suggest that the structural requirements of the receptors mediating rotational behavior and inhibition of DOPA accumulation are similar and therefore may involve only one receptor type.

1938 ADENOSINE AS A MEDIATOR OF THE EFFECTS OF LITHIUM AND OTHER PSYCHOTROPIC DRUGS. A. Sattin, E. Einterz* and N. Whicker*, Institute of Psychiatric Research, Dept. of Psychiatry, Indiana Univ. Sch. Med., Indianapolis, IN 46223.

The accumulation of cyclic AMP in chopped brain tissue has served historically as a useful tool for identifying neurotransmitters and their interactions by examining the effects of the test substances upon the tissue content of cyclic AMP. The most striking example of this was the discovery that adenosine activates adenylyl cyclase, that adenosine receptors interact with biogenic amine receptors, and that activation of adenosine receptors is antagonized by methylxanthines (A. Sattin and T.W. Rall, Molec. Pharmacol. 6: 13-23, 1970). Desipramine (T. Kodama et al., BBA, 252: 165-170, 1971) and other tricyclic drugs (M. Huang and J. Daly, J. Med. Chem. 15: 458-462, 1972) have adenosine like effects on cyclic AMP accumulation in chopped cerebral cortex. This has been confirmed and correlated with the effects of a series of antidepressant drugs applied iontophoretically to rat cerebral cortical neurons in vivo (A. Sattin et al., Life Sci. 23: 2621-2626, 1978). Mediation of the drug effects by adenosine was based upon prevention of increase in cyclic AMP in vitro by theophylline and potentiation of the inhibitory effects of the tricyclic drugs by adenosine in vivo. The tricyclic drugs produce decreases in the chopped tissue content of ATP and increases in the content of 5'-AMP and adenosine. The increase in the tissue content of adenosine is sufficient to account for the observed increase in cyclic AMP. This work has now been extended to mice in vivo.

In order to enhance the effect of the drugs on the adenosine compounds chronically drug treated mice were stressed with an electroconvulsive seizure, then allowed to partially recover before freezing in isopentane. The most striking effects were seen following 5 μ equiv./g of LiCl x 7 days in SEC X DBA hybrid mice (60 days old). Five saline-injected control mice gave values for adenosine and cyclic AMP of 3.48 ± 0.28 and 2.64 ± 0.38 (SEM) nmoles/g respectively in whole forebrain. The respective values for ATP, ADP and 5'-AMP were 8.49 ± 0.71, 1.34 ± 0.16 and 0.49 ± 0.04 μmoles/g. The ATP content is more than twice the previously reported value for intact brain and indicates the efficacy of this freeze-fixation method. Following Li^+ treatment ATP increased 50% and ADP increased over 3-fold. Ninety sec. following onset of ECS ATP was still less than half the lithium control and increases were observed in adenosine (10-fold), cyclic AMP (3-fold) and 5'-AMP. A dose-response analysis and an exploration of other post ECS times will be discussed in relation to the possible involvement of adenosine in therapeutic and/or toxic effects of Li^+. (Supported by MH 31137 and the State of Indiana).

1939 INTERACTION OF GABA MIMETICS WITH CENTRAL CHOLINERGIC NEURONS. Bernard Scatton* and Giuseppe Bartholini* (SPON: M. Le Moal). Synthélabo (L.E.R.S.), Research Department, 58, Rue de la Glacière, 75013 PARIS, FRANCE.

The effect of the GABA mimetic agents muscimol and SL 76 002 ({α(chloro-4 phenyl) fluoro-5 hydroxy-2 benzilidene-amino} -4 butyramide) on acetylcholine (ACh) levels were investigated in various regions of the rat brain.

Muscimol (0.2 to 7.5 mg/kg i.p.) and SL 76 002 (10 to 400 mg/kg i.p.) induced a dose-related increase in striatal ACh levels. As ACh esterase activity was not affected by muscimol, SL 76 002 or its metabolites (10^{-4}M) the increase in ACh concentration probably reflects a decreased release of ACh. This effect is unlikely to be connected with an interaction of the GABA agonists with ACh receptors as SL 76 002, its metabolites and muscimol (5 x 10^{-4}M) do not affect the binding of ^{3}H-quinuclidinylbenzylate in rat brain membrane preparations. The enhancement of striatal ACh levels is also unlikely to be the consequence of GABA mimetic-induced changes in dopaminergic transmission : thus, these compounds caused a similar modification of striatal ACh levels after 6-hydroxydopamine induced degeneration of the nigro-striatal dopaminergic pathway. Moreover, SL 76 002 and muscimol increased striatal ACh levels to a similar extent after administration of haloperidol (2 mg/kg) or apomorphine (5 mg/kg). In addition after unilateral transection between substantia nigra and striatum, GABA mimetic agents retain their ability to increase striatal ACh content. Finally, striatal ACh levels were increased after intrastriatal infusion of GABA (750 ng) or muscimol (75 ng). These data suggest that striatal ACh neurons may receive a direct GABAergic inhibitory influence.

GABA also appears to affect ACh neurons in other ACh-rich brain areas. Thus, muscimol (7.5 mg/kg) or SL 76 002 (400 mg/kg) increased ACh levels in the frontal cortex, olfactory tubercle, nucleus accumbens and interpeduncular nucleus. However, the percentage increase was smaller in the latter areas than in striatum. In contrast, no change of ACh levels was observed in the septo-hippocampal system.

The present results suggest that GABA affects brain cholinergic cells in discrete brain areas.

1940 BEHAVIORAL EFFECTS OF THE NOVEL DOPAMINE AGONIST SK&F 38393. Paulette E. Setler, Joseph McDevitt* and Tsuneo Fujita*. Smith Kline & French Laboratories, Philadelphia, Pennsylvania 19101.

SK&F 38393 (2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1H-3-benzazepine) is a dopamine agonist which, in the central nervous system, selectively activates dopamine receptors linked to adenylate cyclase (Setler et al., Eur. J. Pharmacol. 50:419, 1978). The complex interactions of SK&F 38393 with anticholinergic drugs suggest that the behavioral effects of this agonist may not accurately reflect the consequences of activation of cyclase-coupled dopamine receptors. SK&F 38393 produces contralateral rotation when injected systemically or into the dopamine-depleted caudate nucleus in rats with a unilateral 6-hydroxydopamine-induced lesion of substantia nigra. In non-lesioned rats intra-caudate injection of SK&F 38393 produces rotation only when the rats are given a systemic injection of a muscarinic antagonist such as scopolamine. SK&F 38393 which, unlike most dopamine agonists, does not produce stereotyped behavior in intact rats, does produce stereotypy in rats with either unilateral or bilateral depletion of dopamine and causes a mild form of stereotypy in intact rats also given scopolamine. Another animal model in which SK&F 38393 produces a form of stereotyped behavior is the 6-8 day old rat, which may have a functionally weak central cholinergic system. SK&F 38393 also has little effect on the symptoms induced by a combination of reserpine and α-methyl-p-tyrosine. When given with scopolamine, however, SK&F 38393 reverses the postural and motor effects of acute catecholamine depletion.

SK&F 38393 produces few overt effects in intact rats, but behavioral effects traditionally associated with dopamine agonists may be seen in rats with denervation supersensitivity of dopamine receptors or in rats with reduced central cholinergic function. This may indicate a pharmacologic effect of SK&F 38393 on cholinergic function which masks the effect of dopamine receptor activation. Alternatively, these unmasked behaviors could represent a weak activation of another type of dopamine receptor not coupled to adenylate cyclase. This activation would be observable only when the dopamine receptors are made more sensitive by denervation or suppression of cholinergic activity.

1941 EVIDENCE THAT AMPHETAMINE-INDUCED MYDRIASIS IN THE DOG IS DUE PARTLY TO INHIBITION OF THE EDINGER-WESTPHAL NUCLEUS (EW) BY A MUSCARINIC CHOLINERGIC INPUT. Lawrence G. Sharpe and Wallace B. Pickworth. NIDA Addiction Research Center, Lexington, Kentucky 40583.

Amphetamine produces pupillary dilation in several species by the simultaneous activation of sympathetic and inhibition of parasympathetic impulses which reach the dilator and sphincter pupillae of the iris. Recent evidence showed that microinjections of cholinergic muscarinic agonists in the EW caused mydriasis in the dog, suggesting that the pupilloconstrictor neurons receive tonic muscarinic inhibitory input (Sharpe and Pickworth, SN Meeting, 1978). The purpose of this study was to test the hypothesis that amphetamine produces mydriasis partly through an increased release of acetylcholine (ACh) in the EW to inhibit the pupillary light reflex path.

Five dogs were acclimated to a sling restraint. Atropine N-methyl nitrate or haloperidol (2.73nmol in 1.0 µl) were injected via chronically indwelling cannula in a region of the oculomotor nuclear complex that yielded pupilloconstriction to electrical stimulation. Thirty minutes after the antagonist microinjection, amphetamine (1.0 mg/kg) was infused intravenously for 10 min. Pupillary diameter was measured photographically before and for 2 hr after the infusion.

That microinjections of atropine, but not haloperidol, produced pupilloconstriction, is consistent with the hypothesis that the EW receives tonic muscarinic inhibitory input. Pretreatments with microinjections of atropine, but not haloperidol, partially and significantly antagonized amphetamine-induced mydriasis when compared to saline pretreatment (area under time action curve, $P = 0.01$, $t = 4.35$, 4 df).

Because we have previously shown that when microinjected into the EW (1) catacholamines produce miosis, (2) cholinomimetics produce mydriasis and (3) amphetamine produces no change in pupillary diameter, we conclude that EW is not the site of action of this portion of amphetamine-induced mydriasis. One explanation is that amphetamine may increase ACh release in the EW indirectly by increasing first the release of catecholamines distant from the EW to excite the cell bodies of those cholinergic inhibitory pathways leading to the EW.

1942 BENZODIAZEPINE BINDING IN SUBSTANTIA NIGRA; INCREASED STIMULATION BY GABA AFTER STRIATONIGRAL LESIONS. Haruo Shibuya*, Karen Gale* and Candace Pert. (SPON: J. Neale) Nat'l. Inst. Mental Health, Bethesda, MD 20205 and Dept. of Pharmacol., Georgetown U. School of Med. and Dent., Wash. DC 20007.

Striatonigral GABA-containing pathways were unilaterally destroyed by a discrete electrolytic lesion in the crus cerebri, rostrolateral to substantia nigra (SN). Four weeks postoperatively, the GABA concentration of the SN from the lesioned hemisphere dropped to below 40% of that on the intact side. At this time, specific [^{3}H]diazepam binding (per mg protein) in the SN on the lesioned side was significantly reduced (by 40%) when compared with either the intact contralateral SN or with SN from unoperated control rats.

Addition of GABA (10^{-6} to 10^{-5}M) to benzodiazepine binding assay preparations has been shown to increase benzodiazepine binding by increasing the apparent affinity of the benzodiazepine site for its ligands (Tolman et al., Nature 274: 383, 1978). We observed that the [^{3}H]diazepam binding sites remaining in the SN of the lesioned side exhibited an enhanced response to the addition of GABA to the binding assay. In membranes from the SN of the unlesioned side, 10^{-7}M GABA did not stimulate [^{3}H]diazepam binding, while the same GABA concentration elicited a significant increase (37%) in [^{3}H]diazepam binding to membranes from the SN of the lesioned side. At 10^{-6}M, GABA elicited a 30% enhancement of [^{3}H]diazepam binding in the intact SN and a 77% enhancement of [^{3}H]diazepam binding in the SN from the lesioned hemisphere.

Acute depletion of GABA by injection of isoniazid (400 mg/kg i.p. 1 hr. prior to sacrifice, which results in a 60-70% decrease in nigral GABA) had no significant effect on the ability of GABA to enhance [^{3}H]diazepam binding in SN. Thus it is unlikely that a reduced tissue level of GABA per se can account for the augmentation of the GABA effect on benzodiazepine binding. Instead, the effect which we have observed is most likely a result of the chronic loss of GABA or GABAergic terminals after the lesions.

It has been previously shown that 2-4 weeks after electrolytic lesions of the striatonigral pathways, there is an increase in the amount of high affinity binding sites for [^{3}H]GABA in SN (Guidotti et al., Brain Research, in press). Our observation that these same lesions result in an enhancement of the effect of GABA on benzodiazepine binding suggests that one population of benzodiazepine receptors in SN may be closely coupled to GABA receptors in such a way that supersensitivity of GABA receptors manifests itself on the binding of benzodiazepine receptors as well.

1943 IN VITRO BINDING STUDIES WITH 3H-N-METHYL ASPARTATE. S. Robert Snodgrass (SPON: L. W. Kneisley), Dept. of Neuroscience, Children's Hospital Medical Center, and Dept. Neurology, Harvard Medical School, Boston, MA 02115

What is the "endogenous ligand" for excitotoxins such as kainic acid (KA)? Do the large number of excitotoxins all bind to the same receptor? N-methyl-D-aspartic acid (NMDA) is one of the most potent excitants known. McCulloch, Watkins, and others have suggested that NMDA and KA act at different kinds of receptors. Evans et al (Experientia 33:489, 1977) reported that micromolar concentrations of Mg^{++} antagonized NMDA excitation in frog and rat spinal cord, but not that due to KA or glutamate (glu). They suggested that these Mg^{++} effects were probably post-synaptic as they were not reversed by Ca^{++}.

Binding of 3H-NMDA (80 Ci/mmol, New England Nuclear Corp.) to membrane fractions from rat and mouse brain was studied. The binding was greater in buffers lacking Na^{+} ion and 50 mM Tris acetate or Tris citrate, pH 7.2, were routinely used. Incubation with 1.7 nM 3H-NMDA was for 2 min at room temperature and was terminated by filtration through GF/B glass fiber filters. Non specific binding was estimated with 62 uM nonradioactive NMDA. Binding was found to be saturable, with a Kd of 9.8 nM estimated for mouse cerebellar membranes. No evidence of metabolism of the NMDA was detected by TLC with butanol-acetic acid-water (3:1:1). The binding was not altered by Ca^{++} but was decreased in a dose-dependent manner by Mg^{++}. The IC_{50} for this Mg^{++} inhibition was estimated at 450 uM. Using the same membranes, buffer, and 3H-KA, no effect of Mg^{++} could be detected.

As with other amino acid ligands, binding reaches equilibrium very quickly. KA and glu were weak inhibitors of NMDA binding, while DL-homocysteic acid and D-aspartate (asp) were more potent antagonists. For both glu and asp, the "unnatural" D-isomers had greater potency than the L-isomers. DL-α-aminoadipate was able to block NMDA binding with a potency similar to that of DL-homocysteic acid and D-asp. In parallel studies, nonradioactive NMDA was a poor displacer of KA binding. The number of binding sites for NMDA appears to be somewhat less than the number of KA sites, although both show significant regional variation and the number of binding sites for 3H-L-glutamate, in Na^{+} free media is 8-10 fold greater. The binding sites for KA and NMDA differ in several important ways and it is likely that there are at least two separate receptors which bind excitotoxins. They differ in sensitivity to Mg^{++} ions as first noted in physiological studies.

1944 THE EFFECTS OF CHOLINE AT THE MUSCARINIC CHOLINERGIC RECEPTOR IN BRAIN. Robert C. Speth and Henry I. Yamamura. Department of Pharmacology, University of Arizona Health Sciences Center, Tucson Arizona 85724.

Choline is both a precursor and a metabolite of acetylcholine (ACh). Until recently, little thought was given to the possibility that choline might also interact with cholinergic receptors. We have tested choline for its ability to inhibit specific [^{3}H]-quinuclidinyl benzilate [QNB] binding to muscarinic cholinergic receptors in brain. Choline inhibited [^{3}H]QNB binding with an IC_{50} value of 1.3 mM which is about 1000 fold less potent than ACh (IC_{50}=1.5 μM). Respective K_i values for choline and ACh were 0.34 mM and 0.39 μM.

One mM choline competitively inhibited [^{3}H]QNB binding, shifting the K_D apparent of [^{3}H]QNB from 45 pM to 109 pM with no effect on the maximal binding capacity. The effects of choline appear to be specific to cholinergic receptors since one mM choline did not significantly affect the binding of [^{3}H]spiroperidol or [^{3}H]-flunitrazepam to their receptors on brain membranes. The slopes of log-logit plots for the inhibition of [^{3}H]QNB binding by various cholinergic substances were; 0.44 for ACh, 0.73 for oxotremorine, 0.82 for choline and 1.09 for atropine. Since muscarinic agonists typically have slopes of <1, this suggests that choline may act as an agonist at muscarinic receptors in brain. Brain concentrations of choline are reported to be 15 to 30 μM, however at cholinergic synapses, the choline concentrations may be much higher. If so, it is possible that choline may interact with cholinergic receptors under normal conditions.

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1945 EFFECTS OF MORPHINE (MS), METHIONINE-ENKEPHALIN (ME) AND SUBSTANCE P (SP) ON NEURONAL FIRING IN THE NUCLEUS RETICULARIS GIGANTOCELLULARIS (NRGC) OF THE RAT. (D. D. Spring* and H. J. Haigler, Dept. of Pharmacology, Emory Univ., Atlanta, GA 30322)

The NRGC and surrounding areas (i.e., raphe magnus and nucleus reticularis paragigantocellularis) are responsive to nociceptive stimuli (Morrow and Casey, Adv. in Pain Res. and Ther. 1: 503, 1976). Microinjections of low doses of morphine and enkephalins into the NRGC produce analgesia (Akaike et al, Jap. J. of Pharmacol. 27: 548, 1977; Akaike et al, Neuropharmacol. 17: 775, 1978). Enkephalin and SP containing neurons are found in the region of the NRGC (Hokfelt et al, Proc. Natl. Acad. Sci. 74: 3081, 1977). Since the NRGC and surrounding areas are apparently involved in nociception and analgesia, we microiontophoretically administered MS, ME and SP in these areas to determine the effects on spontaneous neuronal firing and neuronal firing evoked by a nociceptive stimulus (i.e., foot pinch).

Male Sprague-Dawley rats (220-350 gms) were anesthetized with halothane and surgically prepared with a tracheal cannula, paralyzed with gallamine triethiodide; the pCO_2 in the expired air was maintained between 3.5-3.7%. Local anesthetics were administered around all wound edges and on the sterotaxic ear bars. In some experiments, chloral hydrate (80 mg/kg) was periodically administered through a tail vein. A five-barrel micropipette was lowered into the brain through a hole drilled in the skull. The micropipette contained 10 mM ME, 50 mM MS, and 3 mM SP in three barrels; a fourth barrel, filled with 4 M NaCl, was used as a current balance barrel; the central barrel, filled with 2 M NaCl and FG, was used to record single unit activity. Most neurons tested with the nociceptive stimulus responded with a significant ($p<0.05$:1-way ANOVA) increase in firing. Iontophoretically-applied MS and ME usually had no effect on spontaneous firing and failed to block the increase in evoked firing elicited by the nociceptive stimulus. SP had no effect on spontaneous firing; however, in 2 of 18 cells it potentiated the evoked firing. These data do not support the hypothesis that SP is a neurotransmitter in the NRGC of the rat. Systemically-administered MS (2.0-8.0 mg/kg) blocked the increase in firing produced by the nociceptive stimulus but high currents (up to 100 nA) of iontophoretically-applied MS and ME did not. The effect of MS administered systemically was blocked by naloxone (1.0-2.0 mg/kg). Systemically administered morphine apparently acts at sites other than the NRGC to block the increase in neuronal firing evoked by a nociceptive stimulus. MS and ME do not have a direct effect in the NRGC since neither substance blocked evoked firing. (Supported in part by NIDA Grant 1-RO1-DA-01344-03.)

1946 EFFECTS OF UNILATERAL 6-HYDROXYDOPAMINE ADMINISTRATION ON DOPAMINE RECEPTORS IN RAT CORPUS STRIATUM. D.A. Staunton*, B.B. Wolfe, P.M. Groves and P.B. Molinoff. (SPON: W. Hahn) Dept. of Pharmacol., Univ. of Colo. Med. Ctr., Denver, CO 80262

Destruction of the nigrostriatal bundle leads to biochemical, neurophysiological and behavioral alterations in animals. This forms the basis of a model for Parkinson's Disease in humans. Previous research utilizing this paradigm revealed that such lesions lead to an increased sensitivity of striatal neurons to iontophoretically applied dopamine (DA) as well as to an increased binding of ^{3}H-haloperidol to striatal membranes. An increased maximal stimulation of DA-sensitive adenylate cyclase in striatal homogenates from denervated striata has been observed in some studies. In the present experiments, 6-hydroxydopamine (6-OHDA; 8 μg) was injected unilaterally into the nigrostriatal bundle of male Sprague-Dawley rats weighing approximately 150 g. The animals were sacrificed at 1, 2, 3, 5, 14 and 28 days post-lesion and the corpus striata were removed for biochemical analyses. Striatal DA content was reduced by greater than 95% within 2 days following 6-OHDA administration. Nonspecific ^{3}H-spiroperidol binding was defined as the binding in the presence of 2 μM (+)-butaclamol or 10 μM (±)-ADTN. No change in the binding of ^{3}H-spiroperidol was observed 1 or 2 days after lesioning. However, the density of ^{3}H-spiroperidol binding sites was increased by 25-50% in the denervated striata 5 or more days following 6-OHDA treatment. Basal and DA-stimulated adenylate cyclase activity were measured in fresh striatal homogenates from the same animals. Maximal stimulation of enzyme activity occurred in the presence of 100 μM DA. There was no change in either basal or DA-stimulated cyclase activity on the first post-operative day. At longer time points, the ratio of stimulated (100 μM DA) to basal activity was increased by 30-40% on the lesioned side, with no change in the EC50 value. These results demonstrate that lesions of the nigrostriatal pathway lead to similar time-dependent increases in the density of DA receptors and in DA-stimulated adenylate cyclase activity. Increased ^{3}H-spiroperidol binding appears to develop concomitantly with the earliest appearance of apomorphine-evoked contralateral rotational behavior. The present results support the hypothesis that the destruction of the nigrostriatal DA pathway results in a time-dependent development of denervation supersensitivity and that changes in DA receptors are at least partially responsible for the increased sensitivity. Supported by the USPHS (NS 09199).

1947 IS REWARD BEHAVIOR MEDIATED BY AN ENDOGENOUS OPIATE SYSTEM? Elliot A. Stein and Joseph Zerneskie* Department of Biology, Marquette University, Milwaukee, WI 53233.

One of the least investigated, and most unknown aspects of addictive behavior concerns the neuroanatomical and physiological bases of the craving-euphoric effect. Since this behavior is in ways, similar to intracranial self-stimulation(ICSS), it would be of interest to determine if both phenomena are mediated by a set of common central structures. One line of evidence pointing to such a commonality is the remarkable overlap between opiate receptor density with those sites supporting ICSS. If the endogenous opiates play a role in hedonic behavior, then it is conceivable that animals might self-administer opiates into those brain areas subserving reward behavior and containing opiate receptors. To test this hypothesis, male Holtzman rats were stereotaxically prepared with 26 g guide cannulae aimed at various subcortical sites. Following a 2 day recovery period, a 33 g injection cannula is lowered into the brain and the rat is placed into a rectangular plexiglass chamber with a pedal placed at each end. One pedal is arbitrarily chosen as the active one such that each press results in a single drug injection. The alternate pedal serves as a control for non-specific general behavioral activation. Drug doses were 0.1 and 0.5 ug/inj.(volume=10 nl/inj. with delivery time at 280 msec). Experiments were performed overnight for 20 hours.

The highest rates of self administration (in approx. descending order) have been seen for lateral ventricle, lateral hypothalamic area, amygdala and preoptic area. Considerably lower rates were found for the periventricular gray and septum. Finally no significant presses were seen for cortex, cerebellum, reticular formation or fourth ventricle. Preliminary results indicate that 5 ug/inj Met-Enkephalin is also self-administered into LH while 4.5 ug/inj B-Endorphin resulted in an increase in total presses for both pedals.

These results point towards a commonality of reward sites within the central nervous system and are discussed with respect to the role of opiates and the endogenous opiate system in hedonic behaviors. (Supported by grants from the NIDA, Pharmaceutical Manufacturing Association Foundation and the Marquette University Committee on Research)

1948 POWER SPECTRAL ANALYSIS OF REM SLEEP EEG IN THE RAT SELF-ADMINISTERING NARCOTICS. George F. Steinfels, Gerald A. Young and Naim Khazan. Dept. Pharmacol. and Toxicol., Univ. of Maryland Sch. of Pharmacy, Balto., MD 21201

In a previous study we used power spectral analysis to demonstrate that the behavioral states of sleep, REM sleep and wakefulness in the rat are associated with different EEG power spectra (Young et al, 1978a). In a subsequent study we examined the EEG power spectra of the first REM sleep episodes after self-injections of morphine and the last REM sleep episodes before the next injections (Young et al, 1978b). We found that the mean peak EEG frequency during the first REM sleep episodes was higher than that during the last episodes. A similar finding has been reported during methadone self-administration (Steinfels et al, 1978). The present study has extended these findings to include REM sleep EEG power spectral changes occurring throughout the interinjection intervals during self-administration of morphine, methadone, &-alpha-acetylmethadol (LAAM) and the two active N-demethylated metabolites of LAAM, nor-LAAM and dinor-LAAM.

Adult female Sprague-Dawley rats were implanted with chronic cortical and temporalis muscle electrodes and intravenous (iv) cannulas for drug administration. Tolerance and physical dependence were induced by automatic hourly iv injections of increasing doses of morphine. Rats were then trained to lever press to self-administer morphine (10 mg/kg/inj) on an FR-20 schedule of reinforcement (Khazan et al, 1967). For some of the rats, morphine was then replaced with methadone (2 mg/kg), LAAM (1 mg/kg), nor-LAAM (1 mg/kg) or dinor-LAAM (1 mg/kg). At least one week was allowed for the establishment of stabilized self-injection patterns. EEG recordings were stored on FM tape, and, later, EEG samples of successive REM sleep episodes which occurred between self-injections were subjected to power spectral analyses using a Nicolet MED-80 system.

During self-administration of these narcotics, the first REM sleep episode in each interinjection interval had the faster peak EEG frequency. The peak EEG frequencies of successive REM sleep episodes declined in a linear fashion. Differences in the slopes of the linear EEG frequency declines reflected differences in the pharmacokinetic profiles between the narcotics. Lever pressing activity did not appear to be related to these EEG changes since similar EEG frequency changes were seen in rats which received automatically delivered morphine injections. Therefore, these decreases in peak EEG frequencies may reflect a decline in brain levels of the respective narcotic and/or changes in the CNS that precede "drug-seeking behavior." (Supported by NIDA Grant DA 01050.)

1949 STRESS BLOCKS NALOXONE INDUCED HYPOTHERMIA. Jane Stewart and Roelof Eikelboom*. Dept. Psych., Concordia Univ., Montreal, Canada, H3G 1M8.

The specific opiate antagonist, naloxone, produced marked hypothermia in unstressed drug-naive rats. Rectal temperature readings taken 45 min after 2.5, 5, or 10 mg/kg naloxone HCl revealed a significant, dose-dependent hypothermia (> 1^{o} C) in animals thoroughly familiarized with the experimental handling. In contrast, similar doses of naloxone given to experimentally naive rats, or to rats subjected to a specific noise stressor had little, if any, effect on rectal temperature. Because in this first experiment naloxone was administered after initial handling and temperature readings, a second experiment was done in which steps were taken to administer naloxone prior to the onset of stress induced by handling and insertion of the rectal probe. Under these conditions a dose-response suppression of the stress induced rise in body temperature was observed (see also Blässig, Höllt, Bäuerle and Herz, Life Sci. 23:2525, 1978). When similar experiments were carried out in hypophysectomized rats, naloxone produced marked hypothermia even in stressed rats, making it appear that it is pituitary secretions that mediate the masking effects of stress on naloxone induced hypothermia.

The fact that naloxone has hypothermic effects in unstressed intact rats and in hypophysectomized rats suggests that naloxone acts by antagonizing brain endorphin or enkephalin systems that normally participate in tonic thermoregulation. Furthermore, the observation that the hypothermic effect of naloxone is found in the relatively unstressed animal has led us to conclude that the frequent failure to find physiological or behavioral effects of naloxone in drug-naive animals arises, not from a lack of tonic effects of endogenous opioids in normal animals, but rather from the somewhat stressful conditions that characterize acute testing procedures in experimentally naive animals.

1950 INHIBITION OF ACETYLCHOLINE RELEASE FROM THE MYENTERIC PLEXUS BY MORPHINE OR BY DECREASED AVAILABILITY OF Ca^{2+}: DIFFERENCES IN THE KINETICS OF LABELLED ACETYLCHOLINE EFFLUX. John C. Szerb, Dept. of Physiology & Biophysics, Dalhousie U., Halifax, N.S., Canada.

The release of [^{3}H]ACh from guinea-pig myenteric plexus longitudinal muscle preparation (MPLM) evoked by 0.1 Hz stimulation measured in the absence of cholinesterase inhibition has an initial fast and a later slow component. Morphine (1 μM) slows down the initial fast efflux and decreases the releasable pool size but not the rate of the slow efflux (Down & Szerb, Br. J. Pharmac., in press). To see whether a decreased availability of Ca^{2+} for release could be responsible for the effect of morphine on the slow efflux, the kinetics of [^{3}H]ACh release evoked by supramaximal 0.1 Hz stimulation in the absence of cholinesterase inhibition was measured in Krebs containing reduced Ca^{2+} or oxotremorine (OT). During releasing stimulation the concentration of Mg^{2+} was raised to 9.6 mM to avoid the contracture of MPLM which by itself reduced the size of the releasable pool of [^{3}H]ACh. In the presence of 2.5 mM Ca^{2+}, 9.6 mM Mg^{2+} reduced the rate constant of the slow release from 0.023 to 0.017 min^{-1}. The rate constant was further reduced in the presence of 1.75 mM Ca^{2+} or of OT, without changing the size of the releasable pool of [^{3}H]ACh. The effect of OT but not of reduced Ca^{2+} was overcome by atropine. On the other hand, morphine reduced the releasable pool size but not the rate constant of release in the presence of 9.6 mM Mg^{2+}. Results suggest that morphine reduces the size of the releasable pool of [^{3}H]ACh by hyperpolarizing cholinergic neurons as observed by North & Tonini (1977). Because of this hyperpolarization a number of neurons will cease to be excited by field stimulation and their [^{3}H]ACh stores will not be available for evoked release. Those neurons which continue to fire in the presence of morphine will release their [^{3}H]ACh at the same rate as without morphine. On the other hand, reduced Ca^{2+} or OT does not decrease the number of neurons excited but each impulse releases less [^{3}H]ACh, hence the rate of release is slower. Results therefore suggest that reduced availability of Ca^{2+} is not responsible for the depression by morphine of the evoked slow release of [^{3}H]ACh from the MPLM.

(Supported by MRC of Canada).

1951 EFFECTS OF CHRONIC ETHANOL ON CHOLINERGIC AND NEUROLEPTIC BINDING SITES IN BRAIN. Boris Tabakoff, Jeremy Z. Fields, Paula L. Hoffman*, Marietta Munoz-Marcus*, and Chinwuba Okafor*. Dept.Physiol. and Biophys., Univ. Ill. Med. Ctr., & Dept. Pharmacol., Chicago Med. Sch., Chicago, IL 60612

We have previously noted that mice withdrawn from chronic feeding with ethanol were less affected by apomorphine and other dopamine(DA) agonists than control animals. The stimulation of striatal tyrosine hydroxylase activity by neuroleptics was also diminished in the ethanol-withdrawn animals. In the present studies we wished to examine the characteristics of the neuroleptic binding sites in the striatum of ethanol-withdrawn animals and also to examine the muscarinic cholinergic receptors, since the cholinergic systems may participate in the feedback regulation of tyrosine hydroxylase activity. Spiroperidol and haloperidol binding to tissue homogenates were used to investigate the properties of the neuroleptic receptor sites and quinuclidinyl benzylate(QNB) binding was monitored to assess the properties of the muscarinic cholinergic receptors. Ethanol was administered in a liquid diet and control animals received a similar diet in which sucrose was equicalorically substituted for ethanol. The number of QNB binding sites was unaltered in the striatum of ethanol-intoxicated mice(0 time of withdrawal) or at 8 hours after withdrawal, when animals display the most profound withdrawal symptoms. On the other hand, QNB binding in the hippocampus and cerebral cortex was significantly altered in the ethanol withdrawn mice(Table 1).

Table 1. QNB Binding in Control and Ethanol-Withdrawn Mice

	Control		Ethanol Withdrawn*	
Brain Area	K_D(pM)	R_{max}**	K_D(pM)	R_{max}**
Hippocampus(10)	62.6±16.1	596±72	72.3±22.7	703±87^{++}
Striatum(4)	90.8±24.3	981±72	95.6±5.1	888±187
Cortex(6)	74.2±18.9	566±25	76.7±15.9	645±64^{+}

*Withdrawn from ethanol for 8 hours
**fmol/mg protein: $^{++}$, $p<0.01$; $^{+}$, $P<0.1$

Haloperidol and spiroperidol binding were unaltered within the first 24 hours after ethanol withdrawal but further studies at extended time points are now in progress. The results of the binding studies could not explain the decreased pharmacologic effects of neuroleptics in ethanol-withdrawn mice. However, changes in QNB binding may indicate a compensatory response to the ethanol-induced inhibition of acetylcholine release in cortex and hippocampus.

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1952 AGONIST INDUCED INCREASE IN BINDING AFFINITY OF [^{3}H]-MUSCIMOL TO CRUDE SYNAPTIC MEMBRANES OF RAT CEREBELLUM. Rebecca Thomas*, Adrienne Gordon, and Ivan Diamond. Department of Neurology, University of California Medical School, San Francisco, Calif. 94143.

Many investigators have observed specific binding of γ-aminobutyric acid (GABA) to crude synaptic membranes prepared from rat cerebellum. Binding appears to involve two sites, one of high affinity and one of lower affinity. Moreover, Toffano et. al. (P.N.A.S. 75 (8), 4024, 1978) have shown that there is a conversion from low affinity to high affinity sites by removal of an endogenous inhibitor of GABA binding. A major limitation in previous studies is that the ligand was incubated with membranes for up to 30 min. before measurement of the amount bound. In order to elucidate the mechanism of ligand binding to the GABA receptor it is necessary to study the kinetics of the binding reaction. We have therefore been using [^{3}H]-muscimol as a ligand. This GABA agonist has a 10-fold higher affinity for the receptor than GABA itself and a slow off-rate which permits the use of filtration methods to measure muscimol binding. We have observed a biphasic association of [^{3}H]-muscimol with time. Initially there is a rapid phase which is over in less than one minute. This is followed by a slower phase which appears to be linear for at least 20 min. This linear increase in binding correlates with a 2 to 4-fold increase in the affinity and a 50% increase in the number of specific muscimol binding sites. Since the only variable in these experiments is the time the membranes are exposed to [^{3}H]-muscimol, the data suggests that there is an agonist-induced change in binding affinity of the GABA receptor for muscimol.

In order to determine whether this agonist-dependent change in affinity of the GABA receptor is mediated by changes in the endogenous inhibitor or some other mechanism, we investigated the effect of this inhibitor on the agonist-dependent affinity changes. We prepared endogenous inhibitor from frozen membranes which were incubated at 37°C for 30 min. The membranes were then centrifuged at 48,000 x g for 10 min. The supernatant was used as a source of endogenous inhibitor. Membranes to be used for binding studies were washed three times with buffer to remove the released inhibitor. In agreement with others, we find that addition of the inhibitor results in a decrease in affinity for muscimol. However, our results also indicate that a 4-fold change in affinity constant is still induced by muscimol and is not affected by the presence or absence of endogenous inhibitor. It is possible that the agonist-induced change in affinity of the GABA receptor which we report here may account for the phenomenon of pharmacological desensitization of the GABA receptor.

1953 FAILURE OF SYSTEMICALLY ADMINISTERED MUSCIMOL TO ACT AS A GABA AGONIST. James R. Unnerstall* and William J. Pizzi. (SPON: D. Rowland). Department of Psychology, Northeastern Illinois Univ., Chicago, IL 60625.

Muscimol (MUS) is a potent agonist of the putative neurotransmitter gamma-aminobutyric acid (GABA) in vitro and when administered intracranially, in vivo. Systemically administered MUS is also pharmacologically active. However, it is unclear whether the behavioral and biochemical effects are due to the drug per se or to some active metabolite of MUS. While Naik et al. (1976 Neuropharm. 15, 479) and Matthews et al. (1978, Neurosci Abst 4, 428) describe a pharmacological profile for systemically administered MUS consistent with expectations for a GABA agonist, Maggi & Enna (Neuropharm, in press) report that MUS is rapidly metabolized after systemic administration, and that this metabolite is not localized to the GABA receptor. The Maggi & Enna report, along with an observation in our lab indicating that MUS pretreatment protected mice against strychnine induced convulsions, led to the present investigation. A pharmacological profile of MUS ability to protect against the convulsions induced by several chemical agents, which are purported to work by different mechanisms, was determined. The results do not support a GABA mimetic action of systemically administered MUS.

Male Ha:ICR mice, 90-120 days of age, were challenged by CD_{97} doses of bicuculline-HCl (BIC), strychnine-HCl (STRY), 3-mercaptopropionate (3-MP) or pentylenetetrazol (PTZ) following systemic administration of MUS. The ED_{50} of MUS was determined for each convulsant with the method of Litchfield & Wilcoxon (1949). A positive response was defined as a generalized clonic convulsion with loss of righting reflex. MUS was administered 30 minutes prior to the convulsant challenge. All agents were administered intraperitoneally using a 500 ul syringe.

At the highest dose of MUS tested (2.25 mg/kg), animals appeared sedated but maintained a righting reflex. At lower doses, MUS exerted an apparent protective effect against STRY-induced convulsions. However, this trend was reversed at higher doses. Yet, a dose-dependent increase in latencies was observed. On the other hand, MUS clearly exerted a dose-dependent protective effect against 3-MP induced convulsions (ED_{50}=1.36 mg/kg). MUS exerted no protective effect on convulsions induced by BIC or PTZ. In fact, MUS (1 mg/kg administered 30 minutes prior to the convulsant challenge) potentiated BIC & PTZ induced convulsions (BIC: CD_{50}=4.56 mg/kg; MUS+BIC: CD_{50}=3.95 mg/kg; potency ratio= 1.15). (PTZ: CD_{50}=53.92 mg/kg; MUS+BIC: CD_{50}=43.10 mg/kg; potency ratio = 1.25).

These data lend support to the evidence presented by Maggi and Enna demonstrating that systemically adm. MUS is independent of a specific pharmacological action at the GABA receptor.

1954 KAINIC ACID ADMINISTRATION INTO THE SEPTUM ACTIVATES HIPPOCAMPAL THETA RHYTHM WITHOUT ALTERING ACETYLCHOLINE CONTENT. M.R. Vasko, I.L. Crawford, C.N. Allen*. Depts. Pharmacology and Neurology, VA Med. Ctr. and Univ. Texas Hlth. Sci. Ctr. at Dallas, TX 75216.

The neuroexcitatory and neurotoxic actions of kainic acid (KA) in the brain are well established. Therefore, kainate may be a useful tool for the stimulation as well as the destruction of specific neuronal pathways. We studied the effects of low and high doses of KA on physiological and neurochemical parameters of the cholinergic system in the septal-hippocampal pathway. Rhythmic slow activity (RSA, 4-7 hz) was monitored in hippocampus as an index of cholinergic function. In addition, concentrations of acetylcholine (ACh) were measured in discrete brain regions following kainate administration.

Rats (300±25 gm) were implanted with an indwelling cannula guide directed at the medial septum. Bipolar recording electrodes were also implanted on the surface of the hippocampus, bilaterally. Animals were allowed 5 days to recover, then electrical activity was monitored before and after injections of saline or KA. For neurochemical studies, KA (1 and 1.5 µg in 1µl) was injected slowly using a sterotaxically implanted 26ga needle directed at the medial septum. Ten days after the KA injections, the animals were sacrificed by microwave irradiation (1.3 kw) focussed on the head. The brains were removed, dissected, and ACh assayed radiochemically using the enzyme choline kinase.

Low doses of KA (10ng in 1µl) injected into the area of the medial septum caused a rapid activation of RSA bilaterally with a complete loss of desynchronous activity. The RSA was observed in immobile, as well as, moving animals. The desynchrony was abolished for 12 mins. Saline injection (1µl) had no effect on the bursts of desynchrony that occurred at 15-25 sec intervals in control resting animals. High doses of KA (1µg) resulted in electrographic seizure activity characterized by spikes and followed by intermittent abnormal sharp and slow waves. One and 1.5 µg of KA injected into the septum had no significant effect on ACh concentrations in either the septum or the hippocampus. ACh levels in septum and hippocampus were 30.5±6.7 and 26.6±2.8 nmol/gm, respectively, following KA (1µg) as compared to 37.1 ±5.3 and 27.4±4.9 nmol/gm for controls. Minimal, if any, cytotoxicity was observed in the septum following these large doses of KA. The small dose (10 ng) had no demonstrable cytotoxic effects in the hippocampus. Our results show that KA can drive septal-hippocampal RSA at doses that do not produce cytotoxicity or neurochemical changes in ACh. (Supported by VA MRIS 8977 and NIH Service Award GM07062, NIGMS).

1955 THE EFFECT OF CYCLIC NUCLEOTIDES AND A PHOSPHODIESTERASE INHIBITOR ON OPIATE-INDUCED DEPRESSION OF TRANSMITTER RELEASE IN THE MOUSE VAS DEFERENS. Lauren V. Vitek* and R. A. North. Loyola University Medical Center, Maywood, IL 60153.

The amplitude of evoked excitatory junction potentials (e.j.ps) is a sensitive measure of the amount of transmitter released from nerve terminals. Opiates depress evoked transmitter release and hence decrease e.j.p. amplitude in the mouse vas deferens. One hypothesis of opiate action states that the acute effects of opiates are mediated by a decrease in cyclic adenosine-3',5'-monophosphate (cAMP) levels in neurons. According to this hypothesis, an increase in neuronal cAMP levels should prevent or reduce opiate effects. Intracellular recordings were made from smooth muscle cells of the mouse isolated vas deferens. The aim was to determine whether drugs that modify cAMP levels could alter opiate-induced depression of e.j.p. amplitude.

Both cAMP (1mM) and its dibutyryl derivative (dbcAMP; 1mM) decreased the amplitude of the evoked e.j.p. The phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX; 50 or 500μM) increased e.j.p. amplitude; this effect exhibited tachyphylaxis. IBMX (50 or 500μM) and dbcAMP (250 or 500μM) together increased e.j.p. amplitude more than IBMX alone. Neither the cyclic nucleotides nor IBMX altered the resting membrane potential of smooth muscle cells. There was no change in the ability of normorphine to depress the e.j.p. amplitude in the presence of cAMP (1mM) or in the combined presence of IBMX (50μM) and dbcAMP (500μM). Similarly, the dose-response curve for D-ala^2-met^5-enkephalin-amide (DAEA) was not affected by the presence of IBMX (500μM) and dbcAMP (250μM).

The present findings do not support the hypothesis that a reduction in intracellular cAMP levels is an essential step in the inhibition by opiates of transmitter release.

1956 CHOLINERGIC MICROSTIMULATION OF THE PONTINE BRAIN STEM PROVOKES THE DESYNCHRONIZED SLEEP SYNDROME. Ennio Vivaldi*, Robert W. McCarley and J. Allan Hobson. Laboratory of Neurophysiology, Department of Psychiatry, Harvard Medical School, Boston MA 02115.

We have proposed that desynchronized sleep (D) results from the increase in activity of cholinergic gigantocellular tegmental field (FTG) cells and that their recurrent collaterals play an important role in the overall population effect. The fact that local delivery of the long lasting cholinergic agent carbachol to the FTG induces prolonged periods of D supports the idea of a cholinergic state generator mechanism situated in the pontine reticular formation. We have previously documented the similarity of the drug induced state to physiologically occurring D, and established that more confined and less disruptive techniques of drug delivery resulted in more consistent and specific results. It thus seemed possible to adapt the drug delivery system to the cellular level of analysis, and thereby to determine the minimum number of cells which, when activated, could generate the whole D syndrome. We now report preliminary experience with a micropipette system that combines behaviorally effective drug delivery with single unit recording capability.

Two cats were prepared for chronic EEG recording and painless head restraint. Glass micropipettes could then be stereotaxically introduced through a burr hole while sleep-wake behavior was observed and polygraphically monitored. When the tips were positioned at FTG coordinates (P 3.0, HC -7.0, L 1.5) and filled with a 1 M carbachol solution, currents of 300 nA of 10 minutes duration produced a state indistinguishable from D sleep but which lasted for up to 50 minutes, 5 to 10 times the duration of D seen in KCl control experiments.

In one experiment a carbachol micropipette was placed in the peribrachial region of the pons (P 3.0, HC -2.0, L 2.9) from which PGO burst cells may be recorded. Passage of current then induced a dramatic and abnormal state of continuous PGO wave activity without other signs of D sleep; the stereotyped clusters of waves persisted for 15 hours. A unit recorded through the drug-filled pipette showed the tight phase locking of firing and EEG waves that characterizes the PGO burst cell in physiological D sleep.

The microstimulation method thus promises to be a useful tool in our effort to understand the D sleep generator process.

1957 EFFECTS OF CHRONIC DESIPRAMINE TREATMENT AND α-ADRENERGIC DRUGS ON RAT BRAIN NORADRENERGIC NEURONS. W. Warnack, B.A. McMillen, D.C. German, and P.A. Shore. Depts. of Physiol., Psychiat., & Pharmacol., Univ. of Texas Health Science Center, Dallas, TX 75235

The purpose of the present experiment was to study the effects of acute and chronic desipramine (DMI) treatment on the electrophysiology and biochemistry of rat brain norepinephrine (NE) neurons. Locus coeruleus (LC) NE neurons normally fire 1.73 ± 0.14 Hz (N=44) in the chloral hydrate anesthetized rat. After acute DMI (1.25 or 10.0 mg/kg i.p.), LC impulse flow decreased to 0.50 ± 0.11 Hz (N=11) whereas after chronic DMI (5.0 mg/kg b.i.d., i.p., 7-9 days) LC impulse flow partially recovered (1.09 ± 0.24 Hz, N=12). Furthermore, after chronic DMI, LC impulse flow was markedly less sensitive to the acute effects of intravenous DMI. This tolerance to DMI's effects on LC impulse flow, despite continued NE uptake blockade, suggests lessened inhibition of NE neurons. Since the LC has α_2-receptors on cell bodies and/or dendrites which are inhibitory to impulse flow, the present results suggest an α_2-receptor subsensitivity. Consistent with this interpretation are biochemical data showing that both 25 and 250 μg/kg doses of clonidine were equally effective in decreasing whole brain MOPEG-SO_4 in control rats, but only the larger dose decreased it in 12 day DMI rats. To test the sensitivity of α_1-receptors (post-synaptic), prazosin (5.0 mg/kg) in a dose just sufficient to increase MOPEG-SO_4 in control rats was found to be similarly effective in 12 day DMI rats. These data suggest that α_2-receptors become subsensitive during chronic DMI treatment but α_1-receptors do not. (Research supported by USPHS Grants MH-27574, MH-30546, and MH-05831.)

1958 OCCURRENCE AND DISTRIBUTION OF RAT BRAIN 3,4-DIHYDROXYPHENYLETHYLENE GLYCOL (DHPG) DETERMINED BY GAS CHROMATOGRAPHY MASS FRAGMENTOGRAPHY. Jerry J. Warsh, Damodar D. Godse*, Siu Cheung* and Peter Li*. Dept. of Neurochemistry, Clarke Institute of Psychiatry, University of Toronto, Toronto, Canada, M5T 1R8.

In a number of species central nervous system (CNS) norepinephrine is preferentially metabolized to the glycol end products 3,4-dihydroxyphenylethylene glycol (DHPG) and 3-methoxy-4-hydroxyphenylethylene glycol (MHPG). While substantial research has been done on the occurrence and significance of MHPG in the CNS, less is known about the occurrence and biological importance of CNS DHPG. This situation stems from the lack of suitably sensitive and specific methods for quantitation of DHPG in biological samples. We describe here a sensitive and specific GC-MS assay for simultaneous determination of DHPG and MHPG in biological samples and its application to the study of DHPG in rat brain.

Formic acid homogenates of whole rat brain or brain areas were subjected to enzymatic hydrolysis with aryl sulfatase (16 h., 37°C, pH 5.9). Under basic conditions (pH 10-10.5), DHPG and MHPG were simultaneously acetylated and extracted into ethyl acetate containing acetic anhydride (0.5%). The acetylated glycol products were acylated with trifluoroacetic anhydride and quantitated by multiple ion mass fragmentography. For internal standards DHPG-$^2H_2$ was synthesized by reduction of 3,4-dihydroxymandelic acid with $^2H_2$-borane methyl sulfide, while $^2H_3$-MHPG was synthesized by the method of Karoum et al. (J. of Neurochem. 25, 653, 1975).

Whole rat brain DHPG (115 ± 2.6 ng/g) was of the same order of magnitude as brain MHPG (87.0 ± 1.8 ng/g). Unconjugated DHPG and MHPG accounted for 12% and 11%, respectively, of total brain DHPG and MHPG concentrations. Regional brain total DHPG concentrations varied from 963 ± 37 ng/g (n = 6) in hypothalamus and 450 ± 70 ng/g (n = 6) in septum to 76 ± 10 ng/g (n = 6) in the caudate. Total DHPG was significantly higher than MHPG in hypothalamus and septum (molar ratio of DHPG/MHPG = 6.9 ± 0.44 and 3.91 ± 0.31, respectively). Yohimbine (0.5-10mg/kg, i.p.) produced parallel linear increases in both forebrain total DHPG and MHPG. Ten mg/kg yohimbine i.p. resulted in 212 ± 6.1% (n = 8) and 250 ± 5.6% (n = 8) increases in forebrain total DHPG and MHPG, respectively. The present data suggest that in rat brain formation and efflux of conjugated DHPG may account for a major fraction of the metabolic clearance of brain norepinephrine.

1959 EFFECTS OF DIETARY CHOLINE AVAILABILITY AND CHRONIC NEUROLEPTIC ADMINISTRATION ON RAT STRIATAL NEUROTRANSMITTER RECEPTORS. Lynn Wecker, Robert C. Speth and Henry I. Yamamura. Departments of Pharmacology, Louisiana State Univ. Med. Ctr., New Orleans, LA 70112 and Univ. of Arizona Health Sci. Ctr., Tucson, AZ 85724.

Recent investigations indicate that the dietary availability of choline significantly alters the dynamic regulation of acetylcholine metabolism and modifies the responsiveness of central cholinergic neurons to pharmacological manipulation (Neurosci. Abst. 4:436,1978).These effects are generally believed to involve presynaptic synthetic mechanisms, but possible receptor-mediated effects have not been thoroughly investigated. Hence, the present study was designed to determine the effects of dietary choline availability on central muscarinic receptor characteristics. In addition, due to recent evidence indicating that chronic choline administration decreases dopamine agonist-induced stereotypy in rats treated chronically with neuroleptics (Life Sci. 22:1699, 1978), the effects of dietary choline availability on spiroperidol binding in striata were investigated in rats chronically treated with haloperidol. Rats were randomly divided into 3 groups and maintained for 3 weeks on:a standard choline diet (0.2% choline chloride), a choline deficient diet, or a choline supplemented diet (2.0% choline chloride). Animals in each dietary group were injected daily (ip) during the 3 week period with either saline or haloperidol (5 mg/kg) and sacrificed 24 hours following the final injection by decapitation. Striata were removed, homogenized and prepared for receptor binding studies.Muscarinic receptors were characterized by incubating brain samples, in the presence and absence of atropine, with ^{3}H-L-quinuclidinyl benzilate (QNB, 30-1000pM). Specific high affinity binding of ^{3}H-spiroperidol (SP, 30-300pM) was determined in the presence and absence of (+)-butaclamol.Preliminary results indicate that the affinity (K_D) of QNB binding is not altered by dietary choline availability, but a 10% decrease in Bmax was observed in striata from rats on the choline supplemented diet. SP binding in striata from rats maintained on the standard dietary regimen was characterized by a K_D of 71pM, whereas in choline deficient and choline supplemented rats,the K_D was 82pM and 61pM, respectively. The Bmax of SP binding was unaffected by dietary choline manipulation. Chronic haloperidol administration increased the Bmax of SP binding in all dietary groups by approximately 50%. Binding in choline supplemented rats was not differentially affected. Preliminary results suggest that the effects of dietary choline availability on central neurotransmitter mechanisms may be mediated by alterations at receptor sites and we are currently pursuing studies to further characterize the nature of the interactions. (Supported in part by an RSDA #MH-00095 to H.I.Y.)

1960 THERMODYNAMIC EVIDENCE FOR AN AGONIST-SPECIFIC CONFORMATIONAL CHANGE OF THE β-ADRENERGIC RECEPTOR. G.A. Weiland*, K.P. Minneman*, and P.B. Molinoff. (SPON: W. Wickelgren). Dept. of Pharmacol., Univ. of Colo. Med. Ctr., Denver, CO

The effect of temperature on the affinities of β-adrenergic agonists and antagonists was examined by the inhibition of specific ^{125}I-iodohydroxybenzylpindolol binding to turkey erythrocyte membranes. The affinities of agonists increased with decreasing temperature while antagonist affinities were almost entirely independent of temperature. With a decrease in temperature from 37°C to 1°C, equilibrium dissociation constants (Kd's) decreased 56- to 16-fold for full agonists but by less than 3-fold for antagonists. Intermediate decreases in Kd values were observed for partial agonists.

Thermodynamic analysis of the dependence of Kd on temperature demonstrated fundamental differences between agonist and antagonist interactions with the receptor. The binding of agonists was enthalpy-driven with highly unfavorable decreases in entropy (see Table). Antagonist binding, on the other hand, was entropy-driven with only small changes in enthalpy. The binding of partial agonists was associated with intermediate changes in thermodynamic parameters. The changes in enthalpy and entropy were closely correlated with the relative efficacies of the compounds in stimulating adenylate cyclase activity. The results are consistent with the following model: $L + R \overset{1}{\rightleftarrows} LR \overset{2}{\rightleftarrows} LR'$. In this model the binding of agonists or antagonists (step 1) is based on hydrophobic interactions (entropy-driven) with only negligible changes in enthalpy. Agonists, but not antagonists, induce a conformational change (step 2) resulting in a net decrease in entropy and an increase in adenylate cyclase activity. The energy which compensates for this decrease in entropy is provided by a greater decrease in enthalpy. Agonist specific changes in the affinity of the β-adrenergic receptor have been observed following cell disruption and membrane solubilization and in the presence of guanine nucleotides or divalent cations. The thermodynamic approach described here may prove helpful in understanding these changes. Since affinity is a function of changes in enthalpy and entropy, alterations in affinity will reflect changes in these parameters. Thus, thermodynamic analysis may be a useful tool in examining the molecular events involved in hormone receptor interactions. (USPHS NS 13289 and NS 09199).

	ΔH° (kcal/mole) Range	ΔS° (entropy units) Range
Agonists (3)	-18.9 to -13.4	-35.3 to -12.9
Partial Agonists (5)	-10.8 to - 4.1	-13.1 to + 6.7
Antagonists (12)	- 5.1 to + 3.9	+13.0 to +42.3

1961 IDENTIFICATION OF ^{3}H-CLONIDINE BINDING SITES IN RAT BRAIN. P. Weinreich, M. Titeler and P. Seeman. Department of Pharmacology University of Toronto, Toronto, Ontario M5S 1A8.

Previous work has shown that there are two α-adrenergic receptors than can be pharmacologically differentiated (Langer, 1973, 1977; Andén et al., 1976), into a high affinity (Langer, 1973) and a low affinity norepinephrine site. The high affinity site is thought to be located pre-synaptically (Langer, 1973). A current issue is whether or not the high affinity ^{3}H-clonidine binding site in the brain represents this same pre-synaptic adrenergic receptor (Titeler et al., 1978). To test this hypothesis a series of catecholamine derivatives were tested for their ability to compete with ^{3}H-clonidine binding; these binding data were compared with the pre-synaptic cardiac actions of these drugs (Long et al., 1979). The strong correlation between the pre-synaptic cardiac actions of these drugs and their ^{3}H-clonidine IC_{50}'s indicates that the binding site in the brain and the site of action of these drugs in the heart are pharmacologically very similar. Currently we are trying to localize this ^{3}H-clonidine binding site in the brain using specific noradrenergic fibre lesions. (Supported by OMHF and MRC).

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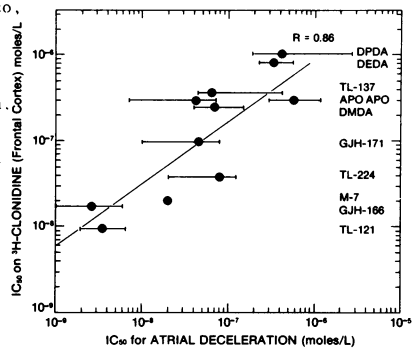

1962 BINDING STUDIES WITH ALPHA AND BETA DIHYDROPICROTOXININ. W. F. White and S. R. Snodgrass. Dept. Neurosci. The Children's Hospital Medical Center, and Dept. Neurol. Harvard Med. Sch. Boston, MA 02115.

The potent convulsant picrotoxinin is known to be a noncompetative antagonist of the GABA receptor. Recently a receptor for picrotoxinin has been characterized by Olsen and his collaborators using the less potent analogue ^{3}H-α-dihydropicrotoxinin. We have investigated the binding characteristics of both the alpha and beta isomers of ^{3}H-dihydropicrotoxinin to membrane fractions prepared from rat brain and also to intact rat cerebral cortical neurons grown in tissue culture. These studies support the findings of Olsen's group on the binding and pharmacological characteristics of ^{3}H-α-dihydropicrotoxinin binding to membrane fractions. Thus we find a small but significant percentage (20%) of the total binding is displacable by excess picrotoxinin and thus is specifically bound to the picrotoxinin receptor. This binding is saturable and is antagonized by the cage convulsant isopropylbicyclophosphoric acid. A number of agents known to interact with the GABA receptor at the Na^+-independent GABA binding site including GABA and the benzodiazepines have no effect on the binding of ^{3}H-α-dihydropicrotoxinin nor do variations of either Na^+ or Cl^- concentration. The development of ^{3}H-α-dihydropicrotoxinin binding sites in the cortical culture system is similar to that reported in situ and parallels the development of other biochemical measures of GABA function in the cultures. We have also investigated the stereospecificity of binding using the beta isomer of ^{3}H-dihydropicrotoxinin. In these studies we find little difference in the binding characteristics of the two isomers. This result is somewhat surprising in light of the marked stereospecificity of binding at most receptors. While this result may indicate a functional equivalence of the two isomers at the physiological picrotoxinin receptor this interpretation must await an analysis of the relative potencies of the two isomers in eliciting convulsions and as antagonists of GABA transmission. Results on these two points will be presented.

1963 SELECTIVE BLOCK OF DEPOLARIZING ACTION OF GABA ON PRIMARY AFFERENTS BY PIRETANIDE. J. Martin Wojtowicz, Roger A. Nicoll. Dept. Pharm. Univ. California, San Francisco, CA 94143.

Using the isolated frog spinal cord in conjunction with sucrose gap recording from the spinal roots it was found that piretanide (10^{-5} - 10^{-3} M) blocked the depolarizing GABA action on the primary afferent terminals, whereas the hyperpolarizing effect of GABA on motoneurones was unchanged. The depolarizing action of β-alanine on primary afferents was also blocked while there was only variable and slight depression of glutamate responses. Piretanide also blocked the dorsal root potential (DRP) generated by ventral root stimulation and reduced the DRP generated by dorsal root stimulation without prolonging its time course. The ventral root potential generated by dorsal root stimulation was little affected by piretanide. The action of piretanide was essentially irreversible since little recovery was seen after washing for 3 hours.

It has previously been reported (Zeuthen et al., Nature 273, 678-680, 1978) that piretanide inhibits chloride transport in intestinal epithelial cells. To examine the mechanism for the GABA blockade in our preparation intracellular recordings from isolated dorsal root ganglia were initiated. Iontophoretic application of GABA produces a marked depolarization of the somata of the dorsal root ganglion cells. The depolarization is associated with a decrease in the membrane resistance and its equilibrium potential (E_{GABA}) is 50-60 mV. Following repeated GABA applications, E_{GABA} can be shifted towards the resting membrane potential suggesting that the ionic gradient responsible for the depolarization is being dissipated. Piretanide accelerates the shift in E_{GABA}. Since Cl^- is the major ion involved in the GABA response (Gallagher et al., J. Physiol. 225, 2-22, 1977), the effect of piretanide can be explained by an inhibition of the inward chloride pump. In addition, a reduction in the conductance change due to GABA occurred in the presence of this drug.

In summary, we have found that piretanide selectively blocks depolarizing GABA responses both by interferring with inward chloride pumping, and by reducing the chloride conductance change elicited by GABA. Such a selective drug should be valuable in separating pre- and postsynaptic inhibitory events. (Supported by GM23478 and RCDA NS00287).

1964 POWER SPECTRAL ANALYSIS OF EEG AFTER ETHANOL ADMINISTRATION: CORRELATION WITH ETHANOL BLOOD LEVELS. Daniel L. Wolf* and Gerald A. Young (SPON: Naim Khazan). Dept. Pharmacol. and Toxicol., Univ. of Maryland Sch. of Pharm., Balto., MD 21201

Dose-related effects upon EEG after acute intravenous ethanol administration were studied in the rat and compared with ethanol blood levels. One group of female Sprague-Dawley rats was implanted with chronic cortical EEG and temporalis muscle electrodes, and indwelling jugular cannulas for ethanol administration. Ethanol (1, 2, and 4 g/kg, i.v.) was given and EEG and behavior were continuously monitored. Analysis of slow-speed EEG polygraph tracings revealed that ethanol had a dose-related effect to increase the duration of individual episodes with high voltage EEG. Peak increases in duration of EEG synchrony occurred at approximately 1.5, 3.0, and 7.0 hours after the 1, 2, and 4 g/kg doses of ethanol, respectively. The synchronized pattern observed in the EEG after ethanol was subjected to off-line power spectral analysis utilizing a Nicolet MED-80 minicomputer. Power spectral densities were determined at 0.1 Hz intervals in the 0-25 Hz range. Power spectral analysis of ethanol-induced EEG synchrony revealed dose-related increases in power in the 0-4 Hz band. Peak increases in 0-4 Hz band spectral power occurred at the same time as peak increases in duration of high voltage EEG episodes. Dose-related decreases in spectral power in the 4-8 Hz and 8-13 Hz bands were observed soon after infusion, but these changes occurred to a much lesser degree. These results indicate that ethanol caused a general shift of the EEG to lower frequencies. Comparison of ethanol-induced EEG synchrony to control slow wave sleep EEG further revealed that ethanol power spectra differed significantly from sleep power spectra in both power and frequency distributions.

A second group of rats bearing only jugular cannulas received the same ethanol treatment. Serial blood samples were withdrawn and the ethanol content determined. The peak blood level occurred at the end of the infusion and was proportional to dose, while the time to eliminate one-half of the peak was 1.3, 2.3, and 6.0 hours for the 1, 2, and 4 g/kg doses, respectively.

Peak changes in EEG spectral power were manifest at a time when approximately 70% of the respective peak ethanol blood level had been eliminated. From these results we conclude that ethanol induces EEG patterns distinct from those observed in the control sleep state and that the peak responses in the EEG synchrony are preceded by, and do not coincide with, the peak levels of blood ethanol. (Supported by NIAAA Grant AA 03659.)

1965 ALPHA ADRENERGIC RECEPTOR SUBTYPES AND CYCLIC AMP ACCUMULATION IN MOUSE CEREBRAL CORTEX. B.B. Wolfe, J.B. Kleiner* and P.B. Molinoff. Department of Pharmacology, University of Colorado Medical Center, Denver CO 80262.

The pharmacological specificity of catecholamine-stimulated cyclic AMP accumulation in mouse cerebral cortex was determined. Both α and β-adrenergic receptors increase cyclic AMP accumulation in slices of cerebral cortex. The difference between the maximal stimulation in the presence of l-epinephrine (EPI) and that in the presence of l-isoproterenol (ISO) was used as a measure of α-adrenergic receptor stimulated cyclic AMP accumulation. Cortical slices from male HS mice from a colony maintained at the University of Colorado were used as a tissue source. These mice were chosen on the basis of the large α-adrenergic component of cyclic AMP accumulation. Typical values for cyclic AMP accumulation were: Basal 25 pmol/mg; l-ISO 150 pmol/mg; l-EPI 500 pmol/mg. The Ki and Kact values were determined for a number of drugs including l-EPI, d-EPI, phentolamine, indoramin, prazosin, yohimbine, clonidine and WB-4101.

Radioligand binding utilizing both (^{3}H)-WB-4101 and (^{3}H)-clonidine was also examined. These ligands appear to bind primarily to α-1 and α-2 receptors respectively. The Kd values of a variety of agonists and antagonists were determined for the inhibition of both (^{3}H)-WB-4101 and (^{3}H)-clonidine binding. The values obtained with (^{3}H)-clonidine did not correllate with Ki or Kact values obtained in studies of cyclic AMP accumulation while the data obtained in studies with (^{3}H)-WB-4101 more closely resembled the cyclic AMP data. The results suggest that α-adrenergic receptor stimulated cyclic AMP accumulation in mouse cerebral cortex is not associated with the α-2 receptor as measured by (^{3}H)-clonidine binding but may be associated with the α-1 receptor as measured by (^{3}H)-WB-4101 binding.

To further examine this question neonatal mice were treated with 6-hydroxydopamine during the first 4 days of life. This treatment resulted in a 70% increase in α-adrenergic receptor stimulated cyclic AMP accumulation in slices of adult mouse cerebral cortex. This was accompanied by a 50% increase in the density of (^{3}H)-WB-4101 binding sites with no significant change in the density of (^{3}H)-clonidine binding sites. Supported by NS13289 and NS09199.

1966 ^{3}H-PERGOLIDE BINDS TO DOPAMINE RECEPTORS IN MAMMALIAN BRAIN. David T. Wong,* Frank P. Bymaster,* Penny T. Lane,* Donald Kau* and Edmund C. Kornfeld.* (Spon: L. Lemberger). The Lilly Research Laboratories, Indianapolis, IN. 46206.

Pergolide, (8β)-8-[(methylthio)methyl]-6-propylergoline, is a potent new dopamine agonist. Pergolide caused turning in rats with unilateral nigrostriatal lesions, lowered serum prolactin, and decreased dopamine turnover in rat brain (Fuller et al., Life Sci. 24, 375, 1979). Pergolide also inhibited the binding of ^{3}H-dopamine and ^{3}H-spiperone in bovine striatal membranes with inhibitor constants (Ki values) of 7.6 and 12 nM, respectively (Wong et al., XI Int'l Congress Biochem. 1979 Abs.).

Specific binding of ^{3}H-pergolide has been demonstrated in corpus striatum and olfactory tubercle of bovine and rat brains. About 70% of the specific binding was localized in the P_2 fraction (crude synaptosomes) of the striatal homogenate of rat brain. Scatchard analysis revealed a single component of ^{3}H-pergolide binding sites with a dissociation constant (Kd value) of 2.8 nM and receptor number of 370 fmole/mg protein in bovine striatal P_2 membranes. The corresponding values for the rat striatal P_2 membranes were 3 nM and 653 fmole/mg protein. Dopamine agonists (apomorphine and the aminotetralin derivatives, M-7 and TL-II-160) were potent inhibitors of ^{3}H-pergolide binding in rat striatal membranes with Ki values of 3.4, 19 and 11.3 nM, respectively. The dopamine antagonist, d-butaclamol, blocked ^{3}H-pergolide binding with a Ki value of 13 nM while its pharmacologically inactive isomer, l-butaclamol, was 1/20 as effective. Dopamine was a relatively weak inhibitor of ^{3}H-pergolide binding (Ki=1125 nM). Since pergolide was over 100-fold more potent in the inhibition of ^{3}H-dopamine binding than dopamine was in the inhibition of ^{3}H-pergolide binding, we conclude that pergolide binds more tightly to the receptors than does dopamine. Binding of ^{3}H-pergolide increased in striatal membranes from rats made supersensitive to apomorphine and pergolide after nigrostriatal lesions with 6-hydroxydopamine or after chronic administration with haloperidol. All of these studies suggest that pergolide produces its dopaminergic responses in vivo by acting on the postsynaptic receptors for dopamine.

1967 REGIONAL BRAIN GLUCOSE UTILIZATION FOLLOWING INTRASTRIATAL INJECTIONS OF KAINIC ACID IN RATS.
G. F. Wooten and R. C. Collins. (Spon. William M. Landau); Dept. Neurol., Wash. U. Med. Sch.; St. Louis, MO 63110

Intrastriatal injections of kainic acid (K.A.) (0.2-3.8 nmol in 0.4 µl of buffered normal saline) resulted in a dose-dependent increase in glucose utilization in the ipsilateral striatum 1-2 hours after injection as determined by ^{14}C-2 deoxyglucose autoradiography. Quantities of K.A. (1.9 and 3.8 nmol) sufficient to cause extensive lesions of the striatum resulted in a marked increase in glucose utilization in deeper layers of the overlying frontal neocortex 1-2 hours after injection. 1 week after injections of 1.9 or 3.8 nmol of K.A. the ipsilateral striatum showed a reduction in glucose utilization associated with a similar reduction in the number of neuronal cell bodies and choline acetyltransferase activity. Even at the lowest K.A. concentrations (0.2 nmol) there was a marked increase in glucose utilization 1-2 hours after injection in ipsilateral globus pallidus, substantia nigra, nucleus accumbens, septum, and ipsilateral greater than contralateral hippocampus; while at the higher concentrations (1.9 and 3.8 nmol) glucose utilization was markedly increased after 1-2 hours in ipsilateral olfactory cortex, pyriform cortex, and ventral tier nuclei of the thalamus as well.

Autoradiographs following intrastriatal injection of 1 µCi of ^{3}H-K.A. in 0.4 µl of normal saline revealed that all detectable tritium remained highly localized in the striatum for at least 2 hours after injection.

These results demonstrate that K.A. causes an increase in glucose utilization in the striatum and in some adjacent as well as far distant brain structures within 2 hours of intrastriatal injection. It appears that the increase in glucose utilization in distant sites may be mediated via direct anatomical connections with the striatum along multisynaptic pathways rather than by diffusion or rapid axonal transport of K.A..

1968 DISTRIBUTION OF PHENYTOIN IN RABBIT BRAIN: IN VITRO STUDY.
T. Yanagihara, Dept. of Neurol., Mayo Clinic and Mayo Med. School, Rochester, MN 55901.

Intracerebral distribution of phenytoin, a commonly used anticonvulsant, has been investigated in the past after systemic or intracranial administration, by incubation with isolated subcellular fractions, and by binding with proteins or lipids extracted from brain. These investigations have shown variable results ranging from covalent binding of phenytoin to macromolecules to reversible binding, and from binding to brain proteins to lipids. At certain periods after systemic administration, nerve cells contained more phenytoin than neuroglial cells. In the present investigations, therefore, binding characteristics of phenytoin were further investigated in vitro by incubation of brain slices or isolated synaptosomes in the presence of 0.25 µM to 100 µM of [^{3}H]phenytoin. Subcellular fractions were subsequently prepared by sucrose density centrifugation, and neuron-enriched and glia-enriched fractions and synaptosomes by Ficoll-density gradient centrifugation. Synaptic subfractions were separated by osmotic shock and subsequent sucrose density gradient centrifugation.

Among subcellular fractions, purified nuclei and microsomes showed high concentration (per protein unit). However, these high activities could be easily reduced by washing in an isotonic sucrose solution. The highest concentration was obtained in the supernatant. There was no significant difference between the neuronal and neuroglial fraction after an incubation period for 30 min. The uptake of phenytoin by isolated synaptosomes did not show high affinity kinetics. Further separation of synaptic subfractions revealed the highest concentration in the soluble fraction. The concentration of the membrane-enriched and other particulate fractions were similar. The uptake to tissue slices or synaptosomes were not saturable within the concentration of phenytoin investigated here. Phenytoin was bound to macromolecules up to 90% in the supernatant fraction. In synaptosomes, a similar extent of binding was observed in the soluble fraction when they were investigated by ultrafiltration technique. However, the bound phenytoin was easily dissociated from macromolecules (proteins) by passing through a Sephadex G-25 column. On the other hand, phenytoin remains bound to serum proteins after the same treatment. The present investigation indicated binding of phenytoin to brain subcellular elements and proteins but did not show any specific high affinity uptake or binding. Binding to brain proteins showed even a lesser degree of affinity than binding to serum proteins. However, more specific binding may exist if the binding characteristics are tested with certain specific proteins. (Supported by the grant NS-52327 from NIH)

1969 THE NEUROPHARMACOLOGY OF A NOVEL γ-AMINOBUTYRIC ACID ANALOG, KOJIC AMINE.
George G. Yarbrough, Michael Williams and Dean R. Haubrich. Dept. of Neuropharm., Merck Institute for Therapeutic Research, West Point, PA 19486.

Kojic amine (KA; 2-aminomethyl-5-hydroxy-4H-pyran-4-one), a compound which shares some structural features and pharmacological properties with γ-aminobutyric acid (GABA) and muscimol, has been examined in a variety of test systems for GABAmimetic activity. In several in vitro central nervous system receptor binding assays employing rat brain membrane preparations, KA exhibited selective activity to displace ^{3}H-muscimol but with a relatively high IC_{50} of 4.4 µM. Iontophoretically applied KA exerted a pronounced (comparable to GABA on the basis of ejection currents) inhibition of the firing of cerebellar Purkinje cells and spontaneously active or glutamate-activated neurons in the cerebral cortex. The inhibitory effects of KA, which were longer lasting than those of GABA, were antagonized by bicuculline and enhanced in the presence of 2,4-diaminobutyric acid. On the isolated amphibian (Bufo marinus) spinal cord, KA was less than 1/3 as potent as GABA in depolarizing primary afferent terminals. In this preparation KA caused a marked decrease in the dorsal and ventral root potentials evoked by electrical stimulation of an adjacent or corresponding dorsal root. KA is a poor substrate for GABA uptake systems into rat brain synaptosomes, has no effect on GABA release in vitro and does not inhibit GABA transaminase activity. Altogether, these data suggest that KA does have some GABAmimetic actions but also exerts other pharmacological effects as well.

1970 METHADONE AND UNDERNUTRITION: A COMPARATIVE STUDY OF THEIR EFFECTS ON BRAIN DEVELOPMENT.
Ian S. Zagon and Patricia J. McLaughlin. Dept. Anatomy, The M.S. Hershey Medical Center, The Pennsylvania State University, Hershey, PA 17033.

The effects of maternal methadone exposure (5 mg/kg methadone-HCl; METH), undernutrition (18 pups/litter; UND), or a combined methadone-undernutrition (METH-UND) treatment, administered during lactation, on body and brain development were studied in 21- and 60-day old Sprague-Dawley rats. At weaning (day 21), the body weights of METH, UND, and METH-UND animals were reduced 19%, 48%, and 58%, respectively, from control levels. METH rats had the most pronounced deficits in brain weight at 21 days; brain weights were reduced 31%, 12%, and 16% from controls in the METH, UND, and METH-UND groups, respectively. Brain/body weight ratios were elevated in the UND and METH-UND groups, suggesting a brain-sparing effect in these rats. Brain length was significantly reduced in METH and METH-UND pups at weaning, while cerebral width was subnormal in METH and UND rats. In comparison to the reductions in brain DNA content of UND and METH-UND rats (9% and 15%, respectively, of control values), 21-day old METH pups had the most marked deficit (43%), while protein contents were 50%, 21%, and 23% lower than controls for rats in the METH, UND, and METH-UND groups, respectively. At 60 days of age, male rats of all experimental groups had subnormal body weights, but female rats were comparable to controls. METH rats were similar to controls in brain weight and brain dimensions on day 60, while METH-UND rats were subnormal in brain weight (25%) and brain length (15%); UND animals had marked reductions in brain length (16%) and cerebral width (6%). Brain DNA and protein contents and concentrations of animals in the METH and UND groups were lower than controls at sexual maturity, with deficits ranging from 6% to 30%. UND rats also had abnormal reductions in brain RNA content (15%) and concentration (17%) at 60 days. METH-UND rats had the fewest number of biochemical alterations, with reductions in DNA (16%), RNA (26%), and protein (25%) contents recorded; these appear to be associated with the decreased brain weights noted at this time. These results suggest that the constellation of neurobiological effects observed in rats subjected to methadone differ from those found in nutritionally-deprived offspring. The magnitude of structural and biochemical changes that occur during preweaning neuro-ontogeny appear to be greatest in methadone-treated offspring, while the most pronounced disturbances in postweaning development are associated with undernutrition. Furthermore, combined exposure to methadone and undernutrition does not produce a cumulative response.

This research was supported by NIDA Grant DA 01618.

1971 OPPOSED EFFECTS OF MUSCIMOL AND PICROTOXIN ON BRAIN STIMULATION REWARD: A ROLE FOR GABA. Peter Zarevics* and Paulette E. Setler (SPON: W. D. Matthews). Smith Kline & French Laboratories, Philadelphia, Pennsylvania 19101.

Rats were trained in a two-lever intracranial self-stimulation (ICSS) paradigm. Responses at the first lever delivered brain stimulation which was decreased in magnitude after every 5 responses. Responses at the second lever reset the current available to its original value. The current level at which the reset responses occurred was defined as the "reward threshold." The rate of responding at each current level during the stimulate-reset sequence was also determined. This paradigm, therefore, allowed simultaneous rate-independent and rate-dependent assessment of ICSS.

Decreased reward, as demonstrated by an elevated "reward threshold," was produced in a dose-related manner by the GABA blocking agent, picrotoxin. Similar effects could be produced by making each stimulation train less rewarding, i.e. by reducing the amount of charge delivered per stimulation. Conversely, increased reward, as indicated by a lower "reward threshold," was produced in a dose-related manner by the GABA-mimetic agent, muscimol, or by increasing the amount of charge delivered by each stimulation. Response rates were not significantly changed at any stimulation intensity following treatment with either drug.

These data suggest that the effects of picrotoxin and muscimol on ICSS are due to changed perception of reward and not to altered performance of the lever pressing task. An important role for GABA in the mediation of reward needs, therefore, to be considered.

NEUROTRANSMITTERS

1972 ^{3}H METARAMINOL UPTAKE IN ISOLATED CEREBRAL MICROVESSELS. Toshiko Abe*, Kozo Abe* and Maria Spatz. Lab. Neuropath. Neuroanat. Sci., NINCDS, NIH, Bethesda, MD 20205.

Norepinephrine, which doesn't cross the blood brain barrier, can be taken up and metabolized in isolated cerebral microvessels showing features of both extraneuronal and neuronal uptake. In order to elucidate further the monoamine's uptake in cerebral microvessels, we investigated the uptake of ^{3}H metaraminol, a norepinephrine analogue which is neither metabolized by MAO nor COMT.

The capillary uptake of ^{3}H metaraminol increased with the time of incubation (30 sec-15 min). The uptake was found to be saturable, because it could be inhibited by addition of unlabeled metaraminol in increasing concentrations to the incubation media containing the labeled substance. The accumulation of ^{3}H metaraminol in the capillaries was stimulated by K^{+} and Na^{+} and inhibited by ouabain, KCN, DPN, adrenergic blocking agents (imipramine, propranolol, dichloroisoproterenol and phentolamine). Moreover, the ^{3}H metaraminol capillary uptake was competitively inhibited by arterenol, 5-hydroxytryptamine and cross inhibited by dopamine, 6-hydroxy dopamine, 5-hydroxydopamine, L-dopa but not by normetanephrine or metanephrine.

These results indicate that ^{3}H metaraminol is taken up by K^{+} and Na^{+} dependent carrier-mediated mechanism (which may be shared by other monoamines) in the cerebral microvessels. This process appears to be similar to the one described for neuronal monoamine uptake especially since extraneuronal uptake of amines was reported to be insensitive to metaraminol but sensitive to normetanephrine and metanephrine.

1973 TRANS-SYNAPTIC EFFECTS OF CHRONIC DORSAL RAPHE STIMULATION ON NEUROTRANSMITTER-SYNTHESIZING ENZYMES. E. Andersen*, N. Dafny, and Z. Gottesfeld. Dept. of Neurobiology and Anatomy. Univ. of Texas Medical School, Houston, TX 77025.

Relatively little is known about the role of neuronal activity in the regulation of neurotransmitter synthesis in the central nervous system. The present work demonstrates the effect of prolonged electrical stimulation of the dorsal raphe (DR) on choline acetyltransferase (ChAT), a cholinergic marker enzyme, and on glutamate decarboxylase (GAD), a GABAergic marker. The brain areas which were tested have been previously shown to receive DR projections. They include caudate nucleus (CN), substantia nigra pars reticulata (SNR), medial habenula (MH), lateral habenula (LH), superior colliculus (SC), ventral tegmental area (VTA), interpeduncular nucleus (IP) and the nucleus of the diagonal band of Broca (NDBB). The stimulation was performed in freely moving animals.

Male Sprague-Dawley rats (200 g) were stereotaxically implanted with chronic electrodes in the DR. After recovery from surgery, the animals were stimulated for 20 minutes daily (20Hz, 6V, 0.2 msec pulse) for 13 days. Sham animals were chronically implanted and were handled identically, but did not receive electrical stimulation. Shortly after the last stimulation, the animals were decapitated, and the brains were quickly removed and frozen on dry ice. Coronal brain sections were cut alternately (60μm and 300μm) in a cryostat at -8°C. The thin sections were stained with 0.1% thionine for microscopic verification of implantation sites and were used as a guide for accurately dissecting discrete areas from the thick sections.

The enzymes ChAT and GAD were assayed in the various brain regions. The activity of ChAT was found to increase by 148% in the LH, 93% in the NDBB, 49% in the VTA, and by 22% in the IP, but did not change in CN, SNR, MH, or SC. GAD activity did not change in the VTA, CN, SNR or IP.

The results suggest that the increase in the activity of ChAT following chronic DR stimulation takes place transynaptically, and that it is modified by serotoninergic neurons located in the DR.

(Supported in part by BRSG-UTMSH to Z.G.)

1974 IMMUNOCYTOCHEMICAL LOCALIZATION OF [LEU^{5}]-ENKEPHALIN AND SUBSTANCE P IN RELATION TO NORADRENERGIC NEURONS IN AREA POSTREMA. D.M. Armstrong, V.M. Pickel, R.J. Miller, T.H. Joh, D.J. Reis. Lab. of Neurobiol., Dept. of Neurol. Cornell Univ. Med. College, New York, NY 10021. (RJM) Dept. of Pharmacol. and Physiol. Sci., Univ. of Chicago, Chicago, IL 60637.

The Area Postrema (AP) in rat contains intrinsic cells which stain with dopamine beta hydroxylase (DBH) and are presumably noradrenergic (Torack et al, Brain Res. 61:235, 1973). We sought to determine by light microscopic immunocytochemistry whether the AP includes dopamine and adrenaline containing cells in addition to the noradrenergic ones. The presence and distribution of the peptides substance P (SP) and enkephalin, which are associated with catecholaminergic neurons in other regions of the brain (Pickel et al, Brain Res. 160:387, 1979), were also examined. Specific antisera to the catecholamine synthesizing enzymes, tyrosine hydroxylase (TH), DBH, and phenylethanolamine N-methyltransferase (PNMT),and to peptides (Leu^{5})-enkephalin and SP were raised in rabbit. Antisera were localized by the peroxidase-antiperoxidase method in 22u Vibratome sections of rat brain fixed by vascular perfusion with 4% paraformaldehyde. TH and DBH but not PNMT were contained in cells of similar morphology throughout the rostrocaudal and dorsoventral extent of the AP. Similarly, cells with enkephalin-like immunoreactivity (ELI) were distributed throughout the AP. ELI containing cells were relatively sparse compared with those labeled with TH and DBH. Varicosities with ELI were present throughout the AP with a heavier concentration ventrolaterally. Unlike ELI, the reaction product for SP was absent in cells and only seen in varicosities located predominantly in the ventrolateral portion of the AP. A few scattered processes containing SP were present in the central AP, although much less dense than the terminals showing ELI. In addition, neuronal perikarya staining with TH, DBH, SP, and ELI were present in lateral regions adjacent to the AP. We conclude the AP in the rat contains intrinsic noradrenergic and enkephalinergic cells. The terminal varicosities in the AP may be derived from these intrinsic cells as well as neuronal perikarya found lateral to the AP. The AP may be a site of interaction of both catecholaminergic and peptidergic neuromodulators.

This research was supported by grants MH24285, NSO6911, HL18974, MH 00078, and LDA-2121-01. (Substance P antiserum generously supplied by S.E. Leeman.)

1975 AMFONELIC ACID AND AMPHETAMINE EFFECTS ON SYNAPTOSOMAL DOPAMINE FORMED FROM PHENYLALANINE. S. P. Bagchi, T.M. Smith* and P. Bagchi* Rockland Research Institute, Orangeburg, N.Y. 10962

Nonamphetamine stimulants amfonelic acid (AA) and cocaine (CO) mimic the central effects of amphetamine (AMT) and all three appear to exert their effects via catecholamines. Reserpine (Res) depletion of brain catecholamines does inhibit the central effects of the nonamphetamines but not of amphetamine. To distinguish between the actions of AMT and those of AA, we have studied the effects of these two drugs alone and in combinations with Res on the synaptosomal (P_2) synthesis/release of dopamine (DA) from phenylalanine (Phe) precursor. P_2 from rat caudate nucleus was incubated with ^{14}C-Phe in tris buffer (pH 7.4) with NaCl (125 mM), KCl (5 mM), $MgCl_2$ (15 mM), pargyline (0.08 mM), glucose (10 mM) and sucrose (0.32 M). After 10 minutes of incubation (37°), the mixture was filtered through a 0.8 mμ Millipore filter to separate the synaptosomes from the medium and the separated fractions were analyzed for labelled DA and synaptosomal level of ^{14}C-Phe. The results show that the addition of either Res, AMT or AA enhanced the release of synaptosomally formed labelled DA into the medium. Res (1.8 micromolar) concomitantly inhibited the total synthesis of labelled DA. AMT and AA in 0.9 to 18 micromolar concentration range stimulated the synthesis and their synthesis and release enhancing effects were comparable. In other experiments, AMT and AA, each in a combination with Res, were incubated with P_2 and labelled DA synthesis/release determined. The results show that both AMT and AA were able to enhance further the synaptosomal release heightened by Res. However, AMT (plus Res) was markedly effective in stimulating the total synthesis of labelled DA over that observed in the presence of Res alone but AA (plus Res) was ineffective. Furthermore, AMT had a greater synthesis stimulating effect in the presence than in the absence of Res. None of the drugs had any significant effect on the synaptosomal level of ^{14}C-Phe substrate. Other results show that the effects of CO on the DA synthesis/release were similar to those of AA. The results suggest that the action of AA and CO may differ from that of AMT on the Res responsive intrasynaptosomal DA pool at the site of phenylalanine hydroxylation. (Kindly supported by the Dept. of Mental Health, State of New York).

1976 IN VITRO EFFECTS OF pH AND PHOSPHORYLATION ON NEOSTRIATAL TYROSINE HYDROXYLASE FROM CONTROL AND HALOPERIDOL TREATED RATS. Charles Bakhit* and James W. Gibb (SPON: W. Stevens). Department of Biochemical Pharmacology and Toxicology, University of Utah, Salt Lake City, Utah 84112

We have recently shown that the haloperidol-induced increase in the activity of tyrosine hydroxylase (TH) is pH dependent (Fed. Proc., 38, 805, 1979). This activation of TH occurs at the higher pH spectrum but not at the optimum pH for TH. A decrease in Km of TH after haloperidol treatment is seen at pH 6.5 but no change is observed at pH 6.0 for the pteridine cofactor. It is possible that haloperidol may produce its effect by causing a phosphorylation of the enzyme. In an attempt to understand the relationship between pH, phosphorylation and the haloperidol-induced effect, neostriatal TH activity from control and haloperidol-treated (1 mg/kg) rats was assayed under normal and phosphorylating conditions at different pH values using the Nagatsu method, with 6 MPH_4 as the cofactor. The apparent Vmax and Km of TH were determined at two pH values, 6.0 and 6.5. As reported previously, haloperidol treatment caused a decrease in Km with no change in Vmax for the cofactor at pH 6.5, but at pH 6.0 haloperidol did not induce a change in the Km or Vmax of TH. With phosphorylating conditions at pH 6.5, TH from haloperidol-treated rats showed an increase in apparent Vmax, and a return of the Km to control value; the haloperidol-induced activation was no longer observed and the kinetics of enzyme were the same as that for normal rats. In control rats, with phosphorylating conditions at pH 6.5, an increase in Vmax of the enzyme was observed but no change in Km occurred. In contrast, neostriatal TH from control animals assayed at pH 6.0 under phosphorylating conditions showed a decrease in apparent Km but no change in Vmax. However, it should be noted that the activity of the phosphorylated enzyme at pH 6.5 is still less than the activity of TH under optimum pH conditions. These results indicate that the haloperidol-activated enzyme can be further activated by phosphorylation, which causes a return to control kinetics. Furthermore, the kinetics of phosphorylated TH from normal rats at its in vitro optimum pH are different than those at higher pH conditions. (Supported by USPHS grants GM 07579 and DA 00869.)

1977 CHARACTERISTICS OF THE GLUTAMATE RECEPTOR FUNCTION OF BRAIN SYNAPTIC MEMBRANES AND OF THE PURIFIED BINDING PROTEIN. R. Belieu, E. Michaelis, M. Michaelis, and H. Chang*. Dept. of Human Development, Univ. of Kansas, Lawrence, Kansas 66045.

Previous studies from this laboratory have shown that brain synaptic membranes possess high-affinity, Na^+-independent glutamate (Glu) binding sites which have many of the characteristics of the physiologic receptor for L-Glu (Michaelis et al., 1974). In addition, it was demonstrated that this high affinity Glu recognition function was associated with a glycoprotein which was solubilized from the membranes and purified to near homogeneity (Michaelis, 1975). However, since all previous studies were conducted in hypotonic, Na^+-free media, it was considered necessary to examine the behavior of this Glu recognition function of synaptic membranes under more physiological conditions of ionic composition of the incubation medium and of temperature of incubation. A microfuge centrifugation binding assay (Michaelis, 1979) was employed in the measurement of Glu binding to isolated brain synaptic membranes prepared according to the method of Kanner (1978). L-(^{3}H)-Glutamate binding to these membranes was determined either in 10 mM KPO_4 buffer or in a Krebs buffer medium. For a single batch of membranes the binding activity under these two conditions was almost identical. Pretreatment of the membranes with the organomercurial p-chloromercuribenzylsulfonate (PCMS) did not affect Glu binding, even though this type of treatment does block 85% of Glu transport carrier activity.

Glutamate binding to the synaptic membranes measured in either phosphate or Krebs and at either 25° or 37° exhibited a very rapid phase of association kinetics followed by a slower phase of binding. Half-maximal association to the rapidly-binding sites at 25° occurred in 37 - 40 sec and in 15 - 20 sec at 37°. On the basis of the kinetics of this binding interaction, the K_D was estimated to be 80.06 nM. Equilibrium binding assays revealed multiple binding equilibria with apparent K_D's equal to 60 - 70 nM, .200 - .295 μM, and 3.40 μM. The most effective displacing agent for bound L-(^{3}H)-Glu was L-aspartate. The potent neuroexcitatory agent kainic acid was only a weak antagonist of Glu binding to the synaptic membranes regardless of whether such binding was measured in phosphate or Krebs media.

All of the above observations on the Glu interaction with the synaptic membranes were found to hold true also for Glu binding to the purified protein, including the lack of sensitivity to PCMS, the presence of multiple binding equilibria, and the absence of any appreciable effect of kainic acid. (Supported by GM-22357 and Institutional Biomedical Research Support Grant RR-50706.)

1978 DL-α-AMINOADIPATE ANTAGONIZES POSTSYNAPTIC ASPARTATE RESPONSES IN CULTURED MURINE SPINAL CORD NEURONS. Gregory K. Bergey*, Michael R. Martin and Manfred Hermes*. (SPON: Phillip G. Nelson). Laboratory of Developmental Neurobiology, NICHD and Laboratory of Neuro-Otolaryngology, NINCDS, Bethesda, MD 20205.

The effects of D,L-α-aminoadipate (DLAA) on the responses to iontophoretically applied amino acids were studied using cultured dissociated fetal mouse spinal cord neurons. Intracellular recordings were obtained from spinal cord neurons grown in culture for five to ten weeks and responses to iontophoretically applied excitatory (asparate, ASP and glutamate, GLU) and inhibitory (glycine, GLY and γ-aminobutyric acid, GABA) were obtained in the presence of tetrodotoxin blockade of spontaneous electrical activity.

DLAA at DC iontophoretic currents of less than 100 nA rapidly and reversibly reduced the responses to 100 ms (0 to 100 nC) iontophoretic pulses of ASP an average of 87% (n = 23). The plot of mean antagonism of ASP by DLAA was an exponential curve with 50% reduction of the control response occurring with 20 nA of DLAA. Log-log plots of ASP dose-response curves showed no change in slope (m = approx. 1) with DLAA, indicating no alteration of ASP receptor cooperativity by DLAA. In contrast to the effects of DLAA on ASP, GLU responses were reduced an average of only 22% (n = 12) by DLAA currents of 80 - 100 nA. No attenuation of GABA (n = 9) or GLY (n = 10) responses was seen with similar DLAA currents. At the iontophoretic currents used (0-100 nA) DLAA produced no change in membrane potential or membrane conductance. The L-isomer (LAA) produced no antagonism of ASP or GLU (n = 4), confirming reports that the D-isomer is the active dicarboxylic acid antagonist.

These results provide additional evidence that D- and DLAA are useful antagonists of dicarboxylic amino acid putative neurotransmitters, most particularly ASP. No presynaptic effects of ASP or GLU were seen, presumably DLAA antagonism occurs at the postsynaptic membrane. This antagonism occurs in the absence of observable direct effects of DLAA.

1979 THYROTROPIN-RELEASING HORMONE MODULATES THE RESPONSE TO SEVERAL NEUROTRANSMITTERS IN SOMATOSENSORY CORTEX OF CAT. D. J. Braitman, C. R. Auker, and D. O. Carpenter. Neurobiology Department, Armed Forces Radiobiology Research Institute, Bethesda, MD 20014.

Synaptic modulation, the nonlinear summation of responses to two transmitters, has been described for a variety of putative neurotransmitters including the neuroactive peptides. Thyrotropin-releasing hormone (TRH, Glu-His-Pro), a peptide present in mammalian cortex, has been reported to specifically enhance the excitatory action of acetylcholine (Ach) on cerebral cortical neurons (Yarbrough, European J. Pharmacol. 48: 19-27, 1978) although Winokar and Beckman (Brain Res. 150: 205-209, 1978) were not able to confirm these results in a small sample of cells.

A number of questions need to be answered concerning the role of TRH as well as neuromodulators in general. (1) Can TRH have both a direct and modulatory effect on the same cell? (2) Is the modulatory effect of TRH specific for the Ach response? (3) If TRH modulates the response of one neurotransmitter on a given cell, does it also modulate the response of other neurotransmitters on that same cell? (4) Does TRH modulate specific response, e.g., the slow excitation frequently seen with Ach and histamine (Hist)? (5) Does TRH exert its effect on all cortical cells or only on specific populations of cells?

We have studied the modulatory effects of TRH on pyramidal tract (PT), nonpyramidal tract (NPT), and unidentified cells in somatosensory cortex (area 4) of cloralose-anesthetized cats with seven-barreled microelectrodes. In each electrode, the center barrel was filled with 2M NaCl for recording single-neuron activity. Each of the other barrels was filled with Ach, Hist, glutamate (Glu), or aspartate (Asp) for ionophoretic application. One barrel was filled with TRH for microionophoretic and/or pressure injection.

The following experimental findings are pertinent to the questions posed above: (1) TRH had a direct excitatory effect on only five of 32 cells tested. In three of these cells there was no modulation of the Ach response. (2) TRH modulated some responses to glutamate, histamine, and aspartate as well as to acetylcholine. In all instances of TRH modulation of Asp the response to Asp was enhanced. The response to Ach was enhanced in four cells and inhibited in one. Hist response was enhanced in two cases and inhibited in one. TRH modulation of Glu was always inhibitory. (3) In one of nine cells tested, TRH was found to enhance the excitatory response to both Ach and Hist. In five other cells, TRH altered the response to one transmitter but not another. (4) TRH potentiated both slow and fast excitatory responses, but not in all cases. In addition, it inhibited four fast excitatory Glu responses and two slow excitatory responses by Ach and Hist. (5) The excitatory Ach response on NPT cells was never potentiated by TRH. All TRH inhibition was on NPT cells. Our results indicate that TRH can modulate both fast and slow excitatory responses and responses to other neurotransmitters as well as Ach on two identified populations of cortical neurons.

1980 IMMUNOCYTOCHEMICAL AND AUTORADIOGRAPHIC LOCALIZATION OF THE γ-AMINOBUTYRIC ACID SYSTEM IN FROG RETINA. C. Brandon, D.M.K. Lam*, and J.-Y. Wu. Departments of Cell Biology and Ophthalmology, Baylor College of Medicine, Houston, Texas 77030.

Several lines of evidence have implicated GABA as a neurotransmitter in the retina of the frog. Graham (Brain Res. 36, 476-479 (1972)) showed levels of GABA and its synthetic enzyme (glutamate decarboxylase, GAD) to be highest in the inner plexiform layer (IPL), and Voaden et al (ibid 67, 115-132 (1974)) demonstrated the presence of a high-affinity uptake system for GABA in horizontal cells and certain amacrine cells. Using a specific antiserum to the mouse brain enzyme (Brain Res. 65, 277-285 (1974)) and a Protein A-Peroxidase-Antiperoxidase (A-PAP) technique, we have examined the GABA system in the frog retina by immunocytochemistry and autoradiography.

Frog (Rana pipiens) retinas were fixed by immersion in 1% formaldehyde containing 0.1% glutaraldehyde or 0.055% acrolein in 0.12M sodium phosphate buffer, pH 7.3. Tissue chopper sections (100um thick) were treated sequentially with rabbit anti-GAD serum (1:200 dilution), protein A (50ug/ml) and PAP; all incubations were carried out in PBS containing 0.1% ovalbumin to minimize non-specific staining.

GAD-positive reaction product was observed to form five discrete laminae within the IPL. The laminae adjacent to the inner nuclear layer (INL) and the ganglion cell layer were the most dense; three evenly-spaced lighter bands were observed between them. In addition, occasional cell bodies lying within the inner third of the INL, presumably amacrine cells, were filled with immunochemical reaction product. Slender, single processes were often seen descending from these cell bodies into the IPL.

For GABA uptake studies, retinas were incubated in vitro with ^{3}H-GABA in frog ringer, then fixed in glutaraldehyde and processed for autoradiography. Grains were observed over horizontal cells, amacrine cells, and ganglion cell bodies.

These results suggest that a family of amacrine cells in the frog retina uses GABA as a neurotransmitter. Ultrastructural analysis of the detailed synaptic relationships of these GAD-containing neurons, thought to be involved in the formation of complex receptive field properties, will be presented.
(Supported in part by NIH grants NS-13224 and EY-02423, and a grant from the Huntington's Chorea Foundation.)

1981 A SYNAPTOSOMAL PREPARATION FROM THE GUINEA PIG ILEUM. Clark A. Briggs*, Rose A. Schulz*, and Jack R. Cooper (Spon: James W. Maas). Dept. of Pharmacology, Yale Univ. School of Med., New Haven, CT 06510.

The myenteric plexus of the guinea pig ileum has characteristics which make it a choice mammalian tissue for the study of neuronal activity. Although part of the peripheral nervous system it bears a remarkable similarity--morphologically, functionally and neurochemically--to the central nervous system (CNS). Perhaps the best known example is the similarity in potency for a wide range of narcotics in inducing analgesia in the CNS and in inhibiting acetylcholine (ACh) release from the myenteric plexus. This similarity of the ileum to the CNS, coupled with the observations that a number of neurotransmitters and neurohormones have been shown to affect the release of ACh, prompted us to develop a synaptosomal preparation from this tissue.

After a considerable number of trials, the following procedure was adopted: Using 250-350g Hartley guinea pigs, strips of myenteric plexus-longitudinal muscle were prepared from the entire ileum, except for the terminal 12cm, by a minor modification of the method of Paton and Vizi. High affinity choline uptake was used as a marker for synaptosomal activity during the isolation. After mincing, the strips were homogenized in 0.32M sucrose-3mM sodium phosphate buffer, pH 7.2, first by Ultra-Turrax homogenization at low speed, followed by Teflon-glass homogenization. A crude fraction ("P_2") containing most of the synaptosomal activity was prepared by initially centrifuging at 1,000g for 10 min. followed by centrifugation of the supernatant at 17,000g for 20 min. The synaptosomal fraction was prepared by applying the P_2 to a discontinuous sucrose-Metrizamide gradient containing 3mM sodium phosphate buffer, pH 7.2; this was centrifuged at 86,000g for 30 min. in an SW50L rotor to yield a synaptosomal band with a 7 to 8 fold enrichment compared to the P_2 fraction. Recovery of activity amounted to 40 to 50%. Electron microscopy of the fraction revealed a fairly homogeneous synaptosomal population with some membrane contamination but no free mitrochondria.

Synaptosomal activity was indicated by a high affinity choline transport system which displayed sodium and temperature dependence and inhibition by hemicholinium-3 and hypo-osmotic shock. In addition, a calcium dependent release of [^{3}H]-ACh, synthesized by preincubation with [^{3}H]-choline, was also demonstrated when the preparation was depolarized with K^+ or veratridine.

In preliminary experiments we have shown an inhibition by oxotremorine of [^{3}H]-ACh release from this preparation.

1982 **MUSCARINIC MODIFICATION OF VOLTAGE-SENSITIVE CURRENTS IN SYMPATHETIC NEURONS.** **D. A. Brown* and Paul R. Adams. Dept. of Physiol. and Biophys., University of Texas Medical Branch, Galveston, Texas, USA.**

Bullfrog sympathetic ganglion cells in vitro were impaled with two microelectrodes (30-40 MΩ, filled with 3 M KCl) and voltage-clamped. In most cases the ganglia were trypsinised and the Ringer supplemented with 10 mM magnesium chloride. A pure muscarinic agonist, dl-muscarine, was applied by bath-perfusion (10 μM) or by iontophoresis. The agonist produced a voltage-sensitive inward current, which was very small (≤0.1 nA) at holding potentials more negative than -50mV and increased to 2-4 nA at holding potentials up to -20mV. Control steady-state current voltage (I/V) curves showed strong rectification at membrane potentials below -60mV. Currents following long (up to 0.5 sec) hyperpolarizing command-steps comprised an ohmic step followed by a slow inward relaxation (time constant ~100msec) in the rectifying range of membrane potential, with an appropriate slow inverse relaxation on restoring the holding potential. The reversal potential of the slowly relaxing currents was near -80 mV. Also, the ohmic current stepping back from a hyperpolarized level was less than that seen at the onset of the hyperpolarizing step. Thus these slow inward currents probably represent deactivation and reactivation of the cell's potassium conductance. Muscarine (i) reduced the rectification of the steady-state I/V curve, (ii) reduced the slow current relaxations following hyperpolarizing voltage-steps and (iii) reduced the slope of the instantaneous I/V curve (measured from ohmic current steps) at depolarized membrane potentials (-20 to -40 mV) by up to 70%. In contrast, the slope of both steady-state and instantaneous I/V curves at more hyperpolarized levels (≤-60 mV) was usually unchanged. Further, muscarine did not clearly reduce the currents generated by small (10-20 mV) depolarizing voltage steps. These observations suggest that muscarine's main action is to inhibit that component of the cell's potassium conductance that becomes evident at a restricted range of potentials around -35 mV, with diminishing effect at more depolarized or more hyperpolarized potentials. **(Supported by NIH grant NS-14984 and a travel grant from the Wellcome Foundation.)**

1983 ACUTE AND CHRONIC ALCOHOL ADMINISTRATION ALTER GABA RECEPTOR SENSITIVITY IN INBRED STRAINS OF MICE. Troie Burch* and Maharaj K. Ticku Dept. Pharmacology, Univ. Texas Health Sci. Ctr., San Antonio, TX 78284.

The molecular basis of action of alcohol (ethanol), and the neuronal components involved in its tolerance, physical dependence and withdrawal are not known. Ability of alcohol to enhance γ-aminobutyric acid (GABA) mediated transmission has been reported (Davidoff, R.A. Arch. Neurol. 28:60, 1973), and GABA mimetics have been reported to reduce ethanol withdrawal symptoms, while GABA antagonists produce symptoms similar to those seen during alcohol withdrawal (Goldstein, D.B., JPET 186:1, 1973). We have examined the interactions of acute alcohol with GABA receptors in DBA (alcohol avoiding) and C57 (alcohol preferring) mice, and of chronic alcohol in C57 mice. DBA and C57 mice like rat brain bind GABA to two classes of binding sites. DBA mice have significantly higher affinities (K_{D1} = 13 nM; K_{D2} = 91 nM) and lower binding capacities (B_{max1} = 713 fm, B_{max2} = 3187 fm) compared to C57 mice (K_{D1} = 27 nM, K_{D2} = 135 nM; B_{max1} = 1421 fm; B_{max2} = 3798 fm) for the two binding sites. Acute alcohol (4 g/kg) produced a significant increase in the binding capacity of the low affinity GABA binding site in both C57 (12%) and DBA (23%) mice, without significantly altering other binding parameters. These results suggest that acute alcohol ingestion may produce a possible facilitatory effect on GABAergic transmission, which may be responsible for alcohol induced behavioral depression. Chronically treated C57 mice (2 weeks on 10% ethanol) exhibited a single class of GABA binding site with an apparent affinity of 89 ± 12 nM and a binding capacity of 2471 ± 360 fm/mg protein. The complex effects of chronic alcohol ingestion on both the affinity and the binding capacities of GABA receptor sites may represent an offsetting neuronal mechanism to the continuous depressant effects of alcohol. These results suggest a possible role of GABA receptor sensitivity in the neuropharmacological actions of alcohol and also in the development of its tolerance.
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1984 RELEASE OF ENDOGENOUS EPINEPHRINE, NOREPINEPHRINE, AND DOPAMINE FROM RAT HYPOTHALAMUS IN VITRO. Susan K. Burgess* and Richard E. Tessel* (SPON: Ronald T. Borchardt). University of Kansas, Lawrence, Kansas, 66045.

The presence and distribution of both epinephrine (EPI) and its synthesizing enzyme, PNMT, in the hypothalamus and brainstem suggest the existence of anatomically distinct adrenergic neurons in brain. Comparative measurements of in vivo pharmacological alterations in EPI, norepinephrine (NE), and dopamine (DA) contents and the activities of their synthesizing enzymes have suggested that EPI-containing neurons may be pharmacologically and biochemically distinct as well. To further characterize these neurons, we examined the ability of amphetamine and two depolarizing agents, veratridine and KCl, to release endogenous EPI, in comparison with NE and DA, from minced hypothalamus in vitro. Release of endogenous amine may be a more accurate indication of relevant synaptic events than the conventional isotope-tracer release technique, which presents particularly severe difficulties (e.g., non-specific tracer uptake) with the relatively sparse EPI neurons.

Minced hypothalamic tissue was exposed to the releasing agent for 30 minutes at 37°C in physiological medium. After centrifugal separation of tissue and media, the catecholamines in each fraction were analyzed by HPLC with electrochemical detection. Data are expressed as % release [media content ÷ (tissue + media contents) X 100], and as pmol/mg protein. In no case was control release above 10%. Each of the three agents released the three catecholamines in a dose-dependent way and each agent was essentially equipotent with respect to release of EPI, NE, and DA. Approximate EC50's are: K^+, 45mM; veratridine, $3X10^{-6}$M, and d-amphetamine, $3X10^{-5}$M. Maximal release after K^+ depolarization was: EPI, 50.3±8.5%; NE, 57.9±3.9%; and DA, 64.0±5.4%, representing respectively 0.98±.21 pmol/mg, 42.6±3.4 pmol/mg, and 13.0±1.1 pmol/mg. K^+-induced release was strongly Ca^{++} dependent for all three catecholamines. Veratridine at 10^{-4}M released EPI (46.5±1.9%) and NE (51.6±3.4%) less than it did DA (65.4±5.1%), (representing 1.33±.19 pmol/mg, 62.8±7.6 pmol/mg, and 19.3±1.4 pmol/mg). Veratridine release was largely blocked by $5X10^{-7}$M tetrodotoxin. Amphetamine also preferentially released DA. At 10^{-3}M amphetamine, EPI release was 47.4±5.3%, NE was 36.5±2.3%, and DA was 78.2±2.0%, or 1.04±.25 pmol/mg, 27.7±2.3 pmol/mg, and 15.5±1.5 pmol/mg respectively. Amphetamine induced release was not Ca^{++} dependent.

The data suggest that EPI is stored in nerve terminals and can be released by both exocytotic and non-exocytotic mechanisms.

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1985 AMYGDALOID KINDLING IS ASSOCIATED WITH MUSCARINIC CHOLINERGIC RECEPTOR REDUCTIONS IN THE DENTATE GYRI OF BOTH HIPPOCAMPAL FORMATIONS. Mary Constant Byrne* and James O. McNamara. Div. Neurology, Duke Univ. and VA Med. Center, Durham, N. C. 27710

Kindling refers to the phenomenon whereby periodic electrical stimulation of the brain at current levels too low to induce a behavioral response initially, ultimately produces a motor seizure. Once established, this enhanced sensitivity to electrical stimulation is permanent. Increasing evidence indicates that modification of neural circuitry remote from the stimulating electrode is essential to kindling. Pharmacological studies suggest that the interaction of acetylcholine with muscarinic cholinergic receptors contributes to the development of kindling. We previously reported significant reductions in muscarinic cholinergic receptors in the stimulated amygdala and both hippocampi in animals sacrificed 12-18 hours following completion of amygdaloid kindling. The goal of these studies was to define the distribution of the muscarinic receptor declines within the hippocampi. Electrical stimulations were administered at hourly intervals to the right amygdala of Sprague-Dawley rats until a single Class 5 kindled seizure consisting of rearing and falling was elicited. Animals were sacrificed 18-24 hours following kindling with paired controls (electrode implanted but not stimulated). Both hippocampi were removed and dissected into three principal parts: dentate gyrus, CA3, and CA1 regions. Membranes were prepared from each area and specific binding (atropine displaceable) of muscarinic receptors measured under equilibrium conditions using [^{3}H] quinuclidinyl benzilate (QNB). The mean values ± SEM (N=10) of specifically bound QNB (femtomoles/mg protein) were: right dentate 684±45 control (C); 583±55 kindled (K); left dentate 658±51 C; 542±48 K; right CA3 551±26 C; 492±33 K; left CA3 614±35 C; 568±49 K; right CA 1 563±37 C; 570±38 K; left CA 1 585±29 C; 581±29 K. Statistically significant reductions (paired t-test) were present in the dentate gyri on both sides (right $p < .01$, left $p < .0025$) but not in other regions. Scatchard analysis demonstrated these reductions in QNB binding to be due to decreased numbers of binding sites without alterations in the affinity of the remaining receptors for QNB. The cholinergic innervation of the dentate gyrus arises from neurons situated in the medial septal region. These muscarinic receptor reductions may reflect altered septal cholinergic regulation of neuronal excitability in the dentate gyri following kindling. The dentate gyri of both hippocampi may represent sites of remote neuronal alterations which contribute to the development of kindling established in the amygdala.

1986 COMBINED IMMUNOCYTOCHEMISTRY AND AUTORADIOGRAPHY AFTER IN VIVO INJECTIONS OF ANTIBODY AND ^{3}H-LABELED COMPOUNDS: LOCALIZATION OF COEXISTENT PUTATIVE TRANSMITTERS AND TRANSMITTER-RECEPTOR SYSTEMS IN THE NERVOUS SYSTEM. Victoria Chan-Palay. Dept. Neurobiology, Harvard Medical School, Boston, MA 02115.

In vivo injections of characterized antibodies into selected areas of the central and peripheral nervous system allow direct visualization of neurons and their connections by subsequent anterograde or retrograde transport of antigen-antibody complexes (Chan-Palay 1979, Chan-Palay, Palay, Wu 1979). Further experiments indicate that another novel approach for transmitter localization is achieved by combined immunocytochemistry and autoradiography after in vivo injections of an antibody and a radioactively labeled substance. Examples will be illustrated from several systems. Studies after injections of ^{3}H-serotonin and monoclonal antibody to Substance P indicate that some neurons in the medullary raphe system contain both serotonin uptake systems and Substance P immunoreactivity, whereas other neurons contain one of the two putative transmitter substances tested for (Chan-Palay 1979). Other investigations into the spinal cord, retina and cerebellum have been made using injections of ^{3}H-muscimol; ^{3}H-isoguvacine; ^{3}H-nipecotic acid or ^{3}H-GABA together with antibody against glutamic acid decarboxylase, the synthetic enzyme for γ-aminobutyric acid (GABA). The cellular locations of GABA synthesis together with the uptake and transport systems for GABA, and muscimol and isoguvacine receptor binding sites are revealed. The method involves in vivo injections in the 0.025-0.05 µl range of undiluted antibody and ^{3}H-labeled substance, fixation by perfusion with formaldehyde fixatives with minimal glutaraldehyde, serial vibratome sectioning, reaction by the indirect peroxidase-antiperoxidase technique and revelation with diaminobenzidine/hydrogen peroxide. Following the immunocytochemical procedures the sections are processed for high resolution autoradiography. The major steps for ensuring or controlling for cytochemical specificity and sensitivity in terms of antigen, antigen-antibody complexes, radioactive label through tissue preservation and subsequent preparations have been taken in each of the studies enumerated. They include the use of injections with antibody pretreated with antigens in excess, normal or preimmune sera; controls for serotonin uptake; and the use of GABA uptake inhibitors, agonists and antagonists. The fundamental scheme of simultaneous injection of multiple labels, one radioactive, another an antibody for the detection of multiple transmitter systems, opens avenues for future investigation of chemically specific cells and their connections.

1987 LOCALIZATION OF DOPAMINE-SENSITIVE ADENYLATE CYCLASE IN THE RAT OLFACTORY TUBERCLE. A.C. Church*, B.S. Bunney and N.R. Krieger, Depts. of Pharmacology and Psychiatry, University of Pennsylvania Medical School, Philadelphia, PA., 19104, and Depts. of Psychiatry and Pharmacology, Yale University Medical School, New Haven, CT., 06510.

Numerous biochemical studies have demonstrated the presence of dopamine-sensitive adenylate cyclase (DSAC) within the central nervous system. The distribution of DSAC activity as a function of the neuronal layers of the olfactory tubercle has been described (Brain Res. 131:303, 1977). This region, because of its laminar organization, is particularly suited to neurochemical localization studies. Here we report the use of selective chemical lesions to localize DSAC within the rat olfactory tubercle. Stereotaxic injections were made directly into the tubercle with kainic acid (1µg in 1µl of artificial CSF) or with 6-OH dopamine (4µg in 1µl of saline with 0.1% ascorbate). Using male Sprague-Dawley rats (150g), unilateral injections were made with a 26 ga. needle over a 1 minute period. Animals were sacrificed 3 days later by decapitation, and the brain was rapidly removed and dissected. Homogenates of the olfactory tubercle were assayed for DSAC by the method of Kebabian et al. (PNAS 69:2145, 1972). The kainic acid lesion reduced the DSAC activity by 70-90% (n=4) as compared to values from sham lesioned or unlesioned controls. Homogenates of 6-OH dopamine treated tubercles did not differ in DSAC activity from untreated controls. The lesions were assessed by light and fluorescence microscopy. Sections from kainic acid treated tubercles showed extensive neuronal losses with increased numbers of glial cells. Sections from 6-OH dopamine treated tubercles appeared normal under the light microscope. Examination of 6-OH dopamine treated tissue by glyoxylic acid-induced-histofluorescence established the loss of dopaminergic terminals. The marked decrease of DSAC accompanying the selective loss of neurons (kainic acid treatment) but not accompanying the loss of dopaminergic terminals (6-OH dopamine treatment) suggests that this enzyme occurs in the neurons and not in the glia or in the dopaminergic terminals of this region. Our findings of a neuronal localization for DSAC in the olfactory tubercle parallel the findings of others in the caudate nucleus (Brain Res. 118:356, 1976; 127:235, 1977) and suggest a similar localization for this enzyme in the two regions. (Supported by Scottish Rite Schizophrenia Research Program, N.M.J., U.S.A. and N.I.H. Grant 1 R01 MH31820-01 PPR).

1988 PENTOBARBITAL AND DIPHENYLHYDANTOIN EFFECTS ON THE EXCITABILITY AND GABA SENSITIVITY OF RAT DORSAL ROOT GANGLION CELLS. Barry W. Connors. Dept. of Physiology, Duke Univ. Med. Center, Durham, N.C. 27710

The anesthetic pentobarbital (PB) and the anticonvulsant diphenylhydantoin (DPH) have each been reported to enhance the inhibitory response to GABA, and to have depressant effects on the excitability of a number of neuronal preparations. Dorsal root ganglion cells provide a simple neuronal system which exhibits a chloride conductance increase to exogenously applied GABA. Ganglia from adult rats were placed in a continuously perfused chamber (37°C) and intracellular recordings were made with 4M K-acetate microelectrodes. All drugs were bath-applied.

PB, at concentrations 0.4-2.0x10^{-4}M, reversibly potentiated the amplitude (up to 180±20% of control) and duration of the GABA response in a dose-dependent manner, without having any effect on the resting potential or input resistance. At 10^{-3}M PB, cells were slightly depolarized and showed a small conductance increase. This is probably an expression of the GABA-mimetic property of the drug (Nicoll. PNAS.72:1460.1975), and at this concentration the responses to GABA were profoundly depressed (20±15% of control), perhaps because of receptor desensitization. Additionally, PB (10^{-4}M) increased the accommodation to long current pulses in some cells, with little change in rheobase.

DPH, up to 2x10^{-4}M, did not affect the amplitude or the time course of the GABA response, nor did it change the resting potential or resistance. It did depress the amplitude and rate of rise of the action potential, as well as increase rheobase current. Repetitive firing was also inhibited. Ganglion cells have variable sensitivity to tetrodotoxin (TTX) (Yoshida et al. J. Neurophysiol. 41:1096. 1978), and the effect of DPH was much greater on those action potentials which could be potently inhibited by TTX. Veratridine, which increases resting sodium conductance, caused a slow depolarization which could be partially reversed by either TTX or DPH. Some spikes exhibited a plateau, which could be greatly prolonged by the addition of 5mM Ba^{++}. DPH had no effect on this presumed calcium component (Dunlap and Fischbach. Nature. 276:837.1978) of the spike.

It is concluded that PB and DPH act through entirely different mechanisms, in this preparation. PB may significantly potentiate inhibitory GABA responses in the central nervous system, as well as decrease repetitive firing of neurons. The properties of DPH are compatible with a TTX-like inhibition of resting and voltage-sensitive sodium conductances, with no visible change in GABA inhibition. (Supported by NS 11933, Training Grant 5T01-GM-02929 and a Duke Univ. Grad. School Research Award)

1989 INVESTIGATIONS OF THE SOURCE OF ENKEPHALIN IN THE RAT GLOBUS PALLIDUS USING KNIFE CUT, ELECTROLYTIC AND KAINATE LESIONS. Fernando M. A. Corrêa, Robert B. Innis, Lynda D. Hester*, Steven R. Childers and Solomon H. Snyder. Dept. Pharmacol., Sch. Med., Johns Hopkins Univ., Baltimore, MD 21205

The pentapeptides leucine- and methionine-enkephalin are believed to act as neurotransmitters at brain opiate receptors. Sensitive radioimmunoassay and immunohistochemical techniques have demonstrated that the globus pallidus contains the highest concentration of these opioid peptides in the rat brain. Cuello and Paxinos (Nature 271:178, 1978) have suggested that enkephalin in the globus pallidus of the rat is synthesized in the caudate-putamen and then axonally transported to the globus pallidus. They found that knife cuts between the globus and caudate-putamen eliminated enkephalin immunohistofluorescence in the globus, although knife cuts between the cortex and caudate-putamen had no effect. However, we have found too few enkephalin reactive cell bodies in the caudate of colchicinized rats to account for the very high concentration of enkephalin in the globus pallidus. In addition, the technical difficulties of making a knife cut between the caudate and globus raised the possibility that the enkephalin depletion following such cuts may not simply result from axonal transection. We have investigated the source of enkephalin in the globus by comparing effects of knife cuts, electrolytic and kainate lesions.

Large electrolytic lesions in anterior regions of the caudate do not decrease enkephalin immunohistofluorescence in the globus pallidus, suggesting that little enkephalin in the globus comes from the anterior caudate. Knife cuts between the caudate and globus eliminate enkephalin fluorescence in the region of astroglial proliferation adjacent to the knife cut. This result suggests that some of the decrease of enkephalin immunofluorescence is due to generalized tissue damage rather than interruption of a pathway from the caudate to the globus. The intensity of staining for enkephalin in the globus is so great that it is unclear whether there may not also be enkephalin containing cell bodies as well as fibers. Results from kainate lesions of the globus pallidus suggest that some of the enkephalin in the globus arises from intrinsic neurons.

A major limitation of the immunohistofluorescence technique is that it is not quantitative. For this reason we have attempted to correlate results from immunhistofluorescence and radioimmunoassay of the globus pallidus dissected free from surrounding brain tissue. (Supported by USPHS grant DA-00266)

1990 EFFECTS OF ACETYLCHOLINE ON CULTURED MAMMALIAN CORTICAL NEURONS. Marc Dichter. Dept. Neurology, Harvard Medical School, Boston, MA 02115.

Rat embryo cortical neurons grown in dissociated cell culture develop morphological, electrophysiological and pharmacological properties similar to cortical neurons in situ and the neurons form new excitatory and inhibitory synaptic connections with one another. The cultures contain cholineacetyl transferase (CAT) and acetylcholinesterase (AChE), both of which start at very low values and increase dramatically during the second and third weeks in culture. Histochemical staining reveals that AChE is contained in approximately 5-15% of the neurons and not in non-neuronal cells. These AChE positive neurons exhibited no specific morphology.

Acetylcholine (ACh) applied by microperfusion at 1 to 500 µM produced no significant change in neuronal membrane potential, membrane conductance or action potential configuration. In approximately 50-70% of neurons, ACh produced a delayed (several seconds) and prolonged increase in spontaneous synaptic potentials, either excitatory or inhibitory, which was generated by a presynaptic mechanism. The ACh effect was blocked by concomitant application of tetrodotoxin, and therefore probably involved the activation of Na channels in axon terminals. The ACh effect was also blocked by atropine at 10-100 µM but not by nicotinic blocking agents.

It can be concluded that ACh does not act as either an excitatory or inhibitory synaptic transmitter between cortical neurons in vitro, but probably acts as a presynaptic modulator of ongoing neurotransmission.

1991 THE EFFECT OF RESERPINE ON THE UPTAKE AND RELEASE OF TRITIATED meta-TYRAMINE (m-TA) AND para-TYRAMINE (p-TA) IN RAT STRIATAL SLICES. L.E. Dyck* and A.A. Boulton, Psychiatric Research Division, University Hospital, Saskatoon, Sask., Canada S7N 0W8.

We have shown that slices of rat striatum preloaded with tritiated m-TA or p-TA can be induced to release labelled amine by 50 mM KCl and also by removal of calcium ions from the incubation medium. The latter confounds efforts to determine the calcium-dependency of the release process. Thus, to determine the source of released amine, we have examined the effect of 10 µM reserpine on the uptake of 10 nM m-TA-^{3}H, p-TA-^{3}H and DA-^{3}H and on the subsequent release stimulated by 50 mM KCl, calcium removal or unlabelled m-TA, p-TA or DA (10 µM). Pargyline (10 µM) was present at all times and release was studied by a rapid transfer technique. Presumably, release stimulated from reserpine-pargyline treated slices originates from cytoplasmic sites; whereas in pargyline treated slices, release could originate from vesicular sites as well. Reserpine is a well known depletor of endogenous stores of catecholamine and has been shown to reduce endogenous m-TA and p-TA, too. The uptakes of p-TA-^{3}H, m-TA-^{3}H and DA-^{3}H, expressed as fmol/mg slice/15 min, were 324 ± 20, 373 ± 17 and 332 ± 12 (mean ± S.E.M.) in the pargyline treated slices; and were significantly reduced ($P < 0.01$) to 219 ± 15, 216 ± 35 and 230 ± 9, respectively, in the reserpine-pargyline treated slices. Since reserpine reduced the total uptake, the amount released into each fraction was expressed as a percentage of the total of the amounts released into each fraction and the amount left in the slices at the end of the experiment. Reserpine attenuated the release of all three whether the stimulus was 50 mM KCl or calcium removal. It could be concluded, then, that both types of stimuli caused a release of m-TA-^{3}H and p-TA-^{3}H as well as DA-^{3}H from a vesicular site.

The effect of reserpine on release of tritiated amine by unlabelled amines (10 µM) was also investigated. The magnitudes of the releasing effects of the unlabelled amines on preloaded m-TA-^{3}H, p-TA-^{3}H and DA-^{3}H were similar, the release of m-TA-^{3}H by DA being the least. Reserpine had no effect on the amine-induced releases of m-TA-^{3}H, but it attenuated the amine-induced releases of DA-^{3}H. Reserpine did not alter the m-TA-induced release, potentiated the p-TA-induced release and attenuated the DA-induced release of p-TA-^{3}H. It could be concluded that m-TA-^{3}H is not released from vesicles by exogenous amine, p-TA-^{3}H is released from vesicles by DA only, and DA-^{3}H is released from vesicles by all three amines.

Supported by the MRC of Canada and Saskatchewan Health.

1992 ACTIONS OF ANTI-CONVULSANTS ON GABA-DEPOLARIZATIONS AND ACTION POTENTIALS RECORDED FROM A MAMMALIAN SENSORY NEURON. Joel P. Gallagher, Hiroe Inokuchi* and Patricia Shinnick-Gallagher, Dept. of Pharmacology & Toxicology, Univ. Texas Med. Br., Galveston, TX 77550.

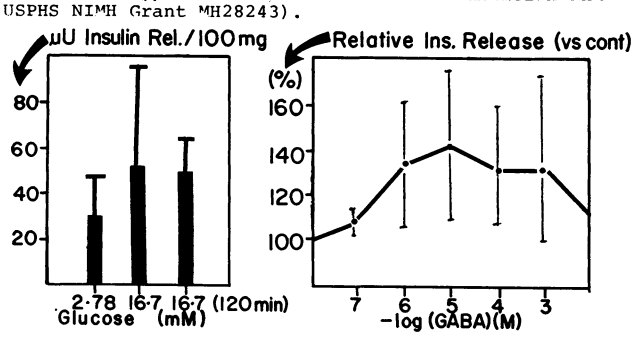
The knowledge that gamma-aminobutyric acid (GABA) is an inhibitory substance in the mammalian brain has resulted in a great amount of research to suggest that an alteration of the GABAergic system may be involved in the etiology or treatment of epilepsy. With this suggestion in mind, we have begun an investigation using three anti-convulsant drugs to examine their potential interactions with a GABA receptor which we have previously characterized on the cell body of cat dorsal root (sensory) ganglia (DRG). This in vitro system is especially suitable since interactions of drugs with receptors can be studied in the absence of synapses. Three structurally different anti-convulsants have been examined: phenobarbital (PB), phenytoin (DPH) and valporic acid (V). Two different types of active responses were recorded from impaled DRG cells: 1) a direct action potential (AP) was obtained by passing a brief cathodal pulse through the recording electrode; and 2) a membrane depolarization was obtained by iontophoretic application of GABA (GD). The AP and GD were recorded before and after superfusion of the anti-convulsants. It was possible to separate a general depressant effect (block of AP) from a specific GABA-receptor effect (alteration in time-course and/or amplitude of GD). PB at concentrations of 5×10^{-5}M to 2×10^{-4}M enhanced the amplitude and prolonged the half-fall of GD without altering AP. At 5×10^{-4}M to 5×10^{-3}M, PB had a dual action, altering the GD as in lower concentrations, but also depressing or completely blocking the AP. DPH (1×10^{-5}M to 1×10^{-4}M) did not affect the GD but either depressed or completely blocked the AP. At 2×10^{-4}M, DPH also depressed GD. V (1×10^{-4}M to 1×10^{-3}M) produced only a slight (10%) depression of GD, but did not affect AP. These results suggest that these three anti-convulsants employed in generalized epilepsy may produce their clinical efficacy by different mechanisms. (Supported by NIH Grant, NS 13727.)

1993 SOLUBILIZATION AND SEPARATION OF NEUROTRANSMITTER RECEPTORS IN THE BRAIN. Moshe Gavish, Ted Usdin, Raymond S. L. Chang, Solomon H. Snyder. Dept. Pharmacol., Sch. Med., Johns Hopkins Univ., Baltimore, MD 21205

Binding sites for neurotransmitter receptors in the brain have proved more difficult to solubilize than peripheral neurotransmitter and hormone receptors. Using a variety of detergents and techniques for assessing binding to soluble proteins, we have been able to solubilize several neurotransmitter receptors under conditions in which binding of the ligands is maintained in the soluble state. Receptors successfully solubilized include benzodiazepine receptors labled with ^{3}H-flunitrazepam, GABA receptors labeled with ^{3}H-muscimol, dopamine receptors labeled with ^{3}H-spiroperidol, histamine H_1 receptors labeled with ^{3}H-mepyramine and histamine H_2 receptors labeled with ^{3}H-cimetidine. Affinity chromatography utilizing supports to which appropriate drugs have been linked covalently has permitted extensive purification of some of these receptors. Molecular sieving techniques have enabled us to separate different populations of binding sites in order to ascertain possible interactions between various receptors. The possibility of multiply receptor sites for various transmitters has been explored by efforts to separate distinct macromolecular entities binding individual ^{3}H ligands.

1994 GABA RELEASE FROM, AND ACTION IN THE ENDOCRINE PANCREAS: EFFECT ON INSULIN AND GLUCAGON RELEASE. J.C. Gerber, III and T.A. Hare. Thomas Jefferson University, Philadelphia, PA 19107.

γ-Aminobutyric acid (GABA) is thought to be the major inhibitory neurotransmitter in the mammalian central nervous system (CNS). As in areas of the CNS, (where it is present at a level of 1-10 nMols/mg wet weight) it is also possible that GABA may act in an inhibitory or excitatory manner in peripheral systems. Using the sensitive (lower limit of sensitivity = 1 pMol) and specific (confirmed by GC/MS and radioreceptor assays) Ion Exchange/Fluorometric assay for GABA, we have recently reported (Fed. Proc. 38: 375, 1979) GABA's presence in various peripheral organs of the rat. Because GABA was present in isolated pancreatic islet tissue at a level roughly one-tenth that of brain (190 pMols/mg wet wt.) and at a similar level in catfish brockman bodies we have begun investigation of its role in endocrine pancreatic function by examining its relationship to insulin and glucagon secretion. We report here a significant ($p < 0.05$) increase in GABA release from perifused rabbit pancreata in response to 16.7 mM glucose as compared to 2.78 mM glucose. Rabbit pancreata were found to have a GABA content very similar to that of the rat. Further, GABA was found to facilitate insulin release into the perifusate with maximum insulin release occurring in response to 10^{-5}M GABA. Preliminary experiments suggest significant uptake of GABA into perifused pancreata. (Supported in part by the HD Foundation and USPHS NIMH Grant MH28243).



1995 INCREASED GLUTAMATE DECARBOXYLASE ACTIVITY FOLLOWING IN VITRO DEPOLARIZATION OF RAT BRAIN CORTICAL SLICES. Barry I. Gold, Barbara C. Bailey*, and Francis P. Huger* Dept. Pharmacology, Uniformed Services Univ. Sch. Med., Bethesda, MD 20014.

Changes in glutamate decarboxylase activity (GAD) in striatal slices have been reported following in vitro depolarizing stimuli (Life Sci. 22:187-194 [1978]; J. Neurochem. 32:883-888 [1979]). This report describes a persistent increase in GAD activity following in vitro depolarizing stimuli.

Rat frontal cortices were dissected fresh daily and two-hundred micron slices were prepared with a Sorval tissue chopper. The slices were suspended by vortexing in 5 ml of normal Krebs-Ringer-Phosphate medium (KRP), a high potassium KRP (K^+-KRP) or KRP containing 100 μM veratridine. Slices were preincubated 15 min at 37° and were recovered by filtration onto nylon mesh. Filtered slices were homogenized in Sorensen's buffer (10 mM, pH 6.5) containing 0.5 mM dithiothreitol and 0.5% (v/v) Triton X-100. The homogenates were centrifuged at 49000 g for 30 min and the supernatants aspirated and stored on ice for assay within two hours. GAD activity was estimated by the ability of the supernatants to liberate $^{14}CO_2$ from [1-^{14}C] glu.

Increased GAD activity was seen in supernatants prepared from high K^+- or veratridine-depolarized frontal cortex slices when assayed in the absence of added pyridoxal phosphate (PLP).

	PREINCUBATION MEDIUM		
	KRP	K^+-KRP	VERATRIDINE-KRP
GAD activity (nmoles/mg/15min)	4.9	11.2	5.9

These results are consistent with the hypothesis that GAD activity may be regulated by cofactor availability (Nature 266:847-548 [1977]). We also found, however, a twofold increase in GAD activity in supernatants prepared from K^+-depolarized slices assayed in the presence of saturating (50 μM) PLP concentrations. This would suggest that in vitro depolarization does not merely increase the saturation of GAD by PLP. Attempts to increase in vitro GAD activity by added calcium were unsuccessful. When supernatants prepared from fresh frontal cortex were assayed in the presence of several concentrations of $CaCl_2$, a slight inhibition (15%) was seen at 30 μM.

Experiments are in progress to study the mechanism by which depolarizing stimuli cause a persistent increase in GAD activity.

1996 REGULATION OF ACETYLCHOLINE RELEASE IN THE CAT CAUDATE NUCLEUS. Jay M. Gorell *, Michael J. Callahan *, and Eugene P. Schoener. Depts. of Neurology and Pharmacology, Wayne State Univ. Schl. Med. Detroit, MI 48201.

Adult cats were prepared under halothane anesthesia, and each had a push-pull cannula stereotaxically implanted in the right caudate nucleus. Wound edges and pressure points were infiltrated with lidocaine, and halothane was withdrawn. Gallamine was given IV and ventilatory support was provided to the conscious animal throughout each experiment. Caudate perfusion with normal saline was stabilized at 50 µl/min for one hour prior to collection of samples. Subsequent perfusates were collected during 10 - 15 min consecutive periods.

Basal samples were taken during the first 50 min of each experiment. Thereafter, atropine in saline was perfused at 10^{-6}, 10^{-5}, 10^{-4}, and 10^{-3} M for 50 min at each dose level. Finally, normal saline alone was perfused for 50 min. At the end of each collection period, the sample was stored at -80 C for later acetylcholine (ACh) assay. At the end of each experiment the perfusion site was marked for subsequent identification; verification of placement in the caudate nucleus was made in each case.

150 µl triplicates of each 10 - 15 min perfusate sample were assayed by a modification of a radioenzymatic method (Goldberg and Mc Caman, 1973).

Basal ACh release levels were 10.3 ± 0.3 nanomolar (6 cats; 23 samples). Sequentially perfused atropine 10^{-6} and 10^{-5} M produced no change, 10^{-4} M increased release 3.4 fold, and 10^{-3} M rapidly decreased ACh release to 26 % of basal levels. Saline perfused immediately after atropine 10^{-3} M rapidly increased ACh release beyond basal values.

These data suggest that probable post-synaptic muscarinic block occurs in the caudate with atropine 10^{-4} M, and that possible pre-synaptic cholinergic block occurs with atropine 10^{-3}M

This research was supported by a grant to the Dept. of Neurology, Wayne State Univ. by the Detroit General Hospital Research Corp.

1997 DEAFFERENTATION OF GABAERGIC PROJECTIONS TO THE HABENULA: IMMUNOCYTOCHEMICAL DEMONSTRATION. Zehava Gottesfeld, Christopher Brandon and Jang-Yen Wu. Dept. Neurobiol. and Anat., Univ. of Texas, Med. Sch. Houston, and Dept. Cell Biol., Baylor Coll Med., Houston, TX 77025.

Biochemical studies employing glutamate decarboxylase (GAD) as a marker have identified GABAergic neuronal elements in the medial (MH) and lateral (LH) habenular nuclei. GAD activity in the LH was higher than in the MH, and was partly attributed to GABAergic projections via the stria medullaris (SM) (Gottesfeld et al., Brain Research 130:184, 1977). The present work has been designed to accurately localize the GABAergic system in the habenulae by immunocytochemical visualization of GAD.

Unilateral high frequency lesions were placed stereotaxically in the SM of adult rats. Following 4 months survival the animals were anaesthetized and fixed via intracardiac perfusion with solutions containing periodate-lysine-paraformaldehyde (McLean and Nakane, J. Histochem. Cytochem., 22:1077, 1974). Coronal brain sections (50-100µm thick) were stained by the peroxidase-anti-peroxidase method of Sternberger (Petrali et al., J. Histochem. Cytochem. 22:782, 1974) as modified by Brandon et al., (PNAS, in press). Both the antigen (purified mouse brain GAD) and antiserum have been extensively characterized (Wu et al., J. Biol. Chem. 284:3029, 1973). The sections were embedded in Spurr's resin and observed by light and electron microscopy.

GAD-positive reaction product appeared within axon terminals, seen as punctate structures, in both habenular nuclei. The intensity of the stain was more striking in the LH, particularly in the mediolateral aspect of the nucleus, and was greatly diminished in the controlateral, lesioned side. Furthermore, the prolonged lesion seemed to cause marked shrinkage of the habenular complex. A dense, narrow streak containing GAD-positive axon terminals was observed underlying the ependyma within the MH. These nerve endings appeared in close contact with a single layer of large cell bodies which lined the third ventricle. These terminals were not affected by the SM lesion.

The disappearance of GAD immunochemical staining from the LH in response to SM lesions corroborates the biochemical data showing that the SM contains GABAergic projections to the LH. Also, it provides new information on the localization of GABAergic terminals in the habenular nuclei.

(Supported in part by BRSG-UTMSH to Z.G. and NS-13224 to J.-Y.W.)

1998 BLOCKADE OF GLUTAMATE EXCITATION AND GABA INHIBITION OF BRAIN STEM NEURONS BY CURARE. R. W. Greene* and D. O. Carpenter (SPON: M. I. Varon). Neurobiology Department, Armed Forces Radiobiology Research Institute, Bethesda, MD 20014

Curare is a competitive antagonist of the nicotinic acetylcholine receptor, where it causes a shift to the right in the dose response curve, and will block the binding of α-bungarotoxin, a specific nicotinic receptor antagonist. Recent studies on Aplysia neurons in our laboratory have demonstrated, however, that two different types of response, due to conductance increases to Na^+ and Cl^-, respectively, are blocked by curare when these responses are elicited by any of nine neurotransmitters acting at different and specific receptors (J. Neurobiol. 8: 119, 1977). We suggested that these effects of curare are mediated by blockade of channels, not receptors. In these experiments we have investigated the effects of curare on the glutamate Na^+-dependent excitation and GABA Cl^--dependent inhibition of reticulospinal neurons of the brain stem of the cat to determine if these, like the comparable responses on Aplysia neurons, are blocked by curare.

The floor of the IV ventricle of cats was exposed by aspiration of the cerebellum, and recordings were made from antidromically identified reticulospinal units of the pontine medial reticular formation. We used seven-barrelled micropipettes, filled with saturated curare at pH 7, 1 M glutamate at pH 8, 1 M GABA, 1 M acetylcholine, picrotoxin (saturated), and 0.5 M NaCl, all at pH 4. The center barrel contained 2 M NaCl for recording. All the reticulospinal cells encountered were silent but could be driven with a constant background current of glutamate. The glutamate-induced excitation was completely and reversibly blocked when curare was applied by pressure injection or by ionophoresis. Sometimes this blockade was preceded by a brief period of increased excitation. The inhibitory actions of GABA were also reversibly blocked by curare, and the transient excitation that sometimes followed curare application probably reflects blockade of natural Cl^--dependent inhibitory potentials. Hill et al. (Brit. J. Pharmacol. 56: 9, 1976) have previously reported that the convulsant effects of curare may result from blockade of GABA and glycine inhibition.

Recent re-analysis of the actions of curare at neuromuscular junction (Manalis, Nature 267: 366, 1977) has shown a channel blocking effect here also. Ascher et al. (J. Physiol. 278: 207, 1978) have applied noise analysis to Aplysia excitatory responses to acetylcholine and have confirmed that curare blocks the channel. The present results suggest that 1) curare can block both Na^+ and Cl^- channels, 2) the ion channels of invertebrates and vertebrates are similar in at least this regard, and 3) blockade of a response by curare does not necessarily indicate that acetylcholine is the transmitter involved.

1999 PRIMARY VS. SECONDARY EFFECTS OF FIGHTING ON DOPAMINE UPTAKE IN ISOLATED MICE M.G. Hadfield, Dept. of Pathology, Medical College Virginia, Richmond, Virginia 23298

The present study was undertaken to separate primary effects on dopamine (DA) uptake produced by fighting from secondary effects that may accompany fighting such as those resulting from motor activity and sensory stimulation. To accomplish this aim, several controls were instituted including animals not exposed to fighting (resting controls), animals exposed to the fighting situation but not permitted to fight (stimulus controls) and animals exposed to stress and motor activity equivalent to that of fighting. To augment the power of the controls, DA uptake was studied in both the nigrostriatal (extrapyramidal) system which is known to modulate motor activity and the mesocortical system which is altered by stress. These two DA systems were also chosen because we have just reported that fighting significantly increases Km and Vmax for DA uptake in the mesorcortical system but not the nigrostriatal system, (VII Int. Mtg. ISN, 1979).

Isolated male ICR mice were placed for five minutes in a combat arena (fighting animals), in a chamber separated from the fighting arena by wire mesh (stimulus controls) or in a tumbling wheel containing cylinders the size of mice (motor/stress controls). The brains were then removed and synaptosome-rich homogenates of striatum and prefrontal cortex were respectively prepared and incubated with varying concentrations of ^{3}HDA. Uptake values were obtained by liquid scintillation counting from which Km& Vmax were calculated (Michaelis-Menten analysis). Each group of experiments was repeated 7-9 times and were compared with matching resting controls by "t" test.

The stumulus control animals showed an increase in Km& Vmax for DA uptake in the mesortical terminals, though of lesser magnitude than that noted in the fighting animals. This group alone also showed a decrease in Km and Vmax for DA uptake in the striatum. The motor/stress controls showed modest increases in values for the uptake constants in the pre-frontal cortex and no change in the striatum.

Fighting, witnessing fighting as a spectator (stimulus controls) and motor activity and pummeling similar to that experiences during fighting (motor stress controls)--all represent forms of stress which alter the neurotransmitter activity (uptake) of the mesortical DA system. Of these, fighting produces the greatest change, but it is interesting that significant changes in DA uptake are produced in the protected stimulus controls. Thus, there may be no specific primary effect of fighting, alone, on DA uptake. The decreased DA uptake in striatum noted in the stimulus controls may be related to inability to "act out" on a stress producing situation.

2000 EFFECTS OF SUBCORTICAL LESIONS ON NEOCORTICAL CHOLINERGIC MARKERS. Stanley L. Hartgraves*, Patricia L. Mensah, Peter H. Kelly. Dept. Physiol. and Biophysics and Dept. of Anatomy, Univ. of Southern California Sch. Med., Los Angeles, CA 90033.

Experimental evidence suggests there are cholinergic pathways to the neocortex. Undercutting of the neocortex in the cat causes a large decrease in choline acetyltransferase (CAT) (Hebb et al., 1963, Nature, 198, 692). Pepeu, et al. (1973, Brain Research, 57, 153) reported a decrease of acetylcholine in the neocortex of the rat following lesion of the septum. On the basis of horseradish peroxidase studies Divac (1975, Brain Research, 93, 385) has described direct projections to neocortex from neurons in the medial septum and globus pallidus region.

Here we have examined changes in the neocortex of CAT and [^{3}H] choline uptake, as markers of cholinergic neurons, one week after electrolytic lesions of the globus pallidus, medial septum and ventral thalamus. Pallidal lesions caused decreases of CAT by 58% and 42% in anterior and middle neocortical regions and a smaller decrease (by 23%) in the posterior cortex. In hippocampus CAT was unaltered. These lesions also decreased [^{3}H] choline uptake in anterior and middle neocortical regions by 35-40%. Septal lesions did not alter CAT in anterior or middle regions but caused decreases of 90% in hippocampus and 30% in posterior neocortex. Hippocampal [^{3}H] choline uptake was decreased 65% by this lesion. Lesions of the ventral thalamus caused no change in CAT activity in the regions studied (anterior and middle neocortex). These results are consistent with the hypothesis that the globus pallidus sends a diffuse network of cholinergic fibers to the neocortex, with preference to the anterior and middle regions, while the medial septal cholinergic innervation of neocortex is only to its posterior regions. Fibers from ventral thalamus contribute little or nothing to the subcortical cholinergic innervation of neocortex.

2001 RECEPTOR BINDING TO ASTROGLIA CELLS: THE QNB RECEPTOR. Fritz A. Henn[1], Barbara Oderfeld-Nowak*[1] and Robert Roskoski[2]. U. of Ia., Iowa City, Iowa 52242.

A preliminary study of QNB binding in astroglial and synaptosomal fractions of bovine caudate revealed an enrichment of QNB binding in astroglial cells. The enzymatic profiles of the fractions suggests that both choline acetyl transferase and glutamic acid decarboxylase are enriched in the synaptic fraction along with GABA or musimol binding. The muscarinic receptor ligand QNB appears to be enriched in astroglial cells. This enrichment is not large enough to account for all QNB binding, suggesting that the ligand may bind both glial and neuronal elements. This is analogous to the diazepam receptor which is clearly present in astroglia in the frontal cortex and neuronal elements in the cerebellum. Data on QNB binding has also been obtained on micro preparations of astroglia from rat hippocampus. These data support a glial localization as do lesion studies of hippocampus.

*Barbara Oderfeld-Nowak was an NIH Fogarty International Fellow.

2002 NEUROTRANSMITTER UPTAKE MECHANISMS IN DEVELOPING AND AGING CHICK IRIS. Douglas W. Hoffman*, Mario Marchi*, and Ezio Giacobini (Spon. S. Maxson) Dept. Biobehavioral Sci., University of Connecticut, Storrs, CT. 06268.

Age-related changes in the characteristics of accumulation of norepinephrine (NE) and choline (Ch) in chick iris in vitro were studied throughout the lifespan of the animal. We have found a specific uptake for NE at 10 days of incubation (d.i.). At this point uptake is sensitive to ouabain, desmethylimipramine (DMI), cocaine and low sodium concentrations, but the $^{Q}10$ ($V_{37°}/V_{27°}$) value does not differ significantly from 1 until hatch (21 d.i.). The time course of development is different for each of these individual characteristics of the uptake process,suggesting that they are selectively sensitive to changes in energy metabolism and membrane composition during ontogenesis of the chick. The kinetic values for NE uptake appear to fall into two groups; 10 d.i. to 1 month after hatch (a.h.), K_m=13-19 uM, and 2 months a.h. to 2 years a.h., K_m=3.5-5 uM. The V_{max} increases steadily with age up to 2 years. Other age-related changes in NE uptake are variations in sensitivity to DMI at 2 years a.h., and the appearance and increase in metanephrine sensitivity after hatch.

NE uptake is compared developmentally with Ch uptake, which is first seen at the earliest time of innervation of the iris by cholinergic fibers (4 1/2 d.i., stage 24). The K_m for Ch uptake does not show the same age-related changes as the K_m for NE uptake, but does remain stable from 1 month a.h. to 5 and 7 years of age. The V_{max}, after increasing from 5 d.i. to 3 months a.h., declines at 5 and 7 years of age, paralleling changes in Ch and acetylcholine levels in the iris (see Giacobini et al., Marchi, et al., Abstracts, this meeting).

Changes in neurotransmitter uptake during development and aging reflect changes in the maturing nerve terminals. It is not yet clear if this is due to changes in molecular aspects of the uptake mechanism, or is in response to changes in the basic metabolic and structural processes of the developing and aging neuron.

(Supported by AOA90-A-1039-02 and Univ. of Connecticut Research Foundation).

2003 OSMOTIC LYSIS OF BOVINE CHROMAFFIN GRANULES IN ISOTONIC SOLUTIONS OF SALTS OF WEAK ACIDS AT LOW MEDIUM PH. Ronald W. Holz and Bruce Trock.* Dept. of Pharmacology, Univ. of Michigan Medical School., Ann Arbor, MI 48109.

Chromaffin granules have an intragranular pH of 5.5-5.7. The neutral form of weak acids such as acetic acid should be permeant and attain equal concentrations across the granule membrane. The negatively charged species should be relatively impermeant but should gain access to the granule interior by dissociation of the neutral species within the granule. The intragranular concentration of the charged species will then be determined by the local pH, the intragranular (and extragranular) concentration of neutral species, and the dissociation constant of the weak acid. One predicts that in isotonic solutions of weak acids, that granules would be stable when the medium pH is 7 because the negative species is excluded from the granule interior. When the medium pH is lowered, the intragranular concentration of both charged and uncharged species should increase. The granules should osmotically lyse and release their contents of catecholamines, ATP, and dopamine-β-hydroxylase (DBH) into the medium when the medium pH approaches the intragranular pH. Chromaffin granules in isotonic solutions of $K^+ CH_3COO^-$ (acetate), $K^+ CH_3CHOHCOO^-$ (lactate), and $K_2^+ OOCCH_2CH_2COO^-$ (succinate) are stable at pH 7 but release their contents of catecholamines, ATP and soluble DBH when the medium pH is less than 6. The effect is inhibited by increasing the osmolality of the solution with sucrose. Granules in isotonic solutions of salts of strong acids such as $K^+ CH_3SO_4^-$ (methylsulfate), $K^+ CH_2OHCH_2SO_3^-$ (isethionate), and K_2SO_4 are stable between pH 7.0-5.4.[2] These data provide further evidence that the intragranular pH is acidic. Supported by grants from the Michigan Heart Association and the NSF (#BNS-7824494).

2004 ACETYLCHOLINE METABOLISM IN THE NEMATODE ASCARIS. Carl D. Johnson* (SPON: A.O.W. Stretton). Dept. of Zoology, Univ. of Wisconsin, Madison, WI 53706.

We have been studying the distribution of choline acetyl transferase (CAT) and acetylcholinesterase (AChE) in the nematode Ascaris. The tissues of nematodes are arranged in three layers: hypodermis, muscle and gut plus gonad. The nervous system which contains some 250 cells is embedded in the hypodermal layer. In comparison to more complex animals it is a significant simplification that neither neurons nor neuronal processes extend into the muscle layer or into gut or gonads; therefore cholinergic enzymes found in these tissues cannot result from neuronal synthesis.

Among the neurons, we have previously described the selective localization of CAT in excitatory as compared to inhibitory motorneurons (Neuroscience Abstr. 4:197, 1978). Pharmacological experiments suggest that interneurons which drive excitatory motorneurons may also be cholinergic since their input is blocked by curare (I.S. Kass, unpublished results). CAT activity in neurons, however, represents only ~1% of the total activity in the animal. Most of the CAT is localized in the hypodermis and is particularly concentrated in the tip of the head. The function of ACh synthesized in hypodermis is not yet understood but if it is releasable it could provide additional, presumably tonic, excitatory input to muscle.

The muscle layer contains two forms of AChE separable by velocity sedimentation (5S and 13S) with different Kms for ACh (5S ~100 μm; 13S ~20 μM). They are also differentially affected by detergents; the 5S form is irreversibly inactivated by Na deoxycholate whereas the larger form is inhibited by TRX-100. Comparable forms of AChE in the nematode Caenorhabditis elegans have been shown by mutant analysis to be the products of separate genes. Analysis of the distribution of separable forms within different muscle regions reveals that the 5S form is high in the front half of the animal and declines posterior to the vulva. The 13S form is more evenly distributed. In the most common locomotory behavior in Ascaris waves of contraction are initiated at the vulva and move anteriorly, so the muscle region with high 5S activity is more active. Thus 5S AChE may be regulated by muscle activity.

Finally, both CAT and AChE have been located in gonads. CAT levels are high in the vas deferens and in the anterior end of the uterus whereas AChE is concentrated in the seminal vesicle and in the vagina. We are currently investigating the possibility that ACh may play a role in the activation or guidance of Ascaris sperm.

(Supported by a postdoctoral fellowship from M.D.A.A.)

2005 ACTION OF KAINIC ACID IN THE TOAD AND GOLDFISH RETINA. Jochen Kleinschmidt*, Charles L. Zucker* and Stephen Yazulla*. (SPON: B. M. Twarog). Dept. Biology, SUNY Stony Brook, Stony Brook, NY 11794.

Kainic acid (KA) is an analog of L-glutamic acid (GLU) and has a potent neuroexcitatory and neurotoxic action in the goldfish retina. In order to investigate the mechanism of action of KA in the retina and to find out if it involves GLU receptors, we have studied the early neurotoxic effects of KA in isolated retinae of goldfish and toad (Bufo marinus). Retinae were isolated from dark-adapted animals and incubated in 200 μl of Ringer with and without KA and other agents for various times. After incubation, retinae were processed for routine histology and investigated by light and electron microscopy.

One of the earliest visible signs of KA neurotoxicity in the retina is the formation of clear, membrane bound vacuoles in the outer plexiform layer (OPL). Vacuoles are found within photoreceptor synaptic invaginations as well as more proximally in the OPL and appear to originate mostly from distal and proximal segments of horizontal cell dendrites. The extent of vacuolation in the OPL is graded with KA concentration and with length of incubation; half-maximal swelling occurs at 5μM KA for 15 min incubations, or at 5 - 10 min incubation for 20μM KA. In goldfish but not in the toad, rod-connected dendrites are less sensitive to KA than are cone-connected dendrites. KA-induced vacuolation in the OPL: (1) is not mimicked by other excitotoxic amino acids including 50mM GLU, by convulsants, or by a variety of agents which cause nonspecific cellular swelling elsewhere in the CNS or in other tissues, (2) is not blocked in zero Ca Ringer or by 20mM Mg or 2mM Co, precluding a presynaptic action via transmitter release from photoreceptor terminals, (3) is Na-dependent and is greatly reduced by 0.5mM pentobarbitone which at similar concentrations blocks Na-mediated synaptic excitation in other preparations.

These findings suggest that the action of KA in the OPL is specific, is the result of a direct action of KA on postsynaptic membranes, is mediated by a Na ionophore similar to synaptic ionophores, and appears to involve binding of KA to a receptor which either has a very low affinity for GLU or which readily desensitizes or inactivates to GLU but not to KA.

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2006 INTRACELLULAR ACTIONS OF GABA, GLYCINE AND SOME RELATED AGENTS. K. Krnjević, A. Constanti* and A. Nistri*. Anaesthesia Research and Physiology Departments. McGill Univ. Montreal PQ Canada. H3G 1Y6.

GABA and glycine have well-known inhibitory actions when applied to many central neurons. There is good evidence that these are mediated by specific receptors, situated on the external surface of the neuronal membrane, which activate anion-permeable channels. Under normal conditions, the predominant ion involved is Cl^-, and since in central neurons the Cl^- equilibrium potential is usually more negative than the resting potential, both GABA and glycine have a hyperpolarizing action associated with a fall in membrane resistance.

There is some evidence, however, that GABA can depolarize glia (Krnjević and Schwartz, 1967, Exp.Br.Res.3,306) and that a depolarizing effect is superimposed on its predominant hyperpolarizing action on motoneurons (Krnjević et al.,1977, Can.J.Physiol. Pharmac. 55,653). These depolarizations (which have not been observed with glycine) could be due to electrogenic uptake of GABA in association with Na^+. If such electrogenic transport indeed takes place, one might expect it to be reversible, as in some cells that utilize electrogenic transport systems for neutral amino acids (Johnstone, 1979,Can.J.Physiol.Pharmac. 57,1). To test for this possibility, we have injected GABA and some related agents into lumbosacral motoneurons in cats under Dial, using balanced iontophoresis with the return current through a KCl- or K citrate-containing barrel of the 4-barrelled glass micropipettes.

The majority of cells into which GABA was injected (5-15 nA, for 10-100 sec) showed repeatably a small hyperpolarization (typically by 2-5 mV) which persisted for 1 min or more after the end of the injection, was not readily reversible by hyperpolarization, was not potentiated by an intracellular injection of a benzodiazepine, and was not associated with a fall in membrane resistance - on the contrary, intracellular injections of GABA often increased the input resistance.

These characteristics indicate that this hyperpolarizing action is probably not mediated by the "classical" external GABA receptors; apart from the surprising increase in resistance, they are consistent with a reversed electrogenic outward transport of GABA.

We conclude that if motoneurones indeed have Na^+ coupled neutral amino acid (and closely related) transport systems, only that for GABA (of the agents tested) seems to have the characteristics (rate, stoichiometry of Na^+-coupling, etc.) required for clear electrogenic manifestations of transport.

Supported by the Canadian Medical Research Council.

2007 CHLORIDE DEPENDENCE OF CHOLINE UPTAKE, ACETYLCHOLINE SYNTHESIS AND RELEASE. H.Ksiezak* and A.M.Goldberg. The Johns Hopkins Univ. School of Hygiene and Public Health, Baltimore, MD 21205.

The role of chloride (Cl^-) in the functioning of the cholinergic nerve terminal has only recently been described. Rossier et al. (J.Neurochem., 29:1007, 1977) demonstrated that lack of Cl^- inhibited the activity of choline acetyltransferase. Further, Kuhar et al. (J.Neurochem., 31:251, 1978) have shown that the sodium dependent high affinity choline transport is also Cl^- dependent. In addition, Gennaro et al. (J.Physiol., 280:237, 1978) demonstrated that propionate substitution for Cl^- in elevated K^+, depletes the frog neuromuscular junction of its synaptic vesicles content. We therefore examined the effects of propionate substitution for Cl^- on the uptake of choline, synthesis and release of ACh in rat brain synaptosomal preparations (P2 fraction). The kinetics of transport were studied over a range of choline concentrations 1-100 μM in 5 mM K^+ Krebs-Phosphate buffer (5 K-P) or in the same medium in which all Cl^- was replaced with propionate (125 mM; 5 K^+ Cl^- -free). In 5 K-P two components, high and low affinity uptake of choline, were observed. In 5 K^+ Cl^- -free medium only a low component could be detected and at 10 μM choline, the amount of newly synthesized ACh found in the pellet was reduced by 75%. However, in 35 K-P newly synthesized ACh was maintained but again only a low affinity component could be observed (Carroll and Goldberg, J.Neurochem., 25:523, 1975). The total amount of ACh (released plus remaining in tissue) was greatly increased by incubation in 35 K-P over 30 min. (270 to 690 pmol/mg prot.) but was only slightly increased in 35 K^+ Cl^- -free medium (235 to 290 pmol/mg prot.). In 35 K-P release of ACh was linear for at least 30 min. and was 400 pmol/mg prot. In 35 K^+ Cl^- -free medium release was also linear for 30 min. but was reduced to 200 pmol/mg prot. Therefore, the process of release in 35 K^+ Cl^- -free medium was not impaired but the amount of ACh released was reduced. Further, in K-P the tissue level was maintained for 30 min. while in the Cl^--free medium the tissue levels were greatly decreased. These results confirm and extend the observation and importance of Cl^- on the uptake of choline and/or synthesis of ACh. However, they do not allow a conclusion to be drawn as to whether the reduction in newly synthesized ACh was the result of a decrease in uptake of choline or in the synthesis of ACh. These results further suggest that the synthesis of ACh and the transport of choline are closely associated, and possibly even linked. They also raise the interesting possibility that propionate may be a useful agent in studying the mechanisms of release of ACh.

2008 ELECTRON MICROSCOPIC LOCALIZATION OF CHOLINERGIC MUSCARINIC RECEPTORS IN RAT BRAIN. Michael J. Kuhar, Naomi Taylor*, Nigel Birdsall* and Edward Hulme*. (SPON: K. Dismukes). Dept. of Pharmacol., the Johns Hopkins Univ. Sch. of Med., Balto., Md., and Mol. Pharm. Section, Natl. Inst. for Med. Res., Mill Hill, London, England.

Propylbenzilylcholine mustard (N-2'- chloroethyl-N- 2",3"-propyl-2-aminobenzilate, PBCM) is a potent, specific, irreversible cholinergic muscarinic antagonist. In its radiolabeled form, it can be used in autoradiographic studies to localize the receptor at the microscopic level. The goal of these studies was to utilize ^{3}H-PBCM to localize the muscarinic receptor at the electron microscopic level.

Rats were perfused with 30 ml 0.1% glutaraldehyde in buffer intracardially. The brains were rapidly removed and dissected in ice cold saline. Slices of hippocampal formation and cerebral cortex (350μ) were prepared with a tissue chopper and incubated with 5 to 25 nM concentrations of ^{3}H-PBCM in a Kreb's Ringer solution for 15-25 min at 30°C. Some slices were preincubated with 1 μM concentrations of QNB for 3 min and incubated further with ^{3}H-PBCM to obtain "blanks" for measurement of background values. Specific to nonspecific ratios of binding varied between about 2 to 5. The slices were rinsed with cold Ringer's solution 3 times, and further fixed with aldehydes and osmium. The slices were dehydrated with ethanol and embedded in TAAB resin.

Light microscopic autoradiography of 1μ sections revealed a limited penetration of drug into the tissue and, by grain counts, confirmed the specific to nonspecific ratios observed in biochemical studies. Preliminary electron microscopic autoradiography with thin sections (1-5 month exposures) revealed a significant fraction of grains over synapses in both the hippocampus and cortex. This fraction was reduced in the tissues treated with QNB. These preliminary studies suggest the possibility of studying the ultrastructural localization of muscarinic receptors. They were supported by USPHS grants MH25951 and MH00053, and a grant from the McKnight foundation.

2009 DIFFERENTIAL BINDING TO DOPAMINE AND NEUROLEPTIC RECEPTORS. J. Y. Lew* and M. Goldstein. New York University Medical Center, Department of Psychiatry, 550 First Ave., New York, N.Y. 10016.

To determine whether dopamine (DA) receptors with high affinity for DA agonists have different properties than those with a high affinity for DA antagonists, we have investigated the thermal stability of these receptors in calf striatum.

The exposure of striatal membranes to 53°C for 2 min. resulted in a decrease of the specific binding of H^{3}-spiroperidol (Spi) by 5-10%, while the specific binding of H^{3}-DA was decreased by 80-90%. Scatchard plot analysis revealed that the dissociation constant for H^{3}-Spi and for H^{3}-DA was the same in the thermal exposed membranes as in the control membranes. The maximum number of binding sites in thermal exposed membranes was decreased for H^{3}-Spi by approx. 10% and for H^{3}-DA by approx. 75%. Thus, the selective decrease in specific binding of H^{3}-DA suggests that thermal exposure results in an inactivation of DA receptors with high affinity for DA.

The potencies of DA agonists and DA antagonists to displace H^{3}-Spi from intact striatal membranes and from thermal exposed striatal membranes were investigated. The displacement of H^{3}-Spi by DA agonists is significantly reduced in the thermal exposed membranes while the displacement of DA antagonist is not altered.

Thus, thermal exposure of striatal membranes makes it possible to differentiate DA receptors with high affinity for DA antagonists (neuroleptic receptors) from those with high affinity for DA agonists. The results of this study indicate that neuroleptic receptors and DA receptors are two distinct molecular entities; one of them is thermal stable while the other is labile.

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2010 RAT TRIGEMINAL MESENCEPHALIC NEURONS ARE RESISTANT TO BOTH EXCITATORY AND NEUROTOXIC ACTIONS OF KAINIC ACID. J.P. Lund and C. de Montigny, Centre de Recherche en Sciences Neurologiques, Université de Montréal, Montréal, Canada.

The "excitotoxic" hypothesis proposes that neurotoxic amino acids exert their toxic effect through neuronal excitation. Colonnier et al. (1979) have reported that neurons of trigeminal mesencephalic nucleus (NMT) of the cat survive kainic acid (KA) injections. In the present study, we confirmed the resistance of NMT neurons to the toxic action of KA in the rat and found addition that these neurons are not excited by iontophoretically-applied KA.

In the first series of experiments, 2.5 μg of KA in 5 μl of saline were injected in the vicinity of the pontine part of NMT on one side. 5 μl of vehicle was injected on the other side. Histological sections were prepared in a conventional manner. Ten and 14 days following KA injections all non-NMT neurons within a radius of 2 mm from the injection sites, including the relatively resistant cerebellar cortical granule cells, had disappeared. However NMT neurons remained and were comparable in number and appearance to those of the control saline-injected side. Thus, these observations in the rat confirm those of Colonnier et al. (1979) in the cat.

In the second series of experiments, the effect of iontophoretic application of KA was studied in NMT. This nucleus contains cell bodies of primary afferent neurons which innervate pressoreceptors around the teeth and muscle spindles in the jaw closing muscles. Sixty-three NMT neurons recorded were identified by mechanical stimulation of their receptors and tested iontophoretically. Thirty non-NMT neurons were tested with the same pipettes. A 0.1 M KA solution at pH 8.0 and a conventional iontophoretic technique with an automatic current "balance" were used. Recording sites were marked with fast green and verified histologically.

None of the NMT neurons responded to KA applied iontophoretically, even when currents of 100 nA or more were used, but all thirty non-NMT control neurons were activated when KA was applied with small currents (1-10 nA).

It is concluded that, in the NMT, the absence of both neurotoxic and neuroexcitatory effects of KA is consistant with the "excitotoxic" hypothesis. The results also suggest that KA requires the presence of receptors to exert both its neurotoxic and neuroexcitatory actions.

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2011 DESENSITIZING EXCITATORY RESPONSES TO PEPTIDES, PURINES, PROTONS AND DRUGS REVEALED USING CULTURED MAMMALIAN NEURONS. J.F. MacDonald, J.L. Barker, D.L. Gruol, L.M. Huang*, and T.G. Smith*. Lab. of Neurophysiology, NINCDS, NIH, Bethesda, Md. 20014

Spinal neurons derived from mouse embryos were grown in tissue culture for 4 weeks or more. Intracellular recordings using conventional and voltage clamp techniques, coupled with extracellular microiontophores or microperfusion were used to study the effects of leucine- and methionine-enkephalin, inosine, substance P, and flurazepam on neuronal membrane properties. 10 mM Mg^{++} was added to the recording medium to prevent spontaneous synaptic activity and allow clearer examination of pharmacologically induced membrane events. Iontophoresis of all of these substances evoked rapidly depolarizing, rapidly desensitizing excitatory responses on spinal neurons. Under voltage clamp the responses were associated with both an increase in inward current and membrane conductance. The responses extrapolated to an apparent reversed potential close to the peak of the spike (+20 mV), suggesting that they are due primarily to activation of sodium conductance. Responsive cells showed a non-uniform distribution of response amplitude over the cell surface.

Because iontophoresis requires charged molecules, which frequently necessitates acidification to pH 3, control pipettes containing HCl at pH 3-5 were also tested and occasionally such pipettes evoked entirely similar rapidly depolarizing, rapidly desensitizing excitatory responses. An increase in the frequency of cells responding to H^{+} ions was observed using pipettes containing 1M HCl (pH < 1). H^{+} ion excitation of spinal neurons may result from rapid titration of protein groups comprising membrane receptors or channels.

In order to ensure that the drug responses were not all due to iontophoresis of H^{+} ions, all of the substances were dissolved in bathing medium (1-10 μM) and applied to single neurons by pressure microperfusion. Rapidly desensitizing excitatory responses were evoked by all of the substances using this alternative method of application which eliminates any contribution of H^{+} ions.

Thus, evanescent excitation of central mammalian neurons is common to a wide variety of endogenous substances. A phenomenologically similar form of synaptic excitation has been reported in invertebrates. While the pharmacokinetics of flurazepam application would prevent drug-induced excitation in the intact animal, part of the therapeutic efficacy of the drug might be due to drug-induced desensitization of naturally occurring excitatory synaptic transmission. In this regard, cross desensitization between pharmacologically applied flurazepam and inosine occurs on these cultured neurons.

2012 BICUCULLINE HAS DIFFERENT ACTIONS ON MAMMALIAN SPINAL CORD AND FOREBRAIN NEURONS IN PRIMARY DISSOCIATED CELL CULTURES. R.L. Macdonald, A.B. Young, L.M. Nowak. Department of Neurology, University of Michigan Medical Center, Ann Arbor, MI 48109.

Convulsants such as bicuculline have been demonstrated to antagonize GABA-mediated inhibition in many in vitro and in vivo experimental preparations and it has been suggested that such antagonism forms the basis for their convulsant activity. We have investigated the action of bicuculline on forebrain and spinal cord neurons in primary dissociated cell culture and report that bicuculline produces paroxysmal depolarization in both neuronal systems but with a different ED_{50}.

Spinal cords and forebrains were removed from 13-14 or 14.5-15.5 day old fetal mice respectively and grown (as previously reported) in normal growth medium for four to sixteen weeks prior to electrophysiological study. Only neurons with spontaneous activity or with evoked action potentials were accepted for study. Paroxysmal depolarizing events (PDE) were defined as abrupt, randomly occurring depolarizations followed by volleys of action potentials and repolarization.

The percentage of forebrain neurons with PDE was a function of bicuculline concentration with about 20% at 100nM, 60% at 200nM and 100% at 500nM giving an ED_{50} for PDE of between 150 and 200nM. The PDE-bucuculline dose response curve was steep with PDE ranging from control levels to 100% over 8-500nM. Also as the bicuculline dose increased, time-to-peak, duration and frequency of PDE declined.

In spinal cord neurons, PDE was also produced by bicuculline but over a different dose range. PDE occurred in about 40% of neurons in 5μM, 80% in 20μM and 100% in 40μM bicuculline with a ED_{50} for PDE between 5 and 10μM. Again the PDE-bicuculline dose-response curve was steep with control to 100% range occurring between 1-40μM. Increasing doses of bicuculline decreased PDE duration, rise time and frequency as in forebrain neurons.

Thus, we have demonstrated that the ED_{50} for bicuculline-induced PDE is higher in spinal cord than in forebrain neurons in culture consistent with the similar shift in ED_{50} for bicuculline displacement of GABA-binding (see abstract Young and Macdonald). Furthermore, the entire PDE-bicuculline dose response curve overlaps the lower 20% of the GABA-displacement curve in both preparations. This demonstrates that displacement of GABA by bicuculline from binding sites occurs at bicuculline doses which are physiologically active in producing paroxysmal activity and supports the hypothesis that antagonism of GABA-mediated inhibition is involved in the production of paroxysmal depolarizing events.

2013 L-THREONINE ADMINISTRATION INCREASES GLYCINE CONCENTRATIONS IN THE RAT CENTRAL NERVOUS SYSTEM. Timothy J. Maher* and Richard J. Wurtman. Lab. Neuroendocrine Regulation, MIT 56-245, Cambridge, Mass. 02139, USA.

Glycine is produced by an enzyme, serine transhydroxy methylase (STHM) that can use either serine or threonine (THR) as substrate. We suspected that STHM might not be saturated with THR at normal brain THR levels; hence we examined the effect of exogenous THR on spinal cord and brain glycine levels. Male rats were killed 1 hour after receiving 0, 50, 100, 200, or 400 mg/kg THR i.p.. Cord and brain THR levels increased in a dose-dependent fashion after THR injection. The concentration of glycine in the spinal cord also significantly increased by 26% in rats receiving 400 mg/kg THR ($p<.01$) and by 17% and 16% in the 200 and 100 mg/kg groups ($p<.05$). Changes in brain glycine followed a similar pattern, but were not increased significantly. Doses of THR that increased cord glycine also tended to decrease its levels of the large neutral amino acid tyrosine; this indicates that THR did in fact compete with other large neutral amino acids for transport at the blood-brain barrier. THR administration (400 mg/kg) increased synaptosomal glycine levels in spinal cords by an average of 16% (from 8.53 to 9.90 nmol/mg protein), compared with a 26% increase in glycine concentrations seen in intact spinal cords.

These observations indicate that THR can be used to enhance glycine levels in rat spinal cord neurons, and also suggest that glycine synthesis may normally be influenced by plasma composition (that is, by the ratio of plasma THR concentration to the sum of the large neutral amino acid concentration).

2014 ASPARTATE AS THE TRANSMITTER OF THE AUDITORY NERVE. Michael R. Martin. LNO, NINCDS, NIH, Bethesda, MD 20205.

One criterion for establishing a transmitter role for a compound is to determine that a substance affecting the natural transmitter affects the putative transmitter in an identical fashion. There is substantial evidence that either glutamate or aspartate (or both) is the natural transmitter(s) of the auditory nerve Wenthold and Gulley, Brain Res. 138: 111, 1977; Martin and Adams, Neuroscience, in press). There is evidence that glutamate diethyl ester (GDEE) is a more selective antagonist of glutamate than aspartate and that the opposite is true for D-α-aminoadipate (DAA) (McLennan and Hall, Brain Res. 149: 541, 1978). Magnesium (Mg) has been shown to be effective in preferentially reducing aspartate and N-methyl-D-aspartate (NMDA, a structural analog of aspartate) responses (Davies and Watkins, Brain Res. 130: 364, 1977). In the present study the actions of the antagonists GDEE, Mg and DAA were compared on glutamate-, aspartate- and NMDA-evoked excitations and on synaptic responses on 11 chopper - type units in the anteroventral cochlear nucleus of the cat. The drugs were iontophoresed from seven barreled microelectrodes, with automatic current balancing control.

The effects of the antagonists GDEE and Mg were compared on glutamate and aspartate responses on 5 units and on glutamate and NMDA responses on 2 units. Neither antagonist was very effective in differentiating between glutamate and aspartate; both were depressed with a tendency for GDEE to depress glutamate and Mg to depress aspartate preferentially. NMDA was not affected by GDEE. In contrast, Mg blocked the NMDA response. Mg depressed and GDEE had no effect on synaptically-evoked responses on 1 of these units.

The effects of the antagonists GDEE and DAA were compared on glutamate and aspartate responses on 1 unit and on glutamate and NMDA responses on 4 units. Again, there was a slight preferential depression of glutamate over aspartate by GDEE while on the same unit DAA preferentially depressed the aspartate response. GDEE had little effect on NMDA responses. DAA at low iontophoretic currents blocked the NMDA response without having any substantial effect on glutamate. DAA depressed the synaptically-evoked responses while GDEE had no effect on any of these units.

The preferential depressions of aspartate, NMDA and synaptic responses by DAA and Mg were in contrast to the preferential depression of glutamate and the inability to affect synaptic responses by GDEE. The data are preliminary but suggest that aspartate or an aspartate-like substance is more likely to be the transmitter of the auditory nerve than glutamate. Further experiments are being conducted to substantiate these initial observations.

2015 GLYCINE AND PEPTIDES IN THE PROCESSES OF THE IDENTIFIED APLYSIA NEURONS R3-R14. D.J. McAdoo, C.H. Price, and M.H. McAdoo*. Marine Biomedical Institute and Dept. of Human Biological Chemistry and Genetics, Univ. Texas Medical Branch, Galveston, TX 77550.

Previous work has shown that the cell bodies of R3-R14, identified neurons in the abdominal ganglion of Aplysia, contain unusually high glycine concentrations and unique small peptides. These substances are candidates for release from R3-R14 as chemical messengers. We have recently located the terminals of the major axons of R3-R14 (Price and McAdoo, this meeting) and this has enabled us to do chemical analyses on nerves containing their preterminal processes. Using gas chromatography-mass spectrometry, we have found glycine concentrations in these nerves that are 10 times higher than in other nerves. By autoradiography, we have demonstrated a specific and rapid glycine uptake system in the axons and terminals of R3-R14.

SDS polyacrylamide gel electrophoresis demonstrated that there are small peptides in the axons and terminals of R3-R14 with molecular weights that are similar to those of the peptides found in the cell bodies. There are large quantitative differences between the gel patterns of peptides in the axons and cell bodies, consistent with peptide processing during axonal transport.

Statistical analysis of electron microscope autoradiographs demonstrated a highly significant association of axonally transported ^{3}H-glycine with the large osmophilic vesicles characteristic of R3-R14. Earlier work (Price et al., J. Neurobiol., in press) has shown that 80% of the radioactivity in such transport experiments is in molecular glycine, not protein. This is the first report of an association of a free amino acid with neuronal vesicles. As glycine is not osmophilic, the vesicles presumably contain peptides as well.

The presence of unique small peptides and high concentrations of free glycine in the R3-R14 terminal regions suggests that both could be released as chemical messengers, but neither case has yet been proven. Morphological studies have shown that R3-R14 have endings on vascular smooth muscle and secretory endings in neurohemal areas. These biochemical and morphological results suggest that R3-R14 may use multiple modes of signalling to their target tissues.

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2016 SLOW EXCITATORY RESPONSE TO DOPAMINE IN APLYSIA. M. J. McCreery* and T. C. Pellmar (SPON: C. M. Woodbury). Armed Forces Radiobiology Research Institute, Bethesda, MD 20014.

Previous reports have demonstrated a slow excitatory response in some neurons of Aplysia to iontophoretically applied serotonin (5-HT) and GABA. Gerschenfeld and Paupardin-Tritsch (J. Physiol. 243: 427, 1974) characterized a slow depolarizing response to 5-HT, the A' response. This response reached peak amplitude in 10-25 sec and was almost abolished when Na+ was replaced by Tris+. Unlike the fast excitatory response to 5-HT due to an increase in sodium conductance (A response), the A' response was unchanged by 10^{-4}M curare. Observation of a similar slow depolarizing response to GABA was made by Yarowsky and Carpenter (Br. Res. 144: 75, 1978) in L11 of the abdominal ganglion. The underlying mechanism of this response appeared to be a conductance increase to sodium and, like the A' response to 5-HT, was curare-insensitive.

We report here that some neurons in the abdominal and buccal ganglia of Aplysia, studied under current or voltage clamp conditions, exhibit a slow depolarizing response to iontophoretically applied dopamine. The duration as well as the time-to-peak of this response is much longer than that of the fast increased sodium conductance response to dopamine which has been observed by several workers. In contrast to the fast excitatory response which peaks in 1-3 sec and lasts 10-20 sec, this slowly developing depolarization peaks in 10-25 sec and lasts as long as 1-2 min. It is minimally affected by exposure to low-Cl^- seawater or to 10^{-3}M curare. The response amplitude is reduced when sucrose is substituted for NaCl. Although it has been difficult to ascertain a change in membrane conductance during the response, it behaves like a conductance increase to sodium in that the response amplitude decreases with membrane depolarization and extrapolates to zero amplitude at a potential between 0 mV and -20 mV.

These observations demonstrate another type of excitatory response to dopamine in Aplysia. The ionic mechanism of this response appears to be similar to the slow depolarizations already reported for 5-HT and GABA.

2017 ACh RELEASE FROM RAT CORTICAL SYNAPTOSOMES AND ITS RELATIONSHIP TO Na^+K^+-ATPase ACTIVITY. Edwin M. Meyer* and Jack R. Cooper. Dept. of Pharmacology, Yale Univ. School of Med., New Haven, CT 06510.

Since a number of problems exist with the vesicular theory of transmitter release, we are exploring the possibility that the release mechanism may involve H^+ generation at the presynaptic terminal via Na^+,K^+-ATPase activity. Accordingly, the efflux of [^{14}C]-acetylcholine (ACh) and [^{14}C]-choline from superfused rat cerebro-cortical synaptosomes was monitored continuously through an anthracene scintillator flow cell. The release of [^{14}C]-ACh but not that of [^{14}C]-choline was increased several fold by short (10-60s) exposures to 60mM K^+, 10^{-5}M veratridine, or electrical field stimulation (20V). Each of these treatments released [^{14}C]-ACh via a Ca^{2+} dependent, reversible mechanism. Ouabain (10^{-4}-10^{-3}M), a specific inhibitor of transport Na^+,K^+-ATPase, also increased [^{14}C]-ACh efflux from these cortical synaptosomes within several seconds of exposure; however, as would be predicted, ouabain-induced transmitter release was not dependent on the presence of extracellular Ca^{2+}. Synaptosomal Na^+,K^+-ATPase activity and [^{22}Na]-efflux were also measured via batch or superfusion techniques, and preliminary evidence from these experiments indicated that ACh-releasing agents or treatments also inhibited synaptosomal Na^+K^+-ATPase activity in a manner that temporally paralleled their action on [^{14}C]-ACh release. These preliminary results support a model which implicates changes in Na^+,K^+-ATPase activity as a trigger for neurotransmitter release.

2018 ROLE OF DOPAMINE AND ITS AGONISTS AND ANTAGONISTS IN CAROTID BODY CHEMORECEPTOR FUNCTION OF THE RAT. J. Mishra, H. N. Sapru, and A. Hess. Dept. Anat., CMDNJ-Rutgers Medical School, Piscataway, NJ 08854, Section of Neurological Surgery, CMDNJ-Med. School, Newark, NJ 07103.

In vivo studies on the inhibitory or stimulatory action of dopamine, its agonists and antagonists on the carotid bodies of cat, dog, rabbit and rat have produced variable and conflicting results. These might occur because of the differing types of anaesthesia employed and the varying levels of anaesthesia achieved in the experimental animals. In the present study, decerebrate as well as chloral hydrate (35mg/kg i. p.) anaesthetized rats were used to study carotid body chemoreceptor function. Injection of the dopamine agonist apomorphine (200µg/kg) by itself in the carotid artery close to the carotid body resulted in minimal excitation or no change in the carotid sinus nerve (CSN) activity or respiration. However, apomorphine potentiated the ventilatory as well as CSN response to NaCN (100µg/kg, i. a.). On the other hand, chlorpromazine suppressed the ventilatory and CSN response to NaCN. Chlorpromazine by itself had no significant effect on respiration and CSN activity. Dopamine (1-6µg/kg close to carotid body) produced by itself small or no excitation of respiration. Increasing the dose up to 10µg/kg did not produce any alteration in respiratory response. These results were confirmed in decerebrate rats, thus eliminating the variability, if any, due to anaesthesia. Injection of high doses of dopamine (20µg/kg), however, produced by itself increase in respiration. Severance of the ninth nerve bilaterally abolished all respiratory responses to exogenous neurotropic substances. To ascertain further that the respiratory response elicited in the present study was indeed mediated by the carotid body, the ventilatory response of the cat was compared to that of the rat. As previously demonstrated by others recording cat CSN activity, dopamine agonists inhibited, while antagonists stimulated respiration. It thus appears that dopamine and its agonists and antagonists have opposite effects on breathing responses of cat and rat, despite the similarities in morphology, innervation, neurotransmitter content, and presumed function of the carotid bodies of these different mammalian species.

2019 CYSTEINE UPTAKE IN CENTRAL NERVOUS SYSTEM: COMPARISON OF UPTAKE IN HOMOGENATE AND CRUDE SYNAPTOSOMAL PREPARATION OF DIFFERENT REGIONS OF THE BRAIN AND THE EFFECTS OF SOME INHIBITORS. C.H. Misra, R.C. Smith and J. Sammeta*

The nerve endings in the brain possess membrane transport mechanisms for the uptake of various substances. The compounds thought to act as transmitter characteristically are taken up by high affinity mechanism [(Logan, W.J. and Snyder, S.H., Nature, 234, 297 (1971)]. The accumulation of L-(^{35}S)-cysteine into synaptosomal preparation of rat cerebral cortex was carried out with the same high affinity uptake mechanisms. [Misra, C.H. and Smith, R.C., Society for Neuroscience. Abstract #1424, Vol 4, 448 (1978)]. The high affinity uptake system appears to be associated with a unique population of nerve terminals which can be separated from the other terminals that concentrate other synaptic transmitter. Therefore, we have examined a variety of amino acids, other putative transmitters, and drugs for their ability to inhibit the high affinity uptake of L-(^{35}S)-cysteine into crude synaptosomal preparation of rat cerebral cortex.

Twenty substances were tested as inhibitors of the uptake of L-(^{35}S)-cysteine in crude synaptosomal preparations (P_2) of rat cerebral cortex. Among cysteine analogous tested, only S-ethyl-L-cysteine had affinity for the uptake mechanism comparable to cysteine. L-(^{35}S)-cysteine uptake was also potentially inhibited by norepinephrine, dopamine, butaperazine, L-glutamic acid, L-aspartic acid, DL-Homocysteine and glutathione. L-(^{35}S)-cysteine uptake was examined in homogenate of cerebral cortex and other regions of the rat brain. The uptake of L-(^{35}S)-cysteine was also studied in the subcellular factions (P_1, P_2 and P_3) of the rat cerebral cortex homogenate. Accummulations of L-(^{35}S)-cysteine were in the order of crude synaptosomal fraction > nuclear fractions > microsomal fractions.

2020 GABA RECEPTOR BINDING IN BOVINE RETINA: EFFECT OF FREEZING TRITON X-100, and $NaClO_4$. Cheryl K. Mitchell* and Dianna A. Redburn. (SPON: E. Simon Sears). Department of Neurobiology and Anatomy. The University of Texas Medical School at Houston, Houston, Texas 77025.

Two different types of ^{3}H-GABA receptor binding assays were performed on retinal subcellular fractions. In one assay, fractions were pretreated by freeze-thawing, and exposure to Triton X-100 before incubation with ^{3}H-GABA and rapid centrifugation as described by Enna and Snyder (Brain Res. 115, 174-179, 1976). These pretreatments were reported to cause an apparent increase in GABA receptor binding in brain synaptosomal membranes: freezing (20°C, 24 hrs)=two fold increase; treatment with 0.05% Triton X-100=five fold increase. Two retinal synaptosomal fractions were assayed: the first was enriched in large photoreceptor cell terminals (the outer plexiform layer or the OPL fraction), and the second contained conventional sized terminals (the inner plexiform layer or IPL fraction). GABA receptors from both fractions showed similar Triton sensitivity as compared to brain, with maximal stimulation noted at 0.05% Triton concentration. However, unlike brain, freeze-thawing of the tissue prior to the assay had little affect on the apparent binding. In Triton treated material two binding sites were observed: a lower affinity site (K_D=330 nM) with the number of binding sites roughly equal in both fractions (10 pmoles/gm tissue), and a higher affinity site (K_D=38 nM) limited primarily to the OPL fraction.

In the second type of ^{3}H-GABA binding assay, $NaClO_4$ was included during the incubation period. Preliminary results indicate that, like Triton pretreatment, $NaClO_4$ increases the specific ^{3}H-GABA receptor binding in both IPL and OPL fractions. However, the two differences were noted: 1) the overall affinity of the GABA receptor sites is significantly lower than in Triton treated material, 2) more binding sites were observed in the IPL fractions after $NaClO_4$ treatment than with Triton treatment. Experiments to determine the pharmacological specificity of these sites are currently underway. (This study was supported by USPH Grant EYO 1655-03 and RCDA 1K04 EY 00088-02 to DAR.)

2021 β-ALANINE INHIBITION OF POTASSIUM INDUCED ^{3}H-γ-AMINOBUTYRIC ACID RELEASE FROM MOUSE CORTICAL SLICES. James V. O'Fallon and Joseph W. Harding. Dept. Vet. Microbiology/Pathology, Wash. State U., Pullman, Wa 99164.

Mouse cortical slices were preloaded with ^{3}H-γ-aminobutyric acid (GABA) and superfused with 115mM NaCl, 5mM KCl, 1.2mM $MgCl_2$, 1.2mM Na_2HPO_4, 5.0mM glucose, 10mM animooxyacetic acid (AOAA) and 40mM glycyglycine (pH 7.4). The introduction of 40mM KCl with 2.5mM $CaCl_2$ caused a release of ^{3}H-GABA which was 3-5 times higher than the spontaneous efflux rate. No significant release was seen in the absence of added $CaCl_2$. The release of ^{3}H-GABA was virtually eliminated by superfusion with 50μM β-alanine. β-Alanine added to the uptake medium alone had no effect on ^{3}H-GABA release. The introduction of 50μM 2,4-Diamino-n-butyric acid (DABA) had no effect on K^+-stimulated, Ca^{++} dependent release when introduced into both the uptake and superfusion mediums. 50μM glycine also had no effect whereas 50μM unlabeled GABA increased the spontaneous efflux rate of ^{3}H-GABA 3-4 fold but had no effect on K^+ induced release. In the absence of AOAA no K^+ induced release of ^{3}H-GABA was observed.

2022 BLOCKADE BY α-AMINOADIPATE OF THE NEUROTOXIC AND LH-RELEASING ACTIONS OF N-METHYL ASPARTATE. J.W. Olney, M.T. Price, T. Fuller, T. de Gubareff*, M. Anglim*, J. Labruyere* and V. Mitchell*. Washington University School of Medicine, St. Louis, MO 63110.

When administered subcutaneously (sc), the excitotoxic amino acids - glutamate (Glu), aspartate (Asp) and certain analogs - selectively penetrate a specific region of the endocrine hypothalamus, the arcuate nucleus (AH), and induce disturbances in neuroendocrine function. When neurotoxic doses are employed, AH neurons are destroyed and a permanent neuroendocrine deficiency syndrome results, which includes impaired reproductive capacity and reduced size of the gonads and accessory reproductive organs. Subtoxic doses induce reversible perturbations in endocrine functions, the most studied of which is a transient rise in serum luteinizing hormone (LH). Price et al. (Neuroendocrinol. 26, 352, 1978), who recently evaluated several of the more potent excitotoxins and found each to be effective in releasing luteinizing hormone (LH), considered N-methyl aspartate (NMA) the most promising for investigating the LH axis. When administered sc in nontoxic doses (15-40 mg/kg) to weanling or young adult male rats, NMA induces LH release by an action which is reversible, rapid of onset, brief in duration, and is dependent upon AH neurons, since rats bearing an excitotoxin-induced AH lesion do not respond to the LH-releasing action of NMA (Price, M.T., et al., Neurosci. Abstr., 1979).

We have proposed that both the AH neurotoxic and LH-releasing actions of NMA stem from excitatory interaction between NMA and synaptic receptors on the dendrosomal surfaces of AH neurons. NMA is thought to act primarily at "Asp-preferring" receptors and its excitatory action at such receptors is reportedly blocked specifically by the antagonist, α-aminoadipate (αAA). We will present evidence that αAA blocks both the LH-releasing and the AH neurotoxic activity of NMA, whereas GABA, a putative neuroinhibitory transmitter, blocks the LH-releasing but not the AH neurotoxic action of NMA. This suggests that αAA blocks NMA specifically at its AH excitatory receptor whereas GABA blocks at some other locus along this LH release pathway.

From these and other findings we are beginning to suspect that AH neurons are a major link in an LH release pathway that is driven by aspartergic excitatory input to synaptic receptors on the dendrosomal surfaces of AH neurons and is subject to GABAergic inhibition at some point between the AH neuronal perikaryon and median eminence storage terminals from which luteinizing hormone releasing hormone is secreted in response to aspartergic activation of the pathway. Supported by grants DA-00259, MH-14677, NS-09156, a Huntington's Chorea Fdn. grant and RSD Award MH-38894 (JWO).

2023 EVIDENCE FOR TWO-INTERCONVERTIBLE FORMS OF THE NICOTINIC ACETYLCHOLINE RECEPTOR FROM GOLDFISH BRAIN. Robert E. Oswald and John A. Freeman. Depts. of Biochem. and Anat., Vanderbilt Univ. Med. Sch., Nashville, TN 37232.

In the central nervous system of the goldfish, intracellularly recorded EPSP's elicited by the iontophoresis of ACh can be blocked by the application of α-bungarotoxin (α-Btx; J. A. Freeman, ARVO Abstracts, 1979), indicating that α-Btx is a useful probe for the nicotinic acetylcholine receptor (nAChR) in this system. We have previously reported some aspects of the kinetic and equilibrium analysis of ^{125}I-α-Btx binding to the goldfish nAChR as well as some of its molecular properties (R. E. Oswald and J. A. Freeman, J. Biol. Chem., 1979). In this report we present evidence for the presence and interconvertibility of two forms of ^{125}I-α-Btx binding sites.

Equilibrium binding studies using high specific activity ^{125}I-α-Btx (1 Ci/μmole) have demonstrated the presence of two distinct binding sites: A high affinity site with a K_D of 0.1 to 0.2 nM, and a low affinity site with a K_D of 6 to 10 nM. A Hill plot indicated the presence of apparent negative cooperativity, yielding a Hill coefficient of 0.89. Only one association rate constant was detectable; however, dissociation kinetics revealed the presence of two distinct components. When a 1000-fold excess of cold α-Btx was added to ^{125}I-α-Btx-nAChR complexes, approximately 20% of the sites dissociated with a half-time of 17 minutes and 80% with a half-time of 45 hours. The addition of d-tubocurarine (d-TC) and carbamylcholine produced the same dissociation kinetics as cold α-Btx when used in low concentrations (0.01 mM d-TC and 1 mM carbamylacholine). However, when high concentrations were used (0.05 to 1 mM d-TC and 25 to 100 mM carbamylcholine), the percentage of rapidly dissociating sites increased up to a maximum of 75% in a dose-dependent fashion without affecting the dissociation rate constant of either site. Because this effect occurs at concentrations of 10^4 to 10^5 fold higher than the K_I of each ligand (10^{-8} for d-TC and 10^{-6} for carbamylcholine), we believe that d-TC and carbamylcholine bind to an allosteric site which mediates a conformational change from a form of the nAChR having a high affinity for α-Btx to a form having low affinity for α-Btx. Thus, the goldfish brain seems to contain one type of α-Btx binding protein (the nAChR) with two interconvertible sites rather than two distinct binding proteins.

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2024 GABA RECEPTORS: AUTORADIOGRAPHIC LOCALIZATION IN RAT CEREBELLUM. José M. Palacios*. W. Scott Young III and Michael J. Kuhar. Dept. of Pharmacol. The Johns Hopkins Univ. Sch. Med., Balto., Md. 21205

A new technique involving the in vitro labeling of mounted tissue sections has been used to localize GABA receptors at the light microscopic level. ^{3}H-muscimol (^{3}H-M) was incubated with mounted tissue sections and the binding kinetics of the drug had all of the characteristics associated with a GABA receptor. The binding was rapidly reversible (t1/2 at 2°C of 20 min), of a high affinity (Kd of 6-13 nM) and saturable(Bmax of about 200 fmol/mg tissue). The pharmacological specificity was also that of a GABA receptor. Specific ^{3}H-M binding was displaced by GABA (Ki about 10 mM), imidazole acetic acid (Ki about 100 nM) and (+) bicuculline (Ki about 10 μM). The binding was insensitive (-) bicuculline, picrotoxin and the GABA uptake inhibitor diaminobutyric acid. The specific to nonspecific binding was about 6:1.

Autoradiographic studies revealed a very high density of receptors over the granule cell layer, a low level over the molecular layer and negligible receptor binding over the white matter. These results were highly reproducible and found in several experiments. A similar distribution was observed in the cerebella of mice and guinea pigs. The results were same whether we used 1,5 or 30 nM concentrations of ^{3}H-M, and the same results were found when ^{3}H-GABA was used to label the receptors. While there were significant levels of receptors in all areas, these results suggest a high localization of GABA receptors to granule cells. This suggestion is confirmed by a variety of neurochemical studies in the literature and in press. For example, removal of granule cells by viral treatment or by utilization of mutant mice results in a 60-70% loss of GABA receptor binding in vitro. These results are compatible with the view that Golgi II cells as well as other cells are GABAergic.

High receptor densities were observed in many other regions of the rat brain as well. These include the substantia nigra zona reticulata, the dorsal lateral geniculate and parts of the dorsal horn of the spinal cord.

These autoradiographic studies can contribute to localizing elements of the GABAergic system in neuronal tissue. They are sensitive and provide light microscopic resolution. They could be valuable adjuncts to other histochemical methods for localizing GABA synthesizing enzymes and GABA uptake sites. These studies were supported by USPHS grants MH25951, MH00053.

2025 INVOLVEMENT OF CYCLIC AMP IN VOLTAGE-DEPENDENT CALCIUM CURRENT ELICITED BY SEROTONIN. T. C. Pellmar and D. O. Carpenter. Department Neurobiology, Armed Forces Radiobiology Research Institute, Bethesda, MD 20014.

In neurons of the lower left quadrant of the abdominal ganglion in Aplysia californica, iontophoretic application of serotonin elicits an extremely slow, voltage-dependent calcium current (Pellmar and Carpenter, Nature 277:483, 1979). The transmitter-induced current, studied under voltage clamp conditions, has a time-to-peak of 10-30 sec and a duration of 1-4 min. The response is of maximum amplitude when the membrane potential is about +10 mV. At more depolarized and at more hyperpolarized membrane potentials, the amplitude is smaller. The transmitter-induced calcium current is not present at potentials more hyperpolarized than -40 mV.

In the abdominal ganglion of Aplysia, perfusion of serotonin produces an increase in cyclic AMP (cAMP) (Cedar and Schwartz, J. Gen. Physiol. 60:570, 1972). It has been proposed that serotonin mediates heterosynaptic facilitation by inducing a presynaptic calcium current through a cyclic nucleotide mechanism (Klein and Kandel, PNAS 75:3512, 1978; Shimahara and Tauc, J. Physiol. Paris 74:515, 1978). We tested the actions of cyclic nucleotides and phosphodiesterase inhibitors on this voltage-dependent calcium current. Perfusion of 10^{-5}M to 10^{-4}M cAMP or dibutyryl cAMP (dBcAMP) has little effect on the amplitude and duration of the voltage-dependent response to iontophoresis of serotonin. In some experiments, a slight and transient reduction in amplitude is observed. On occasion, cAMP and dBcAMP induce a transient inward current while the membrane potential is at a depolarized potential. Adenosine (10^{-5}M to 10^{-4}M) has similar effects, occasionally inducing an inward current and occasionally reducing the amplitude of the response to serotonin. The phosphodiesterase inhibitors isobutylmethylxanthine (IBMX) (1 mM), theophylline (2 mM), and RO 20-1724 (about 1 mM) all cause a reduction in the amplitude of the voltage-dependent calcium current elicited by serotonin. This action is reversible by washing with normal seawater.

Based on these preliminary data, it appears that cAMP does not mediate the action of serotonin to open calcium channels involved in the voltage-dependent response seen here. However, it is conceivable that the serotonin-activated conductances observed by others, including a presynaptic calcium conductance, may operate through a cAMP mechanism. The possibility that the cyclic nucleotides can modulate or directly induce a similar current cannot be excluded.

2026 TURNOVER OF BIOGENIC AMINES IN THE HYPOTHALAMUS OF RATS DURING PYROGEN FEVER. P. E. Penn+ and B. A. Williams*. NASA-Ames Research Center, Moffett Field, CA 94035

Many pharmacological studies have implicated the biogenic amines in the hypothalamus as playing a role in the production of fever, but few investigations of endogenous neurochemicals have been made during fever. Turnover rates of transmitters utilizing radioactive precursors may be one of the most accurate measurements of activity in brain regions. The present study was designed to measure the turnover of 5-hydroxytryptamine (5-HT), norepinephrine (NE) and dopamine (DA) in the hypothalamus of rats during pyrogen fever. Salmonella typhosa (Wyeth, 8 units) was previously found in our laboratory to produce a significant hyperthermia in most rats by 2.5 hours. This pyrogen (N=12) or saline control (N=8) was injected intraperitoneally and the rats killed 2.75 hours later. Rectal temperatures (Tr) were monitored continuously with thermocouples taped to the tail and recorded automatically every 3 minutes. Half of each group received an injection of radioactive precursors, ^{3}H-tryptophan (0.5 mCi) and ^{3}H-tyrosine (1.0 mCi), via an indwelling jugular catheter 60 minutes before killing, and the other half at 90 minutes. The rats were killed by near freezing in liquid nitrogen and the brains dissected in the cold. Turnover was measured by the method of Lane et al. (Life Sci 21, 1101, 1977). At the time of killing most of the pyrogen group showed a significant ($p<.02$) increase (mean ± s.e.m.) in Tr above pre injection levels (0.75 ± 0.13°C, N=10). The saline group showed no change (-0.025 ± 0.16, N=8), and the difference between groups was also significant. No significant differences were found in the levels of the amines between the pyrogen and saline groups. A significant difference was found in the specific activity of NE between the 60 minute pyrogen and saline groups (4.41 ± 0.41 vs 2.6 ± 0.51 dpm/pmole) but no change in ^{3}H turnover. This suggests an increased accumulation of ^{3}H-NE in the pyrogen group, but no change in utilization. An increased turnover of DA for the pyrogen group (44.5 vs 19.2 pmole/mg protein/hr) was found. However, DA is mainly a precursor in the hypothalamus and measurement was near the limit of sensitivity for the assay; these limitations must be considered in interpreting this data. The most significant finding was an increase in the turnover of 5-HT in the pyrogen group (41.3 vs 7.3 pmole/mg protein/hr), indicating a resynthetization rate of 77% of the total pool per hour. These results suggest that at the time point measured, an increase in the utilization of 5-HT in the hypothalamus is correlated with pyrogen fever. (+NRC Associate at NASA-Ames)

2027 AMINO ACID-DIRECTED ACCUMULATION OF CYCLIC NUCLEOTIDES IN RAT CEREBELLAR SLICES. M.A. Rea* and W.J. McBride (Spon: S.L. Morzorati). Depts. of Psych. & Biochem., Institute of Psychiatric Research, Indiana Univ. Sch. of Med., Indianapolis, IN 46223.

Both the harmaline-induced increase in cerebellar climbing fiber activity and the intraventricular micro-injection of glutamate (Glu) cause 2.5 fold increases in rat cerebellar cyclic GMP content in vivo (Biggio and Guidotti, Brain Res. 107: 365, 1976). These findings have been presented as evidence in support of a role for Glu as the excitatory neurotransmitter released from climbing fibers. However, other data have been reported which suggest that aspartate (Asp) may be the climbing fiber transmitter (Nadi et al., J. Neurochem. 28: 661, 1977). Therefore, we undertook a study of the effects of Asp and Glu, as well as GABA and taurine (two suspected inhibitory transmitters) on the accumulation of cyclic GMP and cyclic AMP in rat cerebellar slices. Under the conditions employed in this study, which were adapted from those described by Schmidt et al. (Brain Res. 112: 113, 1976), the basal levels of cyclic GMP and cyclic AMP were 15.2 ± 0.5 and 20.2 ± 0.5 pmol/mg protein, respectively. Incubation of the slices for 15 min in the presence of either Asp or Glu (1 and 10 mM), or 55 mM K^+, resulted in a 2 to 2.5 fold increase in cyclic GMP content. Furthermore, the effects of both amino acids were potentiated by theophylline (1 mM) and dependent upon the presence of Ca^{++} in the medium, suggesting that both Glu and Asp stimulate cyclic GMP accumulation by a similar mechanism. Similarly, both Asp and Glu stimulated cyclic AMP accumulation. However, in this case, the increase caused by Glu (2 to 2.5 fold) was significantly greater ($p<0.05$) than that caused by Asp (1 to 2 fold). Furthermore, the Asp effect was blocked by theophylline and partially dependent upon the presence of Ca^{++} in the medium while the Glu effect was not, suggesting that the effect of these amino acids on cyclic AMP accumulation involve different mechanisms. Cyclic AMP levels were not altered by 55 mM K^+. GABA (10 mM) caused a 25% decrease in cerebellar cyclic AMP levels but had no effect on cyclic GMP levels. Taurine and alanine did not change cerebellar cyclic nucleotide levels at the concentrations used (1 and 10 mM). The finding that both Asp and Glu stimulate cyclic GMP levels by a similar mechanism and to approximately the same degree does not permit an interpretation which favors either amino acid as the climbing fiber neurotransmitter candidate. (Supported by NS 13925).

2028 SEROTONIN LOCALIZATION BY FLUORESCENT TECHNIQUES IN THE BRAINS OF NORMOTENSIVE AND HYPERTENSIVE RATS.
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Brains of normotensive Wistar Kyoto (WKY) and spontaneously hypertensive rats (SHR) were treated according to the Falck-Hillarp method suggested by de la Torre (Dynamics of Brain Monoamines, Plenum, 1972) for histofluorescence of biogenic amines. The brains of these animals showed marked differences which depend on the degree of blood pressure change. WKY animals showed serotonin deposits in all cerebral areas of the brain not corresponding to those normally associated with serotonin deposition (i.e., the raphe system). The SHR animals showed a greater (2-3x) amount of serotonin in discrete granules spread throughout the brain. There is evidence of large amounts of serotonin in neurons and capillary endothelial cells of SHR rats. There are granule accumulations of serotonin which seem to be localized in glial cells. The majority of serotonin granules in the SHR animals, however, is within the neurons or the capillary endothelial cells. The disparity between the WKY and the SHR suggests that the brain tissues act in the hypertensive state as a storage area for serotonin, or there is an increased ability to take up serotonin. Parachlorophenylalanine (pCPA) does not seem to abolish the serotonin fluorescence in the brain to the extent that is has been reported in peripheral tissues. The presence of serotonin in discrete large granules within cells that in addition also fluoresce with norepinephrine suggests that the SHR animals may have cells capable of containing and storing both biogenic amines at the same time. Thus a cell which may have a designated function to release only one transmitter for action at specific receptor sites could possibly demonstrate a capability for release of "foreign" transmitters as well, whether the foreign transmitter possesses any action at postsynaptic sites remains a future question.

2029 INTERACTION OF GABANERGIC AND CHOLINERGIC SYSTEMS IN RABBIT RETINA. Dianna A. Redburn and Thyon Chentanez*. Department of Neurobiology and Anatomy, The University of Texas Medical School at Houston, Houston, Texas 77025.

Two synaptosome fractions were obtained from rabbit retina, one enriched in photoreceptor terminals (the outer plexiform layer, or OPL fraction), a second enriched in conventional sized terminals (the inner plexiform layer or IPL fraction). High affinity uptake sites for ^{3}H-GABA and ^{3}H-choline were highly enriched in the IPL fraction as compared to the OPL fraction. Uptake in both fractions was temperature and sodium dependent, and had similar affinities; however, the Vmax was four fold higher for ^{3}H-choline and three fold higher for ^{3}H-GABA in the IPL fraction. It is unlikely that the relatively minor GABAnergic and cholinergic components found in the IPL were associated with contaminating synaptosomes from the IPL fraction since these systems in OPL fractions displayed unique characteristics. Specifically, in OPL fractions, the ^{3}H-GABA uptake system was stimulated by addition of exogenous ACh. The stimulation was dose dependent and saturable at micromolar concentrations. Increases in endogenous levels of ACh by preincubation with choline or neostigmine also stimulated ^{3}H-GABA uptake specifically in OPL. The cholinergic stimulation was blocked by both nicotinic and muscarinic receptor blockers, however, nicotinic blockers were 10 times more potent as measured by IC_{50} concentrations. Nicotinic agonists, nicotine and DMPP mimicked the action of exodogenous and exogenous ACh. If the GABAnergic and cholinergic systems in the OPL are associated with photoreceptor elements then these data would be consistent with reports from other species which suggest that cholinergic cone terminals transmit directly to GABAnergic horizontal cell terminals within the cone triad. (The work was supported by USPH Grant EYO 1655-03 and RCDA IK04 00088-02 to DAR.)

2030 EFFECTS OF NEURONAL DELETIONS ON UPTAKE AND BINDING OF CEREBELLAR NEUROTRANSMITTER CANDIDATES. Brooks H. Rohde* and W.J. McBride, Depts. Psych. & Biochem., Inst. of Psych. Research, Indiana Univ. School of Medicine, Indianapolis, IN 46223.

Exposure of the cerebella to x-irradiation on a schedule from day 12 to 15 after birth (12-15X) causes a loss of late-forming granule cells while exposure to x-irradiation from day 4 to 15 (4-15X) causes the loss of granule, stellate, and basket cells. Injection of 3-acetylpyridine (3-AP) causes the loss of the climbing fiber input without apparently causing damage to other cellular elements in the cerebellum. The uptake of 1.0 µM (^{3}H)-glutamate (glu) and (^{3}H)aspartate (asp) was approximately 20% lower while the uptake of 1.0 µM (^{3}H)GABA and (^{3}H)taurine (tau) was unchanged in the crude synaptosomal fraction (P_2) isolated from the cerebella of 12-15X rats relative to control values. More detailed kinetic analysis of the uptake of (^{3}H)glu revealed that the K_m value was not changed but that the V_{max} value was 20% lower in the 12-15X rats relative to the control group. In the 4-15X group, decreases were seen in the P_2 uptake of 1.0 µM (^{3}H)glu (40%), (^{3}H)asp (30%) and (^{3}H)GABA (40%) relative to control values. Uptake of 1.0 µM (^{3}H)tau was not significantly different between the control and 4-15X groups. More detailed kinetic analysis of the uptake of (^{3}H)GABA revealed that the K_m value was not changed but that the V_{max} value was decreased by 40% in 4-15X rats relative to control values. The uptake of 1.0 µM (^{3}H)glu, (^{3}H)asp, (^{3}H)GABA and (^{3}H)tau into the P_2 fraction was not different for 3-AP injected animals relative to control values. Sodium-independent binding of 20 nM (^{3}H)GABA was reduced by 20% and binding of 20 nM (^{3}H)kainic acid was reduced by 29% in synaptic plasma membrane (SPM) preparations obtained from 12-15X group relative to control values. Sodium-independent binding of 1.0 nM (^{3}H)quinuclidinyl benzilate (QNB) was increased by 22% in 12-15X group while binding of 1.0 nM (^{3}H)dihydroalprenolol (DHA) was not different from control values. The data on uptake are consistent with the idea that glu may be the excitatory neurotransmitter released from the granule cells and that GABA may be the inhibitory transmitter released from the basket cells. However, on the basis of the uptake data, no conclusions may be drawn pertaining to the transmitter released by the climbing fibers. The decreased sodium-independent binding of (^{3}H)GABA and (^{3}H)kainate may indicate a loss of GABA and glutamate receptors in the granule cell deficient cerebella. Conversely, loss of granule cells does not appear to result in a decrease of either muscarinic or beta-adrenergic receptors. (Supported by NS 13925).

2031 SOMATOSTATIN AND SUBSTANCE P INHIBIT CATECHOLAMINE SECRETION FROM GUINEA PIG CHROMAFFIN CELLS. Lorna W. Role*, Robert L. Perlman*, and Susan E. Leeman, Department of Physiology, Harvard Medical School, Boston, MA 02115.

We have been studying the secretion of catecholamine from purified guinea pig chromaffin cells. The chromaffin cells are isolated by collagenase digestion of guinea pig adrenal glands, followed by isopycnic centrifugation through a 5%-25% (w/v) gradient of metrizamide. More than 90% of the cells in these preparations are viable chromaffin cells, as judged by fluorescence histochemistry and the exclusion of trypan blue. Purified chromaffin cells contain 400±50 nmol epinephrine (EPI)/mg protein (mean S.E.M., n=8). These cells accumulate [^{3}H]-l-norepinephrine (NE) by a high-affinity uptake system (apparent K_m=2µM). During a 10 min incubation at 37°C, the cells release 1%-3% of their stored EPI or their newly accumulated NE. Acetylcholine (ACh) causes a dose-dependent increase in the secretion of EPI and of NE; 100 µM ACh releases 10%-20% of the stored catecholamines from the cells. The peptides somatostatin and substance P inhibit the secretion of catecholamine from the chromaffin cells. In twelve experiments, somatostatin (10 µM) inhibited basal catecholamine release by up to 50%, and substance P (10 µM) inhibited basal release by up to 40%. These peptides also inhibit ACh-induced catecholamine secretion. Somatostatin (10-100 µM) inhibited catecholamine secretion produced by 100 µM ACh by about 55%. Half-maximal inhibition of ACh-induced catecholamine secretion was produced by 3 µM somatostatin. Substance P (10 µM) inhibited ACh-induced catecholamine secretion by about 40%. Chromaffin cells were extracted with 2M acetic acid. These extracts contain both immunoreactive somatostatin and immunoreactive substance P. The possible release of these peptides from the cells is being investigated.

2032 IRREVERSIBLE INHIBITION OF THE HIGH-AFFINITY CHOLINE CARRIER. B. Jane Rylett and E. Howard Colhoun*, Department of Pharmacology, University of Western Ontario, London, Ontario, Canada.

The high-affinity uptake of choline (HAUC) into cholinergic nerve endings would appear to be essential to, and the rate-limiting step in the synthesis of acetylcholine. A number of structural analogues of choline have been shown to compete with choline for this carrier, with some being incorporated into the nerve ending as false transmitters. The nitrogen mustard analogue of choline, choline mustard aziridinium ion (ChM Az), has affinity for this sodium-dependent high-affinity transport system causing inhibition of choline uptake into rat forebrain synaptosomes (Rylett and Colhoun, Can. J. Physiol. Pharm. 55, 769, 1977; Society for Neuroscience, 7th Meeting, abst. 1319, 1977). HAUC was found to be competitively inhibited in synaptosomes pre-incubated with ChM Az for 10 minutes at 37°C (controls at 0°C); the K_i value was calculated to be 2.63 µM. However, the inhibition produced by this compound increases progressively with time and shows an irreversible component insofar that inhibition of HAUC could not be reversed by washing the synaptosomes in inhibitor-free medium. Further evaluation of the kinetics of the inhibition of HAUC by the nitrogen mustard analogue revealed that when preincubation of synaptosomes with ChM Az was increased from 10 to 30 minutes before the measurement of HAUC the nature of the inhibition produced shifted from competitive to mixed competitive - noncompetitive type. This is indicative of a time-dependent decrease in the maximum velocity with which choline could be transported into the nerve terminals as well as an alteration in the apparent affinity with which the substrate choline binds to the carrier. A corresponding decrease in the K_i value was observed at the longer incubation time thus supporting the idea of a time-dependent development of an irreversible blockade of the high-affinity choline carriers. Analysis of the kinetics of the inhibition of HAUC into synaptosomes by ChM Az and the apparent irreversible binding of this nitrogen mustard analogue to the high-affinity choline carrier seems to provide an explanation for the mechanism of action of this compound at the presynaptic ending as well as important information about HAUC. Thus, the shift in the type of inhibition produced by ChM Az with increasing time could be indicative of an excess of carrier sites on the presynaptic membrane over those which are contributing to the control V_{max} measured. Inactivation of carrier sites by alkylation beyond a critical level could cause an initially competitive substrate to show a noncompetitive type of kinetics since sufficient carriers may no longer be functional to transport choline at the V_{max} rate.

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2033 IMPORTANCE OF THE CHOLINERGIC PATWAYS OF THE SEPTAL AREA AND THE SUBFORNICAL ORGAN IN THE REGULATION OF THE SODIUM AND POTASSIUM RENAL EXCRETION. Wilson Abrão Saad, José V.Menani*, Luiz A.A.Camargo*, José Antunes-Rodrigues* and Miguel R.Covian*. Dept. Fisiol. and Patol. Sch. Dent. - UNESP - Araraquara - 14.800 - SP. Brasil

Evidence has been presented indicating that cholinergic stimulation of the hypothalamus and septal area play an important part in the regulation of sodium and potassium in the urine (Silva-Netto, C.R. et.al. J. Physiol. 72:917,1976 and Saad, W.A. et.al. Pharmacol. Biochem.Behav. 3:985,1975). Recent investigations have shown the importance of the subfornical organ in the control mediation of renal sodium and potassium excretion. (Saad, W.A.et.al. Cienc.Cult. 30:487,1978). The present report was undertaken to provide an analysis of the degree of intensity of participation in the septal area and the subfornical organ in this control. Male Holtzman rats weighing 250-320grams were used in these experiments. All animals had cannulae stereotoxically implanted in the septal area or in the subfornical organ. Carbachol was injected into these areas. The urine was collected over a period of 120 minutes and the concentration of Na^+ was determined by flame fotometry. The results are shown below.

	Carbachol (ug)						
	0.01	0.02	0.1	0.2	0.5		
Na^+	37.4	91.4	191.4	264.0	350.0	-	AS
(uEq/120')	61.4	120.2	286.9	563.7	820.9	-	OSF
K^+	55.4	83.6	88.0	135.0	133.2	-	AS
(uEq/120')	85.4	109.1	145.5	217.7	222.9	-	OSF

In conclusion, our findings show that the subfornical organ is more sensitive than the septal area in inducing renal eletrolites excretion. Further research is under way in our laboratory with the object of studing the interaction between, hypothalamus, septal area, and, subfornical organ in this regulation.

Supported by FAPESP.

2034 REGIONAL HIGH-AFFINITY ^{3}H-CHOLINE ACCUMULATION IN CAT FOREBRAIN: SELECTIVE INCREASE IN THE CAUDATE-PUTAMEN AFTER CORTICOSTEROID PRE-TREATMENT. A. Sastre, D.K. Riker, T. Baker, R.H. Roth and W.F. Riker, Jr. Dept. Physiology, Johns Hopkins U. Sch. Med., Baltimore, MD 21205; Depts. Pharmacology, Cornell U. Med. College, N.Y., NY 10021, and Yale U. Sch. Med. New Haven, CT 06510.

The effect of acute and chronic gluco- and mineralocorticoid treatment on high-affinity ^{3}H-choline accumulation (HACA) was investigated in cat brain synaptosomes. HACA in forebrain synaptosomes was found to be Na^+- and energy-dependent, hemicholinium-3 sensitive ($K_i \approx 2.5 \times 10^{-9}$ M), and of high-affinity (K_T = 0.43 - 0.63 µM). The rank order of regional HACA at 0.04 µM choline (4 min at 37°C) was: caudate-putamen > anterior perforated space (incl. olfactory tubercule, n. accumbens and islands of Calleja) > hippocampus-fornix > prefrontal neocortex. HACA in caudate-putamen synaptosomes was significantly elevated 37 - 75% after daily treatment for one week with either triamcinolone diacetate (8 mg/kg), hydrocortisone acetate (4 or 32 mg/kg), or deoxycorticosterone (32 mg/kg), but not with 11α-epicortisol (8 mg/kg), a biologically inert epimer of hydrocortisone. Treatment-induced increases in caudate-putamen HACA were attributable to an increase (45 - 53%) in the maximum transport velocity (V_{max}) and not in the apparent transport constant (K_T), as determined by Eadie-Hofstee and double-reciprocal analyses.

Significant increases in HACA were not seen in the hippocampus-fornix, anterior perforated space or prefrontal cortex. Acute treatment of cats with a single intravenous dose of methylprednisolone sodium succinate (90 mg/kg) produced 75 - 83% increases in HACA in caudate putamen and hippocampus-fornix three hours after treatment. At 24 hours HACA was increased (70 and 76%) in the caudate-putamen and anterior perforated space. Increases in HACA found in the caudate-putamen after in vivo treatment could not be produced by in vitro addition of steroids or alterations of the Na^+/K^+ environment. We conclude that the velocity of high-affinity transport of choline into cat brain synaptosomes is strongly and selectively increased in the caudate-putamen after acute or chronic gluco- or mineralocorticoid treatment. The possible explanations for this selectivity will be discussed with reference to neurochemistry and neuropharmacology of the caudate-putamen. Supported by NINCDS grant NS-01447 to W.F.R., Jr. and post-doctoral research fellowships NS-00832 to A.S. and NS-05406 to D.K.R.

2035 EPINEPHRINE IN THE BRAIN OF PRIMATES AND RATS: EFFECT OF STRESS. A. Sauter*, K. Ueta*, T. Asano*, J. Pearson and M. Goldstein. Departments of Psychiatry and Pathology, New York University Medical Center, 550 First Avenue, New York, New York 10016.

Using high pressure liquid chromatography we have measured epinephrine (E) levels in the African green monkey and in the rat. In both species relatively high E levels were found in the C_1 and C_2 regions of the medulla oblongata and in the hypothalamus (especially ventromedial areas which include mamillary bodies, suprachiasmatic N., median eminence, lateral N.). In the monkey relatively high levels of E were also found in the thalamus (anterior N.) and in the epithalamus (habenula), while smaller levels were found in the substantia nigra and in the inner segment of the globus pallidus.

A similar regional distribution of E was observed in two human post-mortem brains. Thus, the distribution of E in human and non-human primate brain is wider than that in the rat.

To determine whether brain E participates in regulating some responses to stress we have studied the effects of immobilization stress in rats on central E and norepinephrine (NE) levels. Exposure of rats to stress results in a significant decrease of E and NE levels in the hypothalamus and in the C_1 and C_2 regions of the medulla oblongata. Stress produces a greater % decrease of E levels than of NE levels. These results suggest that E neurons are involved to a greater extent than NE neurons in some responses to stress or that the synthesis rate of brain E is slower than that of NE.

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2036 ELECTROPHYSIOLOGICAL AND PHARMACOLOGICAL CHARACTERISTICS OF SEROTONIN RECEPTORS ON A NEURONAL SOMATIC CELL HYBRID. William G. Shain and Joseph E. Freschi* (SPON: A. Kobrine). Neurobiol. Dept., Armed Forces Radiobiol. Res. Inst., Bethesda, MD 20014.

The electrophysiology and pharmacology of serotonin (5-HT) responses on a cell line (TCX11) derived from the fusion of mouse neuroblastoma cells (N18TG2) with mouse sympathetic ganglion cells (Greene, L. et al Proc. Nat. Acad. Sci. USA 72: 4923, 1975) were studied. Dopamine (DA) and 5-HT elicit depolarizing, conductance increase responses in these cells (Myers, P.R., J. Cell. Physiol. 91: 103, 1977). The purpose of this study was to characterize the 5-HT response and compare the properties of the 5-HT and DA responses to determine if they are mediated through different receptors. Iontophoresis of 5-HT caused a rapid depolarization of the hybrid cell membrane potential associated with an increase in membrane conductance (Gm). The response rapidly desensitized with repeated pulses of 5-HT. These same results were obtained when DA was iontophoresed, but the cells were 10 to 100 times more sensitive to 5-HT than to DA. When equal pulses were used, the responses to 5-HT and DA appeared mediated by different receptors when examined for reversal potentials, cross desensitization, and antagonist specificity. However, when the iontophoretic pulses were adjusted to give responses of similar amplitude, different results were obtained. DA and 5-HT cross-desensitized. Reversal potentials determined for both DA and 5-HT on the same cell were equal and varied from 0 to +15 mV. Perfusion of the cells with low-sodium medium reduced the 5-HT and DA responses and shifted the reversal potentials in a similar manner. Possible antagonists were bath applied in concentrations of 10 to 100 uM. No drug has been found that blocks one response and not the other. The following drugs blocked both responses: (+)-tubocurarine, chlorpromazine, bulbocapnine, phentolamine, metergoline, bromo-LSD methiothepin, cyproheptadine, cinnanserin, and mersalate. Phentolamine, bromo-LSD, and cyproheptadine, applied by blunt micropipette, caused a depolarization and increase in Gm. The absence of this effect during bath application was presumably due to rapid desensitization. Since the drugs that were only bath applied may have had similar agonist effect, we have not characterized the nature of the blockade mediated by any of these drugs. Pharmacology will be reexamined by establishing dose-response curves of DA and 5-HT in the presence of varying concentrations of test drugs. In addition, the effects on membrane potential and conductance will be assessed by applying the test drugs by micropipette. The 5-HT response of TCX11 appears similar to that on a number of autonomic neurons (e.g., Wallis, D. J. and North, R.A. Neuropharm. 17: 1023, 1978) and on several neuroblastoma clones and neuroblastoma x glioma hybrids (MacDermot, J. et al. Proc. Natl. Acad. Sci. USA 76: 1135, 1979). It is impossible to know how similar the 5-HT receptors are on these various preparations until more detailed pharmacology is performed on all of them.

2037 MICROIONTOPHORETIC STUDIES WITH L-DOPA AND SOME PUTATIVE NEUROTRANSMITTERS ON THE NEURONS OF CAUDATE NUCLEUS OF RAT. J. N. Sharma* and Stanley Fahn (SPON: A.S. Perumal) Department of Neurology, College of Physicians and Surgeons, Columbia University, New York, New York 10032

To investigate the mechanisms of action of L-dopa in Parkinson's disease the effects of microiontophoretic application of L-dopa and its metabolites and dopamine (DA) were studied on spontaneously firing and on glutamate-induced excitatory responses of caudate nucleus (CN) neurons. It was observed that while DA had only inhibitory effect and blocked the glutamate excitatory responses, L-dopa at lower dosage potentiated, whereas at higher dosage blocked the glutamate responses. The interaction between two metabolites of L-dopa, i.e. 3-O-methyldopa and 4-O-methyldopa on glutamate excitatory responses was also studied. Like L-dopa, 3-O-methyldopa, though at lower dosage potentiated the glutamate responses, at higher dosage failed to modify the same. The other metabolite, 4-O-methyldopa, did not have any effect on glutamate responses. On the other hand 3-O-methyldopamine and 4-O-methyldopamine had only blocking effect on the glutamate excitatory responses. The results suggest that L-dopa does not only act by being converted to DA at nigrostriatal nerve terminals, but may also be having its own pharmacological effects one of which may be modification of glutamate responses which has been suggested to be an excitatory neurotransmitter at the cortico-caudate nerve terminals. It is possible that the present observation may be related to the "on-off" phenomenon of patients on chronic L-dopa therapy for Parkinson's disease. Furthermore, the metabolites of DA though less potent also appear to have significant and predominantly inhibitory role in the functioning of CN. It is postulated that the CN neurons have excitatory and inhibitory modulations by cortico-caudate and nigro-striatal neuronal pathways which are glutaminergic and dopaminergic in nature.

2038 IMMUNOCHEMICAL STUDIES OF ANTI-CATFISH GAD IgG. Y. Y. Thomas Su*, Jang-Yen Wu and Dominic M. K. Lam*. (Spon: D. D. Louie) Baylor College of Medicine, Houston, TX 77030

L-Glutamic acid decarboxylase from catfish brain has been purified to homogeneity by the combination of ammonium sulfate fractionation, gel filtration, calcium phosphate gel and preparative polyacrylamide gel electrophoresis. The purity of the enzyme was established by showing that in several gel electrophoresis systems the enzyme migrated as a single band which contained all the enzyme activity. The antibody against purified enzyme was obtained from rabbit after injection of 80 µg of the enzyme. Double immunodiffusion test using this antibody against crude extract showed a sharp precipitin band. The precipitin band from this study showed enzyme activity. The inhibition of the enzyme by antibody was studied. About 65% of the enzyme was inactivated after 4 days incubation of 12 µg of enzyme with 19.2 µg of antiserum. The inhibition of the same amount of the enzyme by 1.64 µg to 19.2 µg of antiserum increased from about 2% to 65% after 4 days of incubation. The enzyme activity presented in precipitate,however, increased to about 80% of the total enzyme activity remained in the reaction mixture. Microcomplement fixation of the antibody and the enzymes from different species were also studied.

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2039 NOVEL GABA RECEPTORS REVEALED ON CULTURED NEURONS. M.K. Ticku, J.L. Barker, J.F. MacDonald and A. Huang*. Dept. of Pharmacology, University of Texas Health Center, San Antonio, Texas; Lab. of Neurophysiology, NINCDS, NIH, Bethesda, Md. 20205.

Neuroblastoma-glioma hybrid cells (108CC15) respond to iontophoretically applied GABA with a rapidly depolarizing, rapidly desensitizing excitatory response which is insensitive to conventional GABA antagonists. To test for the presence of GABA receptors on these cells we measured GABA binding to these cells using centrifugation assays. Binding was measured under Na^+-free conditions, after freeze-thawing and extensive washing of the particulate fraction of homogenates of the hybrid. Specific binding, determined as the difference in the amount bound in the absence and presence of 100 M GABA, represented 80% of the total binding. GABA binding was rapid, reaching equilibrium within five minutes. Binding was saturable and Scatchard analysis revealed the presence of two classes of binding site. The high affinity site had a Kd of 10 nM and binding capacity of 200 fmoles/mg protein, while the low affinity site had a Kd of 63 nM and binding capacity of 500 fmoles/mg protein. Tritated GABA binding was displaced by agonists like GABA, muscimol, isoguvacine and THIP, but very poorly by (+) bicuculline and not by picrotoxin or GABA uptake blockers like nipecotic acid.

The agonist specificity suggests the presence of post-synaptic GABA-like receptors on this clone line. The inability of conventional GABA antagonists to block pharmacologic responses or to displace specifically bound GABA suggest that there may exist a subpopulation of GABA receptors coupled to membrane conductances that are functionally excitatory.

2040 CYCLIC AMP ACCUMULATION IN INTACT CELLS: FACTORS DETERMINING THE DOSE-RESPONSE CURVE FOR AGONISTS. Giancarlo Tonon* and G. Alan Robison. Dept. of Pharmacology, U. of Texas Med. School, Houston, TX 77025.

The accumulation of cyclic AMP (cAMP) in cultured BHK cells in response to β-adrenergic agonists is being studied as a model system for the study of neurotransmitter action in general. Using the adenine prelabeling technique, the following points have been established.

1. In the presence of methylisobutylxanthine (MIX), the level of cyclic AMP rises rapidly in response to epinephrine (EPI) or isoproterenol (ISO), reaching a peak at the same time regardless of the concentration of agonist, during which time the cells become tachyphylactic.

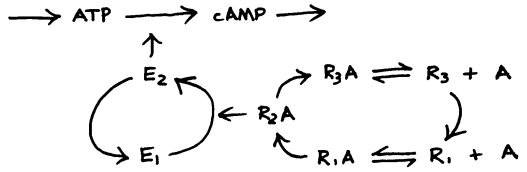
2. Increasing MIX from 0 to 2.0 mM inhibits both PDE and the rate of efflux, and predictably shifts the peak response to the right.

3. The dose-response curve depends not only on the number of agonist molecules per cell and the volume in which these molecules are dispersed, but also in an important way on when the response is measured. At higher agonist concentrations the relationship between dose and response is actually reversed when the response is measured at later times, i.e., the dose-response curve is bell-shaped.

4. The equilibrium binding curve for agonists is shifted several orders of magnitude to the right of the dose-response curve, even though there is no evidence in these cells for the existence of spare receptors.

5. There is a 15 to 30 second lag between the time a supramaximal concentration of propranolol is added and the time the effect can be observed, when the antagonist is added after the agonist.

These and other observations can be understood in terms of a recently developed model (Federation Proc. 38: 532, 1979), the general features of which are shown below.



The general features of this model should be applicable to neuronal and glial cells as well as to fibroblasts, and may be applicable to responses other than the accumulation of cyclic AMP resulting from the activation of adenylyl cyclase.

2041 HISTAMINE: RECEPTORS AND NEURONAL DISTRIBUTION IN MAMALIAN CENTRAL NERVOUS SYSTEM. Vinh T. Tran, Raymond S.L. Chang and Solomon H. Snyder, Dept. Pharmacol., Sch. Med., Johns Hopkins Univ., Baltimore, MD 21205

Histamine H_1-receptors have been successfully labeled in mammalian CNS with [^{3}H]mepyramine (Tran et al., PNAS 75, 6290, 1979). The ability to label the receptor biochemically allows us to study the potential sites of action of histamine. The receptors have wide spread distribution in mammalian peripheral organs. For examples, there are abundant histamine receptor sites in the bronchial tubing and the adrenal medulla. Histamine is a potent mediator of bronchial constriction and releases epinephrine from the adrenal medualla. We have found an heterogeneity of histamine H_1-receptors. Drug specificities differ significantly at H_1-receptors in varying organs and different animal species. Regional variations of H_1-receptors differ in several species and correlate poorly with endogenous histamine levels. To visualize histamine neurons we have purified histidine decarboxylase to apparent homogeneity, and inoculated rabbits and guinea pigs for antibody preparation and immunohistochemical studies. (Supported by USPHS grants MH-18501 and DA-00266 as grants of The John A. Hartford and McKnight Foundations).

2042 DRUG-INDUCED ALTERATIONS IN PLASMA CONCENTRATIONS OF DOPAMINE AND ITS METABOLITES. Glen R. Van Loon, Nathan Appel* Chul Kim* and Doris Ho*. Department of Medicine and Physiology, University of Toronto, Toronto, Ontario, Canada.

The source and significance of dopamine (DA) in plasma remain unknown. Furthermore, the sources of circulating dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), the major metabolites of DA, are also unknown. Although circulating DA and its metabolites may come from peripheral nerve endings, it has been suggested that experimentally-induced alterations in brain DA metabolism may be parallelled by similar changes in plasma concentrations of DA and HVA. In order to investigate this problem further, we examined in 8 fasted (18 hrs.) male dogs the plasma DA, DOPAC and HVA responses to several drugs known to alter DA metabolism. An indwelling cannula for drug administration and blood withdrawal was inserted in a forelimb vein 90 min. prior to drug administration. After each sampling (7 x 3 ml over 3 hrs.), blood was replaced by an equal volume of saline. The dogs lay quietly throughout the experiments. DA was assayed by a radioenzymatic method and DOPAC and HVA by a gas chromatographic method. Basal concentrations of plasma DA, DOPAC and HVA in dogs are 36 ± 2 pg/ml, 9.6 ± 0.6 ng/ml and 9.0 ± 0.5 ng/ml respectively. The following table summarizes the plasma DA, DOPAC and HVA responses to administration of drugs thought to alter transmission in DA neurons. The drugs examined included haloperidol, a DA receptor antagonist; pimozide, another putative DA receptor antagonist, administered in a much smaller dose but which blocked the emetic effect of bromocriptine; bromocriptine, a putative DA receptor agonist.

Drug and Dosage (mg/kg)	DA	DOPAC	HVA
Haloperidol (0.5)	NC	NC	↑
Pimozide (0.025)	NC	↓	NC
Bromocriptine (0.015, then 0.03)	↓	↓	↑then↓
Pimozide (0.025) after bromocriptine (0.015)	NC	↓	↓
Bromocriptine (0.015) after pimozide (0.025)	NC	↑	NC

NC = No change ↑ = Increase ↓ = Decrease

These data are consistent with actions of pimozide and bromocriptine as mixed agonist-antagonists at DA receptors. Clearly, plasma measurements of DA, DOPAC and HVA may be used to monitor the effects of such drugs on DA metabolism. However, precise sites and mechanisms of action of these drugs in altering plasma DA, DOPAC and HVA remain undefined.

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2043 DEVELOPMENT OF GABAnergic FUNCTION IN HIPPOCAMPAL CELL CULTURES. Charles R. Walker* and John H. Peacock (SPON: K. A. Kelts). Dept. of Neurology, Stanford Univ. Med. Sch., Stanford, CA 94305.

Inhibitory synaptic function is mediated by γ-aminobutyric acid (GABA) in the intact hippocampus. Similarly, it is very likely that GABA mediates the abundant inhibitory synaptic activity recorded in hippocampal cell cultures. Thus we have undertaken to determine when GABAnergic function occurs in mouse hippocampal cultures and to examine that function during the first 2 weeks in culture (equivalent postnatal animal age, 2-14 days).

Intracellularly recorded responses to GABA iontophoresis (0.5-1 M, pH 5) occur as early as 2 days after plating and most cells respond to GABA between days 6-14. Although responses can be elicited from distal processes, the response amplitude from processes is smaller than that recorded from the soma and proximal primary trunks. In older cultures, GABA application to the soma has been found to reversibly silence ongoing spontaneous activity.

Rapid desensitization to GABA occurs in some neurons and can mask the true magnitude of the response which ranges between 20-300 mV per nanocoulomb at a membrane potential hyperpolarized to about -100 mV. Desensitization occurred in 19/33 cells when tested with long GABA currents maintained for seconds to minutes.

GABA responses are usually positive at resting potentials of -70 mV but occasionally negative responses occur. Reversal potentials to GABA can be demonstrated with both pulse and steady iontophoresis at membrane potentials between -30 to -55 mV. During steady GABA application, membrane conductance to test electrical pulses increases 50-60% over a -20 to -100 mV membrane potential range.

The hippocampal cultures have Na^+ dependent ^{3}H-GABA uptake that is neuronally specific as shown by autoradiography and by use of the inhibitors DABA and β-alanine. The presence of Na^+ independent ligand binding has been found in culture with intact cells using both ^{3}H-GABA and ^{3}H-muscimol. The K_D for muscimol binding is approximately 2 nM but the kinetics for ^{3}H-GABA have been difficult to determine because of rapid dissociation of GABA from the receptor.

In sum, GABA receptors are present and functional at early times in culture. The diversity of GABA responses in terms of geographical distribution, polarity, and desensitization may reflect differences in cell type or differentiated function in these young cultures and pose questions for further study. (Supported by NIH grants NS 12151 and NS 07012).

2044 GABA INDUCED POTENTIATION OF NOREPINEPHRINE-STIMULATED CYCLIC GMP ACCUMULATION IN RAT PINEAL GLANDS. Robert A. Waniewski and Amin Suria. Dept. of Pharmacology, George Washington Univ. Medical Center, Washington, D.C. 20037.

Endogenous levels of GABA in the rat pineal gland are about 2 x 10^{-5}M and can be elevated to 4 x 10^{-4}M by inhibition of GABA-transaminase with amino-oxy acetic acid. Autoradiographic localization and uptake studies have demonstrated that GABA is found entirely within glial cells in the rat pineal gland. To determine the functional role of GABA in this tissue, the effect of GABA on pineal cyclic nucleotides was examined. Adenylate cyclase (AC) activity was measured in pineal gland homogenates. GABA at 10^{-4} M had no effect on basal or isoproterenol (2 x 10^{-5} M) stimulated AC activity. Rat pineal glands were made supersensitive by bilateral superior cervical ganglionectomy. Basal and isoproterenol-stimulated AC activity was measured in the pineals from these rats after 4 weeks. Again, GABA had no effect on AC activity in these supersensitive pineals.

Cyclic AMP and cyclic GMP levels were measured in intact pineals incubated in Kreb's bicarbonate buffer containing a phosphodiesterase inhibitor, isobutyl methylxanthine (IBMX) for 30 minutes. GABA from 10^{-6} to 10^{-3} had no effect on cyclic GMP levels. Similarly, no change in cyclic AMP levels after 10^{-4} M GABA was observed. However, GABA potentiated the increase in cyclic GMP produced by 10^{-5}M norepinephrine (NE) in a dose-related fashion between 10^{-6} and 10^{-4}M. At 10^{-4} M, GABA significantly increased cyclic GMP levels from 170.1 ± 55.8 picomoles /mg protein with NE alone to 477.0 ± 126.4 picomoles/mg protein in the presence of 10^{-5}NE. GABA produced no effect on NE-stimulated cyclic AMP levels in these same pineals. The potentiation of NE-induced accumulation of cyclic GMP by GABA was also dependent upon the concentration of NE used. GABA evoked a potentiation of cyclic GMP accumulation only in the presence of NE concentrations producing submaximal stimulation (2 x 10^{-6} and 10^{-5}M). These results suggest that endogenous levels of GABA in the pineal gland, presumably of glial origin, may enhance the increase in cyclic GMP elicited by NE. Thus, GABA possibly may play a role in the regulation of NE release from the sympathetic nerve terminals in the rat pineal gland. Interestingly, ascorbic acid (1%) prevented the rise in cyclic GMP produced by 10^{-5} NE. (Supported by NIMH grant #MH 30024-03)

2045 INHIBITION OF [^{3}H]-SPIROPERIDOL BINDING TO RAT STRIATAL MEMBRANES BY S-(+)-APOMORPHINE - COMPARISON WITH R-(-)-APOMORPHINE, (+)-BUTACLAMOL, AND (-)-BUTACLAMOL. R.E. Wilcox, M.E. Goldman*, J.A. Anderson*, P.J. Davis*, S. Seyhan*, R.V. Smith*, and W.H. Riffee* College of Pharmacy, University of Texas, Austin, TX 78712

R-(-)-Apomorphine (-APO) is the focus of much current interest because of its potential use in ameloriating the symptoms of tardive dyskinesia, Huntington's disease, Gilles de la Tourette's syndrome, and schizophrenia as well as its documented value in the treatment of Parkinsonism. These effects appear to be associated with the specific binding of -APO to dopamine receptors of the central nervous system.

Recently, we have prepared S-(+)-apomorphine (+APO), verified to be both > 99% enantiomerically pure and 99% chemically pure (Davis et al., submitted) and have now determined its percent inhibition (IC_{50}) of specific [^{3}H]-spiroperidol ([^{3}H]-SPIRO) binding to rat striatal membranes using the basic procedure of Creese (Creese et al., 1977). IC_{50} values were also established in the same tissue for inhibition of 0.15 nM [^{3}H]-SPIRO by -APO, (+)-butaclamol (+BUT), and (-)-butaclamol (-BUT) as controls since these have been documented in the literature (cf. Fields et al., 1977). In accord with the literature, IC_{50} values were determined to be 10^{-6}M for -APO, 10^{-9}M for +BUT, and 10^{-5}M for -BUT. The IC_{50} value for +APO was found to be 10^{-6}M, suggesting that +APO and -APO are equipotent in inhibiting [^{3}H]-SPIRO binding. Relative potencies of +APO vs. -APO are being further explored via inhibition of [^{3}H]-APO binding and by behavioral studies to account for this apparent lack of stereoselectivity. [Supported by grants NS-06114 (REW) and NS-12259 (RVS) from the National Institute of Neurological and Communicative Disorders and Stroke.]

2046 EFFECTS OF LITHIUM ON EXCITATORY RESPONSES TO SEROTONIN AND DOPAMINE IN APLYSIA. A. M. Williamson*, T. C. Pellmar, and D. O. Carpenter. Neurobiology Department, Armed Forces Radiobiology Research Institute, Bethesda, MD 20014.

Aplysia californica neurons can exhibit similar ionic conductance increases to several neurotransmitters. That has led to the suggestion that there are common ionophores for each class of ionic response (Swann and Carpenter, Nature 236: 751, 1973). If the ionophores mediating the fast sodium conductance increases in response to serotonin and dopamine are identical, then these ionophores should respond similarly to sodium substitutes. Since a different ionophore probably underlies the slow sodium conductance increase to serotonin, A' response of Gerschenfeld and Paupardin-Tritsch (J. Physiol. 243: 427, 1974), sodium substitutes might act differently on this response.

We tested the effects of substituting for 50% and 100% of the sodium in artificial seawater with lithium (Li-seawater) on the excitatory responses to iontophoretically applied serotonin and dopamine. Preliminary results suggest that the slow excitatory response to serotonin is reduced in amplitude and prolonged by 50% Li-seawater. There is a further reduction of amplitude in 100% Li-seawater, but the response was not completely abolished. The reduction in amplitude was partially reversible. The fast conductance increases due to serotonin and dopamine were also reduced in amplitude at both lithium concentrations. Those responses were not prolonged by lithium. Normal seawater partially reversed the reduction in amplitude of the responses to both neurotransmitters.

The observation that lithium affects the fast excitatory responses to dopamine and serotonin in a similar manner supports the hypothesis that they are mediated by the same ionophore. Since lithium acts differently on the slow excitatory response to serotonin, a different ionophore may be involved.

2047 RESPONSES OF MORPHOLOGICALLY IDENTIFIED MAMMALIAN, NEOCORTICAL NEURONS TO ACETYLCHOLINE (ACh), ACECLIDINE (ACec), AND CYCLIC GMP (cGMP). C. Woody, H. Sakai*, B. Swartz*, M. Sakai* and E. Gruen*. Departments of Anatomy & Psychiatry, Brain Research Institute, Mental Retardation Research Center, UCLA Med. Center, Los Angeles, CA 90024.

Effects of extracellular application of ACh (2M, 40-400nA, 30 sec), and ACec (1M, 40-50nA, 30 sec) and intracellular application of cGMP (5μm-5mM, 60-80 psi, 0.5-2 sec) were studied in a total of 34 units recorded from the coronal-pericruciate cortex in 26 cats. Unit activity and input resistance were evaluated for periods from 30 sec to 22 min after drug application (for technical details, see Woody et al. Br. Res. 158: 373-395, 1978). 4% HRP was injected intracellularly, coincidental with application of cGMP and at the conclusion of the experiments in which ACh and ACec were applied. (See Sakai et al. Exp. Neurol. 58: 138-144, 1978).

Of 9 cells given ACh or ACec, 6 showed increases in resistance and 3 did not. Of 22 cells given cGMP, 12 showed increases in resistance and 10 did not. Of 3 cells given ACh or ACec and cGMP, 1 showed increases in resistance to both agents and 2 showed no response to either agent. 11 cells responding to ACh, ACec, or cGMP with an increased resistance were identified morphologically as pyramidal shaped cells in layers V (n=6) and VI (n=5). Two additional responsive cells had dendrites in layer V and were antidromically activated by PT stimulation. The other responsive cells had only dendrites stained by HRP. The magnitude of increase in resistance in the responsive cells averaged more than twice their initial mean input resistance. The resting potentials of all cells averaged 45 mV. Their action potentials averaged 36 mV. These and initial values of input resistance did not differ significantly between responsive and unresponsive cells.

Results of further studies in which HRP alone or HRP plus 0.1mM cyclic AMP were injected indicate that the increases in resistance described above were not attributable to mechanical effects of pressure injection. Earlier studies of iontophoretic application of ACh, cGMP and 5' GMP indicate that the effects are attributable to the pharmacologic action of these agents when applied to cells of the mammalian sensorimotor cortex. (Supp. by HD 05958 and AFOSR 76-3074.)

2048 BICUCULLINE DISPLACES ^{3}H-MUSCIMOL AND NA-INDEPENDENT ^{3}H-GABA BINDING IN CULTURED MAMMALIAN SPINAL CORD AND FOREBRAIN NEURONS. A.B. Young and R.L. Macdonald. Department of Neurology, University of Michigan Medical Center, Ann Arbor, MI 48109.

The neutral amino acid γ-aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the central nervous system and has a wide distribution in brain as well as in spinal cord. Electrophysiological and neuropharmacological studies using varied in vivo and in vitro preparations have suggested that many convulsant and anticonvulsant drugs interact with GABAergic systems to produce their pharmacological effects. Of particular interest is the relationship between convulsant-induced paroxysmal activity and antagonism of GABA-mediated inhibition. To approach the mechanism of action of these agents, we have used primary dissociated neuronal cultures derived from fetal mouse forebrain and spinal cord to investigate the acton of convulsant and anticonvulsant drugs on the uptake, release and binding of putative amino acid neurotransmitters. Such studies permit direct comparison of neurochemical and electrophysiological data using the same mammalian preparation. We report here that the convulsant alkaloid bicuculline displaced sodium independent GABA-binding in both brain and spinal cord cultures but that the ED_{50} for GABA-displacement was substantially different in the two culture systems.

Spinal cords and forebrains were removed from 13-14 or 14.5-15.5 day old fetal mice respectively, mechanically triturated and plated in 35 mm collagen-coated culture dishes. Spinal cords were plated at 1/2 cord/dish while forebrains were plated at 1/3 hemisphere/dish. At three to eight weeks, the cultures were suspended in 50 mm tris-citrate buffer at room temperature. Following addition of triton, homogenization and incubation for 30 min at 37°, the membranes were centrifuged at 48,000g for 20 minutes. The membranes were then twice resuspended in buffer, homogenized and centrifuged. GABA receptors were measured by ^{3}H-muscimol binding or Na-independent ^{3}H-GABA binding. Binding was displacable with GABA, imidazole acetic acid and muscimol but not by glycine or 2,4 diaminobutyric acid (a GABA uptake inhibitor). Bicuculline displacement of GABA-binding had an ED_{50} of 5-7μM in brain cultures. In spinal cord, the ED_{50} for bicuculline was more variable but appeared to be between 20 and 100μM. To confirm these data, we performed similar experiments on tritonized adult rat brain and spinal cord membranes and report that the ED_{50} for bicuculline displacement of GABA-binding was 5 and 40μM respectively. Thus these studies demonstrate that the convulsant alkaloid bicuculline has different effects on brain and spinal cord receptors both in vitro and in vivo. These differences determined biochemically in vitro have been compared to electrophysiological studies of bicuculline-induced paroxysmal discharges using the same preparation (see abstract of Macdonald, Young and Nowak).

2049 LIGHT MICROSCOPIC AUTORADIOGRAPHIC LOCALIZATION OF BENZODIAZEPINE RECEPTORS IN MAMMALIAN BRAIN. W. Scott Young, III, Mary K. Conrad*, and Michael J. Kuhar. Dept. Pharmacol., Johns Hopkins Univ. Sch. Med., Balt., Md. 21205.

We have recently described a novel technique for demonstrating, autoradiographically at the light microscopic level, receptors for diffusable drugs and neurotransmitters. We have used this method to map the distribution of ^{3}H-flunitrazepam (FLU) receptors in the rat and human central nervous system.

Binding of ^{3}H-FLU in 4-10μ slide-mounted cryostat sections at 2°C reaches equilibrium by 40 min and exhibits a half-time of dissociation of 19 min. The binding is saturable (K_D=2.79nM, B_{max}=210 fmoles/mg tissue), is inhibited by benzodiazepines (BZ), and shows appropriate stereospecificity. The binding of ^{3}H-FLU is stimulated by GABA and chloride ion. This method is quantitative as the autoradiography is linear with time of exposure and tissue radioactivity.

Autoradiography of ^{3}H-FLU (1nM for 40 min at 2°C with 10 min wash in Tris-HCl, pH 7.4) reveals striking variations in BZ densities in the rat brain. Highest levels of BZ receptors are found in the frontal cortex (especially the frontal poles), lamina molecularis bulbi olfactorii, insulae Calleja, medial forebrain bundle, nucleus entopeduncularis, nuclei amygdaloideus medialis and lateralis (pars posterior), parts of the hippocampus and basal hypothalamus, caudal zona incerta, nucleus subthalamicus, nucleus ventralis rostralis lemnisci lateralis, substantia nigra (dorso-lateral pars reticulata), molecular layer of the cerebellum, inferior colliculus, nuclei tegmenti dorsalis and ventralis of Gudden, and the substantia gelatinosa of nuclei trigemini and of the dorsal horn of the spinal cord. Other areas show lower levels of receptors. White matter areas display negligible binding of ^{3}H-FLU. Heterogeneity of receptor distribution was also seen in human tissue. Sections incubated in the presence of 1μM clonazepam or diazepam had no binding.

This procedure offers high resolution and should provide greater understanding of the action of BZ's. Similar studies are currently in progress on a number of receptors, including those for α-adrenergic, serotonergic, opioid and neurotensin drugs.

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2050 GTP AND CATION EFFECTS ON RAT STRIATAL DOPAMINERGIC AND MUSCARINIC CHOLINERGIC RECEPTORS. Nancy R. Zahniser and Perry B. Molinoff. Dept. of Pharmacol., Univ. of Colo. Med. Ctr., Denver, CO 80262

Guanine nucleotides and monovalent cations decrease the apparent affinities of agonists for striatal dopamine receptors. In the presence of GTP both the amount of ^{3}H-apomorphine bound as well as the potency of agonists measured by inhibition of ^{3}H-spiroperidol binding to rat striatal membranes were decreased. These GTP dependent decreases in the apparent affinities of agonists for the dopamine receptor are due to an increase in the rate of dissociation of agonists from the receptor. At 15°C the half-time of dissociation of the partial agonist ^{3}H-apomorphine from dopamine receptors in rat striatal membranes was approximately 25 minutes. The addition of 0.3 mM GTP to the incubation medium caused a 3-4 fold increase in the rate of ^{3}H-apomorphine dissociation. At 37°C the half time of dissociation was 2 minutes in control membranes. Thus, in the presence of GTP little specific ^{3}H-apomorphine binding was measurable at 37°C. The presence of monovalent cations (30-150 mM) caused a 2-fold decrease in the ability of agonists or partial agonists to inhibit ^{3}H-spiroperidol binding. The mechanism by which monovalent cations produce their effects has not yet been established. It is interesting to note that the agonist-specific effects of GTP were observed either in the presence or absence of Na^+. Similar effects of monovalent cations were seen in studies of agonist inhibition of ^{3}H-QNB binding to muscarinic cholinergic receptors in rat caudate. GTP, in the presence or absence of monovalent cations, however, had no effect on either the affinity of ^{3}H-QNB or the inhibition of ^{3}H-QNB binding by muscarinic agonists or antagonists. Neither purine nucleotides nor monovalent cations had any effect on the binding properties of dopaminergic or muscarinic cholinergic receptor antagonists. On the other hand, divalent cations increased the affinities of agonists and decreased the affinities of antagonists for both dopaminergic and muscarinic cholinergic receptors. The results of this study together with those from other laboratories support the hypothesis that GTP affects agonist binding to receptors which are linked to adenylate cyclase. Since monovalent cation effects are seen with receptors that are and are not sensitive to GTP, these results suggest that the mechanism by which GTP and monovalent cations produce agonist-specific effects is not the same.

This work was supported by the USPHS (NS 09199) and by an NIH fellowship (NS 055970).

PAIN

2051 DEAFFERENTATION: EFFECTS OF TOOTH PULP EXTIRPATION ON TRIGEMINAL BRAINSTEM NEURONS. G.J. Ball, J.O. Dostrovsky, J.W. Hu and B.J. Sessle. Fac. of Dentistry, Univ. of Toronto, Canada, M5G 1G6.

Recent studies have shown that deafferentation can lead to marked structural and physiological changes in central neuronal organization that have been related to neurological disorders associated with sensory loss. Since it has recently been reported that partial tooth pulp extirpation results in transneuronally induced changes in neurons of the trigeminal (V) brainstem sensory nuclear complex, we wished to determine if the functional properties of V brainstem neurons change accordingly. The tooth pulp also offers an added incentive for such a study since it is predominantly, if not completely, associated with sensory experiences related to pain, and so the central changes induced by removal of an almost exclusive nociceptive sensory input could be explored.

We have initially investigated the effect of aseptic removal of the coronal pulp of the ipsilateral mandibular canine, premolar and molar teeth on the functional properties of single neurons recorded in the V spinal tract nucleus 13-28 days following the pulp extirpation. These recordings were made in adult chloralose-anaesthetized cats and were primarily, although not exclusively, directed at neurons in subnucleus oralis. Neuronal properties in the ipsilateral nucleus were compared with those examined in the contralateral nucleus of the same animal and in the nucleus of other animals which were not subjected to pulp extirpation. These properties included adequate stimulus, receptive field size, von Frey hair threshold, somatotopic pattern, spontaneous activity, inhibitory influences from afferent or nucleus raphe magnus sources, and short-latency excitatory inputs resulting from bilateral electrical stimulation of the remaining canine and premolar pulps, infraorbital and superior laryngeal nerves, and facial skin. Some of these functional properties were modified in neurons ipsilateral to the pulp extirpation. The most obvious changes included an increase in spontaneous activity that often had an erratic bursting character, an increase in receptive field size and number of excitatory inputs, and a partial disruption of the normal "inverted-face" somatotopic pattern of the nucleus; no obvious difference was noted in the inhibitory influences studied, or in the properties of V primary afferents in the nucleus. Most of these changes occurred in neurons located in the dorsal one- to two-thirds of the nucleus, and indicate that considerable functional changes may occur in V brainstem neurons as a result of a partial loss of sensory input from the tooth pulp.

2052 MORPHINE TOLERANCE DECREASES ANALGESIA PRODUCED BY INJECTION OF GLUTAMATE INTO THE PERIAQUEDUCTAL GRAY OF THE RAT. Michael M. Behbehani. Dept. Physiol., Coll. Med. U. Cincinnati, Cincinnati, Ohio 45267

The analgesic action of morphine is in part mediated through the activation of a descending pathway. Major components of this pathway include the periaqueductal gray (PAG) and the nucleus raphe magnus (NRM). It has been shown that injection of glutamate into the PAG increases the firing rate of these cells, leads to an increase in the firing rate of cells in the NRM and produces analgesia (Behbehani & Fields, Brain Res. 1979). To determine the effect of chronic morphine treatment on the analgesia produced by injection of glutamate into the PAG, rats were made dependent on morphine by implanting them with two pellets, each containing 75 mg of morphine. After 72 hours, the animals were anesthetized with urethane and single cells were recorded from the NRM. Glutamate (1µl,50mM) was injected into the PAG with a microsyringe. The analgesic effect of glutamate was measured by recording the EMG from the flexor muscle of a hind leg illicited by noxious heat. The result shows that glutamate injected into the PAG of morphine tolerant animals does not produce analgesia. However, if the animal is pretreated with naloxone and then glutamate is injected into the PAG, it does produce analgesia. In morphine tolerant animals, injection of glutamate into the PAG produces a slight increase in the firing rate of cells in the NRM. When naloxone is iontophoresed near the site of recording in the NRM, it causes a slight increase in the firing rate of these cells. However, after this treatment, injection of glutamate into the PAG produces a significant increase in the firing rate of the NRM cells. In order to determine the effect of glutamate in unanesthetized animals, animals were implanted with an indwelling cannula and after recovery, tail flick latency was measured before and after injection of the glutamate (1µl 50mM). Glutamate injection caused a significant increase in tail flick latency. After this measurement, the animal was implanted with two morphine pellets. 72 hours later, glutamate injection did not produce analgesia but 10 min. after naloxone injection (0.08mg/kg i.p.) glutamate produced significant analgesia. These results suggest that chronic morphine treatment decreases the stimulus induced release of neurotransmitter involved in the interaction between the PAG and the NRM.

2053 ASCENDING AND DESCENDING PROJECTIONS TO THE PERIAQUEDUCTAL GRAY OF THE RAT. Alvin J. Beitz. Dept. Anat., Univ. of South Carolina, Sch. Med., Columbia, SC 29208.

The afferent connections to the midbrain periaqueductal gray region (PAG) in the rat were examined utilizing the technique of retrograde transport of horseradish peroxidase (HRP). Iontophoretic injections of HRP (Sigma Type VI or IX) were made into the PAG of 14 adult rats utilizing a current of 1.9µA over a period of 10-25 minutes. Following the initial fixation with 2% glutaraldehyde-0.5% paraformaldehyde and subsequent perfusion with phosphate buffer, the brains were cut, and alternate sections were reacted with tetramethylbenzidine and diaminobenzidine, respectively. All sections were then carefully scanned and the location of retrogradely labeled cells charted. The predominant descending projections to the PAG were found to arise from the ipsilateral nucleus cuneiformis, the zona incerta, the ventromedial hypothalamic nucleus, and the anterior and lateral hypothalamic areas. A substantial number of labeled cells were also observed in the ipsilateral dorsal premamillary nucleus, the pretectal area, the posterior hypothalamic area, the periventricular gray and the contralateral nucleus cuneiformis. The major ascending projections to the PAG originate from the ipsilateral dorsal parabrachial nucleus, the nucleus reticularis pontis oralis and caudalis, the nucleus reticularis pontis caudalis pars α, the subnucleus reticularis ventralis medulla oblongata, and from the contralateral nucleus prepositus and cerebellum. HRP-labeled neurons were also found in the raphe magnus, the raphe pontis, the pedunculopontine nucleus, the nucleus reticularis gigantocellularis, the substantia nigra and the trigeminal complex. The results of this study indicate that the PAG receives its most predominant input from the hypothalamus and the zona incerta. These two structures may thus, influence the lower brain stem and spinal cord directly or indirectly by way of the central gray. The PAG has been implicated both as a central site of morphine's antinociceptive action and a central locus important to nociception per se. Further study is necessary to determine if the ascending input to the PAG from the reticular formation, the raphe nuclei and the trigeminal complex are involved in this aspect of PAG function.

2054 NEONATAL HYPOTHALAMIC DEFICITS REDUCE ANALGESIA INDUCED BY STRESS AND OPIATES. Richard J. Bodnar, Earl A. Zimmerman, Gary M. Abrams*, Alfred Mansour*, Lucy W. Thomas* and Murray Glusman. New York State Psychiatric Institute and Columbia University College of Physicians and Surgeons, New York N.Y. 10032.

Pain threshold elevations in rats occur following acute exposure to a wide range of severe envionmental stressors. The anti-nociceptive effects of several stressors appear to act independently of the endogenous opioid pain-inhibitory system, but can be altered by deficits in hypothalamic and pituitary function. The present study examined the role of discrete hypothalamic mechanisms in mediating stress-induced analgesia by through the use of selective, non-invassive techniques. In the first experiment, six rats were exposed neonatally to monosodium glutamate (MSG-2mg/g) while six littermate controls received placebo. One hundred and five days later, both groups were tested for basal reactivity to foot shock as well as their analgesic responses to cold-water swims, morphine, food-deprivation and 2-deoxy-D-glucose. MSG treatment altered shock detection but not basal nociceptive thresholds, while producing selective analgesic deficits. The MSG rats failed to display cold-water swim analgesia and exhibited an attenuated analgesic response to morphine. Yet normal analgesic responses were observed following food deprivation and 2-deoxy-D-glucose. In addition to the known deficits in arcuate dopamine and choline acetyltransferase, subsequent immunocytochemistry revealed that there was a marked, loss of arcuate cells containing ACTH and B-lipotropin in MSG rats. In the second experiment, homozygous Brattleboro rats which exhibit diabetes insipidus caused by an absence of vasopressin were examined for basal reactivity to foot shock. In addition the analgesic responsiveness to cold-water swims, morphine and food-deprivation was ascertained. The Brattleboro rats displayed hypersensitive basal nociception and were not analgesic following cold-water swims. Normal analgesic responses were observed following morphine and food deprivation. While peripheral administration of the vasopressin analogue DDAVP reversed the diabetes insipidus and increased basal nociception, it failed to alter the deficit in cold-water swim analgesia. Also, DDAVP did not alter morphine or food deprivation analgesia. These data indicate that the integrity of hypothalamic systems are essential for the full expression of both opiate and non-opiate analgesia. (Supported by NIH Grants #NS 14449, AM 20337 and N.Y.S. Health Research Council Grants #922 and 1518.

2055 PROLONGED ANALGESIA IN RATS AFTER ZINC TANNATE SALTS OF HEROIN, HYDROMORPHONE OR LAAM. B. Brands*, J.C. Baskerville*, M. Hirst and C.W. Gowdey*. Depts. of Pharmacology and Mathematics, Univ. Western Ont., London, Canada, N6A 5C1.

Studies in our laboratory have shown that a single injection of a narcotic agonist given as the zinc tannate will attenuate the severe abstinence syndrome normally seen when rats are withdrawn from repeated codeine administration. It was of interest to test the duration of analgesia induced by these compounds. The zinc tannate salts of heroin (HZT), hydromorphone (DZT) and ℓ-α-acetylmethadol (LZT) were synthesized (method of Gray and Robinson, 1974) and injected in a slow-release vehicle (Collier, 1972), subcutaneously, in adult male Sprague-Dawley rats. The aversive threshold of each rat was determined each day by means of electrical foot stimulation on a titration schedule. Only those rats which had consistent thresholds after saline injections were chosen to continue the experiments; each treatment group consisted of at least 6 rats. The aversive thresholds and body weights were measured every 24 hr after a single injection of either of 2 doses of HZT, DZT, LZT or the vehicle (SRV) alone. Analysis of variance shows that the aversive threshold was significantly increased ($\alpha = 0.05$) over that of SRV group 24 hr after the injection in all groups except low-dose HZT. Significant elevations in threshold persisted for 5 days after high-dose LZT and for 3 days after the low dose. With both doses of DZT significant increases in aversive threshold occurred for 4 successive days. Analysis of body weight changes revealed that whereas HZT rats gained as did SRV, significant decreases occurred in LZT and DZT groups, the greatest decreases occurring 2 days after administration of DZT and 3 days after LZT. These results demonstrate long-lasting analgesia after a single injection of 3 narcotic zinc tannate preparations.

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2056 PERIAQUEDUCTAL GRAY LESIONS AND NON-NARCOTIC ANTI-NOCICEPTION. Martin Brutus, Dennis D. Kelly, Murray Glusman and Richard J. Bodnar. N.Y.S. Psychiatric Inst. and Columbia University, New York, N.Y. 10032.

The ventrolateral periaqueductal gray (PAG) has been implicated in the mediation of opiate and stimulation-produced analgesia. The present study examined whether this region also subserves the antinociceptive properties of stress and select non-narcotic psychotropic drugs. Alterations in pain thresholds were determined both by an operant liminal escape procedure which reflects both an animal's evaluation of the relative aversiveness of a given stimulus and its motivation to terminate its presence, and by a reflex tail-flick test measuring reactive latency to radiant heat. In Experiment 1, twenty-one rats were tested for baseline escape thresholds and then were exposed both before and after PAG lesions to the following variables, each of which had been previously shown to induce dose- or intensity-dependent elevations in escape thresholds: chlordiazepoxide (CDP: 15 mg/kg, ip, 30 min pre-test), haloperidol (HAL: .16 mg/kg, ip, 20 min pre-test), cold-water swims (CWS: 2°c for 3.5 min, 30 min pre-test) and 2-deoxy-D-glucose (2-DG: 600 mg/kg, ip, 60 min pre-test). Appropriate placebo and control tests preceded each determination. Rostral PAG lesions (n=9) decreased significantly the escape threshold elevations induced by HAL, CWS and 2-DG without affecting either basal escape thresholds or the CDP threshold elevations. By contrast, caudal PAG lesions (n=12), which have been shown to eliminate opiate and stimulation-produced analgesia, failed to alter either basal escape thresholds or threshold elevations induced by any of these drugs or stressors. In Experiment 2, stable baseline tail-flick latencies were determined for 26 naive rats which were then subdivided into three matched lesion groups: rostral PAG, caudal PAG and sham. Following surgery, each group was retested for post-operative tail-flick latencies and for sensitivity to CDP, HAL, CWS and 2-DG. First, the anti-nociceptive effects of HAL noted on the liminal escape test were not apparent on the tail-flick test, even in sham controls. Second, rostral PAG lesions (n=6) produced similar time-dependent increases in latency as shams (n=7) following CWS, prolonged latencies following 2-DG and decreased latency elevations following CDP. Third, as with escape thresholds, caudal PAG lesions (n=12) showed normal anti-nociceptive responses to CDP, 2-DG and CWS. These data indicate that the caudal PAG, while apparently important for both opiate and stimulation-produced analgesia, plays no role in the anti-nociceptive properties of stress or psychotropic drugs.

2057 PATTERNING OF NEURONAL INTERSPIKE INTERVALS IN THE THALAMUS OF THE CAT FOLLOWING MORPHINE. L.L. Burns* and T.J. Marczynski, Dept. Med. Pharmacol., Univ. of Ill. Med. Center, Chicago, Ill. 60612.

Single unit activity was recorded chronically in the centro-median-parafascicular (CM-Pf) complex of the feline thalamus using fine wire semimicroelectrode bundles. Animals were awake and freely moving.

Morphine was injected (1.0 mg/kg IM) and produced the classic signs of feline morphine mania including desynchronized electrocorticogram, widely dilated pupils and hyperreactivity to environmental stimuli. Analysis of neuronal interspike intervals was performed with an inequality testing pattern detection technique which made statements about consecutive interspike intervals; an interval was either longer (-) or shorter (+) than the previous interval. Occurrences of patterns composed of 3 through 6 signs were tabulated and empirical frequency distributions constructed. Empirical distributions of patterns were compared with those expected from a theoretical model based on independence of sequential signs, using the chi-square test (see Brudno and Marczynski Brain Res. 125: 65, 1977; Marczynski and Burns Neurosci. Abst., 1978). Neurons showed an increase in patterning throughout the course of action of morphine (3½-4 hours). ensembles of patterns developed which were invariant during the morphine effect. In the same neurons during other behavioral states such as slow wave sleep, quiet wakefulness and bar pressing for milk, different patterns developed usually with greater variability than those observed following morphine. The onset of the patterns occurred gradually during the first 40-60 min following the injection, remained stable for 1½-2 hours, thenslowly declined. Naloxone (0.015 mg/kg) was injected 60 min after morphine in two animals. Within 5 min, the patterns so prevalent with morphine either decreased or disappeared entirely, along with the signs of morphine mania. They were absent for 15-20 min, then the same pattern ensembles reappeared in concert with the signs of morphine mania and persisted for another 1½-2 hours. All cells recorded also showed dramatic increases in their firing rates during the action of morphine. Injections of fentanyl (0.0125 mg/kg) gave similar results, but with a much shorter duration of action (30-45 min). Smaller doses of morphine (0.3 mg/kg) and fentanyl (0.00375 mg/kg) did not show these effects.

It is suggested that if the CM-Pf is an integrating area for pain, entrainment of the firing patterns of neurons by morphine may preclude transmission of impulses encoding painful stimuli and thus inhibit the integration of such stimuli. The appearance of invariant patterns may be related to morphine mania or the euphorogenic properties of the drug.

2058 LATERAL HYPOTHALAMIC GATING OF THE AVERSION PRODUCED BY STIMULATION OF NUCLEUS RETICULARIS GIGANTOCELLULARIS. Kenneth D. Carr and Edgar E. Coons*. Dept. of Psychology, New York University, New York, N.Y. 10003.

Rats were implanted with two monopolar stimulating electrodes. One was aimed at the lateral hypothalamus (LH) and the other at medullary nucleus reticularis gigantocellularis (NGC). These rats were found to leverpress for 3-second trains of LH stimulation during continuous, aversive, stimulation of NGC. Because the LH-currents used in this experiment were too low to support self-stimulation in the absence of continuous NGC stimulation, we concluded that pressing was being reinforced by LH-mediated reduction of the NGC-aversion. However, in order to control for the alternative explanation that the LH-trains had been made rewarding by summation with some component of the continuous NGC stimulation, another test was conducted. By using raised currents, rates of pressing for LH-trains in the absence of NGC stimulation were equated with those obtained by using the previous, lower, currents in the presence of continuous NGC stimulation. It was found that gastric-loading depressed responding for LH-trains in the absence but not in the presence of continuous NGC stimulation. Thus, it would seem that leverpressing for LH-trains during NGC stimulation is maintained by aversion-ameliorating effects of LH stimulation which are not identical with the reward effect.

Rates of leverpressing for LH-trains during continuous NGC stimulation are greatest when the two trains are phased so that each pulse in the 25 pps NGC train is preceded by a pulse to LH at an interval of .1-.5 or 10-15 msecs. These two peaks of inhibition in the leverpressing function suggest that this aversion-ameliorating effect represents a specific interaction between outputs of the two stimulated brain regions.

A second experiment was conducted in which the reverse of the above experimental procedure was followed. That is, rats leverpressed for 3-second periods of escape from NGC stimulation during continuous stimulation of LH. The continuous LH stimulation reduced rates of NGC-escape. Most interestingly, escape reduction was greatest when each pulse in the 25 pps NGC train was preceded by a pulse to LH at an interval of .1-.5 or 10-15 msecs.

That the same bimodal function emerged in both experiments suggests that LH-mediated reduction of NGC-escape and working for LH-trains during NGC stimulation are two behavioral reflections of the operation of a common integrative mechanism. The dissociation of LH-responding from LH-reward in the first experiment, complemented by the LH-mediated reduction of escape in the second experiment, suggests that this integrative mechanism mediates a gating of aversion.

Pharmacological analyses of this bimodal gating will be discussed. (Supported by NIMH predoctoral fellowship #5F31 MH07302)

2059 PHARMACOLOGICALLY DISTINCT SYSTEMS IN MEDIAL AND LATERAL MIDBRAIN MEDIATING DESCENDING INHIBITION OF SPINAL NOCICEPTIVE TRANSMISSION IN THE CAT. E. Carstens and M. Zimmermann. II. Physiol. Inst., Univ. Heidelberg, D-69 Heidelberg, Im Neuenheimer Feld 326 (GFR).

Electrical stimulation of both the midbrain periaqueductal gray (PAG) and lateral reticular formation (LRF) strongly inhibits spinal dorsal horn neuronal responses to noxious skin heating. The mechanisms of inhibition from these two midbrain areas appear to be functionally separate (Pflügers Arch. 377:R52, 1978). To determine if these inhibitory mechanisms are also pharmacologically distinct, we tested whether manipulations of two putative transmitter/modulator systems-endogenous opiates and serotonin- have differential effects on spinal neuronal inhibition from PAG and LRF.

Lumbar dorsal horn neurons with A- and C-fiber input from hindlimb cutaneous nerves were recorded in anesthetized cats. Units responded to noxious radiant heat stimuli (50°C, 10 sec) applied at 3 min intervals either without or during stimulation of PAG or LRF (30 Hz). Inhibition was expressed as the ratio: heat-evoked discharge during midbrain stimulation/discharge in the absence of midbrain stimulation.

If descending inhibition is mediated by endogenous opiates, blockade of opiate receptors with the opiate antagonist naloxone should reduce inhibition. Naloxone (1-3 mg/kg i.v.) has no effect on inhibition of spinal neuronal heat-evoked responses by PAG stimulation (Neurosci. Lett. 11:323, 1979). However, naloxone partially reduced inhibition by LRF stimulation in 4 of 8 units studied to date. Inhibition was never completely blocked by naloxone.

Blockade of serotonin receptors by the serotonin antagonist methysergide (0.5 mg i.v.) greatly reduced or abolished inhibition from PAG in each of 5 units. Higher doses only partially reduced inhibition from LRF in 2 of 4 units. In cats pretreated with the serotonin synthesis inhibitor para-chlorophenylalanine (PCPA, 500 mg/kg), inhibition from PAG was significantly reduced while inhibition from LRF was enhanced.

These results indicate that serotonin mediates inhibition from PAG but not LRF. Endogenous opiates may play a modulatory role in descending inhibition from LRF which, however, is mediated by some other neurotransmitter. The role of catecholamines in descending inhibition from midbrain is currently under study.

2060 NUCLEUS RETICULARIS GIGANTOCELLULARIS: A COMMON NEURAL SUBSTRATE FOR MORPHINE AND STIMULATION PRODUCED SUPPRESSION OF DENTAL PULP EVOKED TRIGEMINAL RESPONSES IN THE CAT. Samuel H.H. Chan, Dept. of Life Sci., Indiana State University, Terre Haute, IN 47809.

A parallelism has been revealed in recent years between morphine-induced and stimulation-produced analgesia. Areas along the periventricular-periaqueductal axis are generally accepted to be critically involved in these analgesic processes. The present study attempts to demonstrate that the nucleus reticularis gigantocellularis (NRGC) in the medulla is also intimately related to opiate and stimulation induced antinociceptive actions, using dental pulp evoked trigeminal responses as experimental indices.

Experiments were performed on adult cats that were either lightly anesthetized with pentobarbital sodium (30 mg/kg, i.p.) or precollicularly decerebrated. Intradental stimulation was delivered via a pair of nichrome electrodes implanted into the left upper canine. The resultant evoked field potential was recorded from the ipsilateral subnucleus oralis (oralis potential) and the jaw-opening reflex (JOR) as EMG signals from the left digastric muscle. Microinjection of drugs (at a volume of 1 µl) to the NRGC was delivered by means of a 27-gauge syringe needle which is connected to a microinjection device. Electrical activation of NRGC was induced via a stereotaxically placed bipolar concentric electrode.

Bilateral microinjection of morphine to NRGC, at a dose of 6.5 µg/kg, elicited a small, though insignificant depression of the JOR. Significant inhibition of the evoked JOR, however, resulted when a dose of morphine at 10 µg/kg was introduced, sustaining for 30-60 min. Such suppression was reversed by naloxone, either administered systemically (1 mg/kg) or microinjected bilaterally into the NRGC (1 µg/kg).

The effect of NRGC stimulation on dental pulp evoked trigeminal responses was studied using the conditioning-testing technique. NRGC activation invariably elicited almost 100% suppression of the intradentally evoked oralis potential in all animals within 5-10 msec after the beginning of the reticular activation. This was followed by gradual and varying degree of decline in inhibition. Complete restoration of the control amplitude in most cases did not take place until 500-800 msec following the reticular activation. Similar temporal effect has been observed on JOR.

The present study thus demonstrated that the NRGC is a common neural substrate for morphine and focal stimulation suppression of dentalgia. Parallel investigations in this laboratory suggest that upon activation by the opiate or electrically, neurons in NRGC may prevent the transmission of nociceptive signals from the dental pulp by promoting a depolarization of the pulpal afferents, in a process that may also involve the opiate receptors and enkephalins.

2061 REVERSAL OF AUTOANALGESIA BY YOHIMBINE. William T. Chance and Martin D. Schechter. Prog. Pharmacol., N.E. Ohio Univs. Col. Med., Rootstown, Ohio 44272.

Recent research has delineated the existence of intrinsic pain inhibitory mechanisms which may be activated by acute stress, lesion-induced hyperemotionality or intense fear. These analgesic states have been termed autoanalgesia in that the antinociception is behaviorally-induced and therefore must result from the incipient neuronal activity of endogenously-synthesized molecules. Although initial observations suggested that autoanalgesia may result from activation of an endorphin system within the CNS, additional investigations have de-emphasized endorphin modulation by demonstrating the ineffectiveness of naloxone in reversing the analgesia as well as an absence of cross tolerance between morphine and autoanalgesia. The involvement of centrifugal inhibitory pathways in autoanalgesia has been demonstrated by its obviation upon spinal cord transection, however, no pharmacological treatment has successfully antagonized autoanalgesia, as assessed by tail-flick tests. Since recent research (Koss et al., Neuropharmacology 18: 295, 1979) has indicated that yohimbine may block tonic descending inhibition, we investigated the efficacy of this drug in antagonizing autoanalgesia and morphine analgesia.

Antinociception was assessed using the rat tail-flick procedure with basal latencies of 2-3 sec. and a cut-off criterion of 8 sec. being maintained. In the first experiment (n=20), the effects of yohimbine HCl (5 mg/kg; i.p.) on basal tail-flick latencies was assessed. In the second experiment (n=26), the effect of the drug (5 mg/kg) on analgesia acutely-elicited by footshock (1.0 ma; 15 sec.) or by the classical conditioning of fear to the tail-flick procedure (Chance et al., Brain Res. 141:371, 1978) was investigated. The last experiment (n=27) examined the effect of yohimbine (5 mg/kg) on analgesia elicited by morphine (8 mg/kg; s.c.). Yohimbine decreased basal tail-flick latencies by 47% 15 min after injection, with this hyperalgesic response lasting for at least 3 hr. Analgesia elicited by acute footshock was reduced to control levels following administration of the drug. As in previous research, classical fear conditioning elicited analgesia which incremented to an asymptote by day 5 (6.2 vs 2.6 sec). Yohimbine dramatically reversed this autoanalgesic effect to 1.7 sec on day 6. Morphine analgesia was also reduced 52% by yohimbine, 15 min after administration of the opiate. This reduction, however, declined to 32% at the time of maximum opiate analgesia (60 min). These observations demonstrate effective antagonism of autoanalgesia and suggest that this antagonism is due to removal of inhibitory influences descending to the spinal cord. This suggestion is supported by the drug's hyperalgesic effect and its partial antagonism of morphine analgesia. NIAAA Grant: 5 RO1 AA03157-02.

2062 PROTECTION OF ENDORPHIN DEGRADATION CAUSES ANALGESIA AND ENHANCES ELECTROACUPUNCTURE EFFECTS IN MICE. Richard S.S. Cheng* and Bruce H. Pomeranz. Dept. Zoology, University of Toronto, Toronto, Ontario M5S 1A1.

Recent evidence shows that systematic treatment with antipeptidases (D-leucine and D-phenylalanine) induces hypalgesia (pain reduction) in man and mice and also causes no addiction. These antipeptidases are postulated to act by protecting endorphins from peptidase destruction, since the hypalgesia they produced was naloxone-reversible. To further test the antipeptidase-endorphinergic hypothesis we compared antipeptidase effects in three related strains of mice. One strain (CXBK) is low in opiate receptors and exhibits poor electroacupuncture and morphine analgesia; another strain (Ob/Ob) has abnormally high levels of pituitary B-endorphin. A third strain $B6AF_{1/J}$ is used as control as it exhibits 'normal' electroacupuncture hypalgesia. The results show that Ob/Ob mice exhibit a higher baseline pain threshold level than B6AF, and CXBK, while the antipeptidases show hypalgesia in the order of Ob/Ob > $B6AF_{1/J}$ > CXBK. This correlates with the differences in endorphinergic systems in these 3 strains of mice. In addition, these antipeptidases increase electroacupuncture hypalgesia in $B6AF_{1/J}$ mice. The combination of antipeptidase and electroacupuncture may provide a non-addictive method for clinical pain treatment.

2063 STRESS AND ANTAGONIST POTENTIATION OF OPIATE ANALGESIA. J.E. Comaty, R.L. Borison, H.S. Havdala* and B.I. Diamond. Mount Sinai Hospital, Dept. of Anesthesia, Chicago, Il. 60608.

The endorphins produce morphine-like actions on pain systems, and these actions are blocked by the specific narcotic antagonists naloxone and naltrexone. Although the endorphin systems are well characterized, few practical attempts have been made to manipulate this system, so as to produce analgesia. We were intrigued by the potential analogy between endorphin and other neurotransmitter systems, where chronic administration of the antagonist results in a paradoxical potentiation of the agonist's actions. We have now tested the effects of chronic naltrexone on morphine-induced analgesia. Subjects were either white male Swiss mice or white male Sprague-Dawley rats. Analgesia was measured either by the hot plate test or the tail-flick test. We found in mice that with daily saline treatment and analgesia testing there was a gradual increase in their latency to demonstrate pain, going from a baseline of 4s to an average of 17s prior to withdrawal of treatment. In contrast, animals receiving naltrexone (20 mg/kg) failed to show this increase in latency. On the tenth day of pretreatment withdrawal, animals were challenged with morphine (5 mg/kg), which produces an analgesic response of 13s in naive mice. In saline pretreated mice, latency to response was 14s, however, in animals previously treated with naltrexone, the latency to response was 20s, and at 13 days after withdrawal the time was further increased to 27s. Similarly, in the tail-flick test, we found that one week after withdrawal of chronic pretreatment, the median latency to response to morphine (10 mg/kg) in saline pretreated animals was increased almost two-fold over baseline, whereas the response of animals pretreated with naltrexone was almost four-fold greater than baseline. This apparent hypersensitive response was reversed by two weeks after withdrawal. Our data shows that animals chronically treated with saline and tested for analgesia develop an apparent analgesia over time. This effect is most likely related to a stress-induced analgesia in response to the thermal stimulus used in testing. In contrast, animals receiving naltrexone under similar conditions failed to develop analgesia. This data would suggest that the stress-induced analgesia is most likely mediated via endorphin systems as would be consistent with its reversability by the specific narcotic antagonist naltrexone. The data also demonstrate that chronic naltrexone produces a marked potentiation of morphine-induced analgesia. This phenomenon may be explained by supposing either that naltrexone has produced a sensitization of opiate receptors, either by increasing their numbers or affinity, or by altering morphine's pharmacokinetics. Thus prior treatment of patients with specific narcotic antagonists may lead to an increased efficacy, and thus a decreased dosage of opiates used in clinical situations.

2064 ADRENERGIC AND 5-HYDROXYTRYPTAMINERGIC INFLUENCES ON MORPHINE ANALGESIA: ASSESSMENT BY THREE PAIN TESTS. S.G. Dennis and R. Melzack*. Dept. Psychol., McGill University, Montreal, Quebec, Canada H3A 1B1

The influences of adrenergic and 5-hydroxytryptaminergic (5HT) systems on morphine analgesia (MA) have been explored in many psychopharmacological studies (cf. Takemori, Ann NY Acad Sci., 281: 262, 1976). Unfortunately, the results are highly variable, and a consensus on 5HT and adrenergic mechanisms has been difficult to reach. An important factor in this variability appears to be the pain test. We have been systematically examining the effects of adrenergic and tryptaminergic agents on pain and MA as measured in rats by three pain tests--Tail-Flick, Hot-Plate, and Formalin. These tests differ in the type of noxious stimulation and in the behavioral responses from which pain is inferred. Each test reveals a unique pharmacological profile of analgesia.

In the Tail-Flick test, analgesia was produced and MA enhanced by L-tryptophan (>200 mg/kg), a 5HT precursor. MA was reduced by the β-blocker propranolol at low (1 mg/kg) but not high (>4 mg/kg) doses. In this test, α-adrenergic agents had no measurable effects at the doses used. In the Hot-Plate test, the α-blocker yohimbine (>1 mg/kg) was strongly analgesic and added to MA. Propranolol at 1 and 8 mg/kg, but not at 4 mg/kg, antagonized MA. Propranolol's antagonism was not manifested when methysergide (a 5HT receptor blocker) or yohimbine were present. Adrenergic agonists and L-tryptophan had little or no effect in this test. In the Formalin test, the α-agonist clonidine (>0.03 mg/kg) and the β-agonist isoproterenol (>0.5 mg/kg) were analgesic and synergized MA. Yohimbine and propranolol (both >1 mg/kg) were somewhat analgesic alone and appeared to add to MA. When combined, yohimbine (1 mg/kg) and propranolol (1 mg/kg) occasionally reversed MA. Methysergide (>2 mg/kg), L-tryptophan (>200 mg/kg), p-chloroamphetamine (PCA, 10 mg/kg), and p-chlorophenylalanine (PCPA, 150 mg/kg) enhanced MA, while PCA (20 mg/kg) and PCPA (300 mg/kg) antagonized MA in this test. PCA and PCPA deplete 5HT.

It appears that different pain tests reveal different underlying neural substrates of analgesia, thus making a single pain test insufficient to characterize pain in general. Both the type of noxious stimulation--heat or subcutaneous formalin--and the response required--elevation and licking of the paws or lashing of the tail--contribute to the observed variability. There appear to be multiple adrenergic and tryptaminergic systems--some cooperative and some competitive--that influence pain and analgesia.

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2065 ELECTROPHYSIOLOGICAL EVIDENCE FOR A DIRECT PROJECTION FROM THE PERIAQUEDUCTAL GRAY TO NUCLEUS RAPHE MAGNUS IN THE CAT AND RAT. Jonathan O. Dostrovsky and Yasmin Shah*, Department of Physiology, University of Toronto, Toronto, Ontario, Canada M5S 1A8.

It is widely accepted that stimulation of the periaqueductal gray matter (PAG) can result in analgesia. At least part of this effect is thought to be due to the activation of a descending pathway projecting from the PAG to the nucleus raphe magnus (NRM) which in turn activates a descending inhibitory projection from the NRM to the spinal cord. There is anatomical evidence for a projection from the PAG to NRM and a recent electrophysiological study (Fields and Anderson, Pain, 5: 333, 1978) has demonstrated that PAG stimulation elicits a weak excitation of NRM neurons. This effect may be mediated by a polysynaptic pathway. The present study describes electrophysiological evidence for a direct projection from the PAG to the NRM and adjacent reticular formation obtained by antidromic stimulation.

Experiments were carried out on urethane or chloralose anesthetized cats and urethane anesthetized rats. A concentric bipolar stimulating electrode was stereotaxically placed in NRM. Single unit activity was recorded in the PAG using glass coated tungsten microelectrodes. Antidromic responses were identified by their constant latency and ability to follow two stimuli at intervals shorter than 3 ms. Most of the units could be activated by NRM stimuli of 40-200 μA, 0.1 ms duration. Eighty-four antidromically excited neurons within the PAG were recorded in 5 cats. Conduction velocities calculated from the response latency and stereotaxically estimated inter-electrode distance rangedfrom 0.5 to 23 m/s, with a mean of 6.9 m/s. In 4 rats, conduction velocities of 21 PAG neurons excited from NRM ranged from 0.9 to 10.4 m/s with a mean of 3.3 m/s. Most of the neurons had conduction velocities greater than 2 m/s implying that the PAG to NRM projection is mainly composed of small myelinated fibers. Antidromically activated units were encountered throughout the PAG region but were more numerous in ventral areas. Some neurons outside the PAG could also be antidromically excited. A few neurons were encountered which could be excited orthodromically from NRM.

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2066 INFLUENCE OF ATTENTIONAL PROCESSES ON THE ACTIVITY OF NOCICEPTIVE NEURONS IN THE DORSAL HORN OF THE MEDULLA (TRIGEMINAL NUCLEUS CAUDALIS) OF AWAKE MONKEY. R. Dubner, R.L. Hayes* and D.S. Hoffman* (SPON: S. Gobel). Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20205.

Rhesus monkeys were trained to release a panel when they detected the termination of warming stimuli (37° to 43°C) or the onset of noxious thermal stimuli (45°-49°C) applied to the face. In a second task, the same monkeys released the panel when they detected the onset of a light stimulus while behaviorally non-relevant thermal stimuli were presented. Nociceptive neurons exhibited maximum discharges and monotonic stimulus-response functions to noxious stimuli presented during both tasks. Neural activity also could be correlated with panel press and panel release in both tasks. Movements under the thermode were monitored by EMG recordings from the lip musculature. In many nociceptive neurons, an increase (1/10 to 1/3 peak frequency on 49°C trials) in neural activity was associated with panel release. Similar to the burst response of "attentional" neurons in the dorsal horn of the medulla (Hoffman et al, this volume), this increase in activity occurred when the monkey detected the relevant cue (temperature termination or light onset) that reliably predicted reinforcement. The burst discharge in some nociceptive neurons occurred only during the thermal task. During the inter-trial interval (ITI, 15 sec), in both tasks, there sometimes was a decrease in the level of background activity as the next trial approached. Neural activity during the ITI often was greater in the thermal task than in the light task. The activity of most nociceptive neurons was suppressed immediately preceding panel press. In many neurons this suppression was followed by a phasic or tonic increase in discharge at panel press that sometimes was greater in the thermal task. These changes in neural activity occurred in the absence of thermal stimuli and without mechanical displacement produced by lip movements. Often, the press response was enhanced in early trials of a sequence and was suppressed by preceding noxious thermal trials. These findings demonstrate that neurons, previously studied in anesthetized monkeys, and whose maximum discharge occurs in response to noxious thermal stimuli, exhibit dynamic changes in neural activity related to other events occurring during behavioral tasks. We conclude that attentional mechanisms, most evident in a task using oral-facial stimuli as relevant cues, influence the activity of second order sensory trigeminal neurons participating in the sequence of neural events related to goal-directed behaviors.

2067 APPARENT SAFETY OF HIGH ELECTROANALGESIA CURRENT APPLIED TO INTACT TEETH. R. Wayne Fields, Patrick J. Reynolds, Robert P. O'Donnell* and Richard B. Tacke*. School of Dentistry, University of Oregon Health Sciences Center, Portland, Oregon 97201.

We have demonstrated the effectiveness of electric current, applied to teeth of cats, in lessening the magnitude of trigeminal field potentials (Fields, et al., Exp. Neurol. 47:229-239, 1975) or responsiveness of identified primary afferents (Fields, et al., Exp. Neurol., 53:386-398, 1976) to electrical test stimuli applied to the pulps of those teeth. The presumably analgesic currents have been administered using a remote cathode with the anode applied to the exposed dentin of the tooth in question.

Direct current is theoretically harmful to vital elements of tooth pulp (Fields, et al., Oral Surg. 34:694-703, 1972). We have shown that pulsatile currents with peak levels as low as 70μA can be used as effectively as direct current to lower afferent excitability (Fields, et al., Arch. Oral Biol. in press). When much higher currents, to 1000μA, are used, long lasting hypoexcitability follows administration of direct current, while the effects of pulsatile current appear to be reversible (Fields, et al., Abstr. Soc. Neurosci. 4:459, 1978). In order to establish the clinical safety of electroanalgesia (EA) current we felt it was essential to examine tooth pulps histologically at intervals following administration of current. Such observation requires that the teeth remain intact to insure that pulpal changes are not secondary to invasion of the dentin.

We utilized both dc and ac (rectangular pulses at 1000Hz, 10% duty cycle, capacitively coupled) at peak levels spanning the range known to be effective in lowering afferent excitability and including the potentially damaging value of 1000μA. The anode was a saline soaked cotton wick in contact with a platinum wire, all encased in a length of silastic tubing pushed firmly onto the crown of the intact tooth and sealed in place with wax. The return electrode was an electrocautery grounding pad on a hindlimb. Acute current administration was done under Ethrane anesthesia, and teeth were harvested on days 2, 8, 21 and 60.

In no case, either with direct current or alternating current as high as 1000μA was any histologically recognizable sign of pulp damage apparent. The lack of expected damage with high direct current may be related to diffusion of the current by the highly resistant intact enamel. Unpublished single unit recordings, however, demonstrate that even at low levels, current applied to intact enamel effectively lowers excitability.
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2068 MECHANISM OF ACTION OF DRUGS IN TRIGEMINAL NEURALGIA. Gerhard H. Fromm, Jay D. Glass, Amrik S. Chattha* and Christopher F. Terrence* Dept. Neurol., Sch. Med., Univ. Pittsburgh, Pittsburgh, PA 15261.

One approach to the investigation of trigeminal neuralgia is to examine the mechanism of action of drugs effective against it. Elucidating the effect of these drugs on the trigeminal nucleus should increase our understanding of the pathophysiology of the paroxysms of pain that these drugs can prevent, and also provide us with laboratory tests for predicting the potential usefulness of new drugs. We have therefore studied three drugs in order to compare their effects on the spinal trigeminal nucleus. Two of these drugs relieve trigeminal neuralgia and one does not.

We have previously reported that carbamazepine (CBZ), an anticonvulsant which is currently the drug of choice for the treatment of trigeminal neuralgia, depresses the response of spinal trigeminal neurons to maxillary nerve stimulation. CBZ increases the neurons' latency of response and decreases the number of spikes in response to each stimulus. We have now also found that CBZ facilitates afferent inhibition in the spinal trigeminal nucleus. The afferent inhibition was elicited by delivering a conditioning stimulus to the maxillary nerve prior to the test stimulus.

Phenobarbital (PB), an anticonvulsant which is not effective against trigeminal neuralgia, produced only a small depression of the response to the unconditioned maxillary nerve stimulus. In addition, PB decreased, rather than increased, afferent inhibition in the spinal trigeminal nucleus.

Baclofen (BCL) resembled CBZ both in its ability to relieve trigeminal neuralgia, and in its ability to facilitate afferent inhibition of some neurons in the spinal trigeminal nucleus. BCL depressed the response to the unconditioned maxillary nerve stimulus less than CBZ, but slightly more than PB.

Our results indicate that the two drugs that do relieve trigeminal neuralgia facilitate inhibitory mechanisms in the spinal trigeminal nucleus. This seems to be at least as critical as their ability to depress excitatory mechanisms in this nucleus.

2069 ATTENUATION OF STRESS-INDUCED ANALGESIA BY ANTERIOR HYPOPHYSECTOMY IN THE RAT. Murray Glusman, Richard J. Bodnar, Dennis D. Kelly, Carl Sirio*, Jordan Stern* and Earl Z. Zimmerman. Depts. of Psychiatry and Neurology Columbia Univ., College of Physicians & Surgeons and N.Y. State Psychiatric Inst., New York, NY 10032.

Naive rats subjected to any one of a number of different stressors display temporary analgesia which may last as long as 2 hours after termination of the stress. We reported recently that this phenomenon, generally called stress-induced analgesia (SA), is greatly attenuated by hypophysectomy (Bodnar, R.J. et al, Pain Abstracts, 1:262, 1978; Physiol. Behav. 23: 1979). Similar results, in agreement with ours, have been obtained by others (Pert, A., 10th Annual Winter Conference on Brain Research, 1978; Amir,S. & Amit, Z., Life Sci. 24:439, 1979). We now report that SA is dependent largely on the integrity of the anterior lobe of the pituitary. We studied 40 rats divided into 4 groups of 10 animals each. Group I consisted of rats in which the whole pituitary had been surgically removed; Group II had the anterior pituitary removed; Group III the posterior pituitary removed; and Group IV consisted of sham operated controls. For a period of 2 weeks post-operatively and throughout the experimental test period, Groups I, II and III - the totally and partially hypophysectomized animals - were given daily endocrine supplements of corticosterone (0.2 mg) and thyroxin (2 μg) to enhance their probability of survival in the laboratory environment and under the experimental stress conditions. Baseline nociceptive thresholds, measured by a modification of the Evans flinch-jump procedure, were determined repeatedly for all animals until stable levels were obtained. The rats were then subjected to a single, forced, cold-water swim (2°C for 3.5 min), followed 30 min later by a threshold retest. Prior to the cold-water swim the animals in all groups showed similar thresholds. The stress procedure, however, elicited striking differences among the 4 groups in post-stress thresholds. The Group IV sham operated controls and the Group III animals (posterior pituitary removed, anterior intact), displayed substantial post-stress threshold elevations indicating well-developed SA responses. In contrast, the Group I animals (total hypophysectomy) and the Group II animals (anterior pituitary removed, posterior intact) failed to develop SA, showing no increase in post-stress thresholds over baseline levels. The post-stress thresholds in Groups III and IV were not significantly different from each other, but they were significantly greater than those of Groups I and II. Our results indicate that the anterior pituitary plays a major role in stress-induced analgesia, and a hitherto unrecognized role in the modulation of pain perception. (Supported by NIH Grants MH 15174 and NS 14449).

2070 THE EFFECT OF NALOXONE ON MULTIDIMENSIONAL SCALES OF POSTSURGICAL PAIN IN NONSEDATED PATIENTS. R.H. Gracely*, W.R. Deeter*, P.J. Wolskee*, B.L. Wear*, J.S. Sayer*, M.W. Heft*, J. Sweet*, D. Butler*, and R. Dubner. Neurobiology & Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20205.

The influence of the narcotic antagonist naloxone and placebo on the experience of postsurgical pain was examined in 36 patients following extraction of at least one mandibular third molar. Extractions were performed with a short duration local anesthetic (2% lidocaine without epinephrine) and without the use of intravenous or inhalation sedatives. Visual analog (VAS) and verbal descriptor (VDS) scaling procedures were used to assess responses related to the sensory intensity, unpleasantness and painfulness of postsurgical pain for 2 hrs beginning 1 hour after the initiation of local anesthesia. Two groups of 12 subjects received double-blind intravenous injections of either 10 mg naloxone or naloxone vehicle (placebo) 2 hrs after injection of local anesthesia. A third group received no treatment. Both the VAS and VDS scales showed increased responses following no treatment and decreased responses following placebo. These differences were statistically significant for both the VAS (order X drug interaction, $F(1,22) = 5.29$, $p<0.05$) and VDS (order X drug interaction, $F(1,22) = 6.38$, $p<0.05$) scales of unpleasantness. VAS and VDS scales of sensory intensity and painfulness showed no statistically significant effects. VAS scales did not significantly differentiate between naloxone and placebo on any dimension. The VDS scales, however, showed a significant elevation in unpleasantness responses after naloxone in comparison to placebo (order X drug interaction, $F(1,22) = 6.13$, $p<0.05$). This effect was not significant for VDS scales of sensory intensity or painfulness. This experiment documents a significant difference in the unpleasantness of postsurgical pain following administration of either naloxone or placebo that cannot be explained by reversal of sedative agents used during surgery. The results suggest that naloxone increases the unpleasantness associated with postsurgical pain by reducing the levels of endogenous opiate-like substances present after surgery or after the administration of a placebo. The superior sensitivity of the unpleasantness scales is consistent with previous evidence from factor analyses of verbal pain responses and further stresses the importance of multidimensional assessment of pain experience.

2071 ELECTROPHYSIOLOGICAL EXAMINATION OF PRIMATE SPINORETICULAR TRACT NEURONS. Lawrence H. Haber, Bart D. Moore* and William D. Willis. Marine Biomedical Institute and Depts. of Anatomy and Physiology and Biophysics, Univ. Texas Medical Branch, Galveston, TX 77550.

Various lines of evidence suggest that cells in the nucleus reticularis gigantocellularis (NGc) may serve as a bulbar relay in a spino-reticulo-thalamic nociceptive pathway. The present work was undertaken to elucidate the response properties of spinal neurons projecting to this area of the caudal brain stem.

Experiments were carried out on 11 anesthetized monkeys (Macaca fascicularis). Twenty-nine spinoreticular (SR) neurons in the lumbar (10 cells) and cervical (19 cells) enlargements were identified by antidromic activation from the region of the ipsilateral (9 cells) or contralateral (20 cells) NGc. Three of the cells could also be antidromically activated from the ventro-posterior lateral nucleus of the contralateral thalamus. The threshold stimulus strength to produce an antidromic response varied from 20-300 μA (less than 100 μA in a majority of cases). Conduction velocities of SR neurons ranged from 9-51 m/s (mean = 24.0 m/s ± 9.8). Fifteen of 29 SR neurons could be excited by natural stimulation of one or more limbs. Of these, 1 cell was activated by tactile stimulation, 3 cells were excited by tactile stimuli but their discharge was enhanced still more by noxious mechanical stimulation (i.e., these cells had a wide dynamic range), 9 cells responded only to noxious mechanical stimulation of the skin and 2 cells were excited exclusively by stimulation of the deep tissues. SR cells having peripheral receptive fields were situated in nearly all regions of the spinal gray matter, whereas SR neurons unresponsive to peripheral stimuli were located primarily in laminae VII and VIII. The discharge of SR cells could be facilitated or in fewer cases inhibited by brain stem stimulation.

The implication of these experiments is that spinal neurons which send their projections to (or possibly through) the region of the NGc may be important for the transmission of information necessary for some aspects of pain perception and response.

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2072 ANALGESIA FOLLOWING MICROINJECTION OF PHENTOLAMINE IN THE NUCLEUS RAPHE MAGNUS. Donna L. Hammond and Richard A. Levy, Dept. of Pharmacol., Univ. of Ill. at the Med. Cntr., Chicago, IL., 60612.

The nucleus raphe magnus (NRM) of the brain stem is a source of descending serotonergic fibers which terminate in the dorsal horn of the spinal cord. There is strong evidence that activation of these fibers results in analgesia. Biochemical and histofluorescence studies indicate that the NRM receives a noradrenergic (NA) input which is probably inhibitory, as iontophoretic application of norepinephrine depresses NRM cell firing. It is not known, however, if the NA input modulates the activity of those NRM cells associated with analgesia. In the present study, this input was blocked by microinjection of the NA antagonist phentolamine into the NRM. If the NA input influences those NRM cells which mediate analgesia, then activation (disinhibition) of the NRM by phentolamine would be expected to induce analgesia.

Female rats (220-280 grams) were stereotaxically implanted with a guide sheath positioned 2 mm above the NRM. Following recovery from surgery, 5 or 10 μg phentolamine (P) in 0.5 μl saline was microinjected in the NRM region through an injection cannula extending 2 mm beyond the guide sheath. Alterations in nociceptive threshold were assessed with the tail flick (TF) and hot plate (HP) tests prior to and at fixed intervals following microinjection.

The introduction of both 5 and 10 μg P at sites in the NRM produced a significant elevation in TF latency. The magnitude of the elevation caused by 10 μg P was not significantly greater than that produced by 5 μg P, although the elevation following 10 μg P was more prompt in onset. TF latency was not elevated following microinjection of either tetracaine (4.7 μg, equimolar to 5 μg P) or saline at these same sites, or of P at sites located outside yet close to the NRM. Microinjection of both doses of P into the NRM also increased the nociceptive threshold as assessed by the HP test. HP latency was elevated to a greater extent by 10 than by 5 μg P, although the time to onset of the elevation did not differ. Injection of tetracaine and saline at these same sites did not alter the HP latency. HP latency was elevated following injection of P at sites located close to but outside the NRM, but the onset of this effect was substantially longer than that observed after injection of P at sites in the NRM. Preliminary data indicates that elevation of both TF and HP latencies also occurs following microinjection in the NRM of another NA antagonist, azapetine.

These results suggest that the descending raphe-spinal pathway involved in setting the nociceptive threshold is under tonic inhibitory control by a NA input. (Supported by USPHS NS 12649).

2073 ℓ-BACLOFEN PREFERENTIALLY DEPRESSES NOCICEPTIVE INPUTS IN LUMBAR DORSAL HORN IN THE CAT. James L. Henry, Dept. of Research in Anaesthesia, McGill Univ., Montréal, PQ, Canada. H3G 1Y6

Baclofen, a derivative of GABA best known for its antispastic actions, has also been found to have antinociceptive properties. This latter finding prompted an electrophysiological study of the effects of the d- or ℓ-isomer on single sensory neurones in the L_5-L_7 dorsal horn of the cat (chloralose anaesthetized, n = 8, or unanaesthetized decerebrated, n = 4) spinalized at L_1 and paralysed with pancuronium. In each experiment one single unit was studied; its responsiveness to natural cutaneous stimulation was determined and then one or the other isomer was administered in graded doses via the jugular vein and the effects noted on the single unit. Both forms had depressant effects on dorsal horn units, the ℓ-isomer being approximately 100 x more potent than the d-isomer. The most sensitive modality to baclofen was nociception. Wide dynamic range (WDR) neurones, which responded to automatically controlled periodic applications of noxious radiant heat alternating with an air stream, showed a marked depression of the on-going discharge rate and also of the response to noxious heat with 0.1 mg/kg of ℓ-baclofen, without a change in the response to air. In some cases, where electrical stimulation of the cutaneous receptive field of WDR neurones provoked early and late discharges (conduction velocities corresponded to Aβ and Aδ fibres, respectively), ℓ-baclofen depressed the late discharge at lower doses than those which depressed the early discharge. In other cases nociceptive specific or WDR neurones were excited alternatively by periodic applications of noxious radiant heat and glutamate (applied locally to the neurone by iontophoresis); ℓ-baclofen, at doses which depressed the heat response, had little or no effect on the excitatory response to glutamate, suggesting a presynaptic action. The depressant effects of either isomer could be reversed by the i.v. administration of bicuculline methiodide (1-5 mg/kg) but not of strychnine sulphate (0.1-0.2 mg/kg). These results suggest that baclofen has a presynaptic effect preferentially on small diameter afferent fibres, and acts via a GABA-ergic mechanism.

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2074 EXAGGERATION OF REFLEX GROOMING AND ITS DOMINANCE OVER NOCIOCEPTIVE RESPONSES IN THE BILATERAL DECORTICATED CAT. S.J.Herdman*, V.Cerny, C.N.Liu, W.W.Chambers*, and J.Yu. Dept. of Anat. and Inst. of Neurol. Sci., Univ.of Penna.,Phila., Pa. 19104.

Normal grooming responses consist of licking, nibbling, wiping the face and ears with the forepaws and scratching the head, neck and shoulders with the hindpaws. These responses are transiently lost after bilateral neodecortication or after bilateral removal of the frontal poles including the primary and secondary somesthetic cortical areas. The grooming responses gradually reappear and by 30 to 50 days post-op have become markedly exaggerated demonstrating the effect of cortical release. This grooming may become so marked that the animal cannot stand nor walk being consumed by licking. The reflex zones for the grooming responses in the decorticate cat are essentially the same as in the normal animal. The grooming movements, however, have poor local sign and are frequently ineffective since they are often misdirected. The type of response elicited is also related to the intensity of the stimulus and to a lesser extent to the modality of the stimulus.

Nocioceptive responses such as the flexion reflex, flicking of a limb, tail lashing, growling, and spitting can be completely inhibited by eliciting the licking response. This inhibition persists from 15 seconds to 2 minutes after cessation of reflexly induced grooming. The longer inhibition is related to a longer period of induced grooming. The nocioceptive response then rebounds with even greater intensity but can be inhibited again by eliciting grooming. To determine if the inhibition of the nocioceptive responses by grooming was mediated by endogenous morphine-like substances, we treated the cat with Naloxone (0.4-4 mg/kg body weight) which blocks opiate receptors. This treatment did not alter the grooming nor its inhibition of the nocioceptive responses. We conclude that excessive grooming and the dominance of the grooming response over nocioceptive responses in the decorticate cat is not due to endogenous opiates. Possible mechanisms will be discussed. (Grants 5T32 EY07035-02 and USPHS NS 10464)

2075 PITUITARY-ADRENAL ACTIVITY ASSOCIATED WITH CENTRAL PAIN FOLLOWING COMPLETE FORELIMB DEAFFERENTATIONS IN RATS. J.P. Heybach,* M. Levitt and A. Brodish.* Dept. of Physiol. and Pharm., Bowman Gray Sch. Med., Wake Forest Univ., Winston-Salem, NC 27103.

Adult male rats (SD; 270-350g) underwent bilateral dorsal rhizotomies at C5-T2, inclusive. Postoperatively, withdrawal responses to forepaw pinch and pinprick were absent. Within 17 days following surgery 7 rats with complete forelimb deafferentations displayed a behavioral syndrome characterized by abnormal chewing of the forelimbs. This behavior has been described in the monkey (Levitt, Fed. Proc. 1977, 36, 538) and in the rat and the hypothesis advanced that it represents the response to disturbing or painful sensations referred to the deafferented regions (Basbaum, Exp. Neurol. 1974, 42, 490). In the present study this behavioral syndrome was found to be variable in onset, severity and duration, and was associated with piloerection, squealing when gently handled and hyperreactivity to air puffs directed at lumbar body dermatomes. Control rats were not self-mutilating and consisted of: 2 rats with extensive dorsal rhizotomies; 1 rat with 2 dorsal roots cut bilaterally; and 1 rat with laminectomy and dural incision, but no rhizotomies. Deafferented self-mutilating rats were compared to the control rats with regard to basal plasma corticosterone (B) levels, as an index of pituitary-adrenal activity. Blood samples were taken (0800-1000h) by jugular venapuncture under ether at various pre- and postoperative intervals. Dramatic elevations in plasma B levels corresponded to the day of onset of self-mutilation and remained elevated compared to both pre-mutilation and control group levels while the syndrome persisted. Work in progress is aimed at determining if these elevated plasma B levels are simply a response to peripheral somatic tissue damage or are causally related to the CNS deafferentation which gives rise to the presumed central pain. In support of the latter it was noted that the elevated B levels in self-mutilating rats did not correlate with the degree of tissue damage.

These endocrine changes, which parallel those reported in human cases of phantom limb pain (Pozidaeva, Ortp. Travm. Protez. 1976, 10, 43 [in Russian]), and the associated behavioral changes suggest that this preparation merits further study as a potential model of stressful deafferentation pains.

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2076 NEURAL ACTIVITY RELATED TO ATTENTIONAL PROCESSES IN THE DORSAL HORN OF THE MEDULLA (TRIGEMINAL NUCLEUS CAUDALIS) OF AWAKE MONKEY. D.S. Hoffman*, R.L. Hayes*, and R. Dubner (SPON: J.E. Albano). Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20205.

Unit activity was recorded in the dorsal horn of the medulla (DHM) of rhesus monkeys trained in two reaction time tasks to release a panel when they: (1) detected the termination of a warming (37^{o}-43^{o}C) stimulus or the onset of a noxious (45^{o}-49^{o}C) stimulus applied to the face; (2) detected the onset of a light stimulus while behaviorally non-relevant thermal stimuli were applied to the face. Neural activity could be correlated with the following behavioral events: (1) panel press, which initiated the onset of thermal stimuli in the first task; (2) presentation of the cue for panel release-i.e., termination of thermal stimuli in the first task or onset of light stimuli in the second task; (3) panel release. A subset of neurons located deep in DHM had receptive field response properties that were similar to those of thermal nociceptive neurons in this nucleus. In contrast to the nociceptive neurons, these neurons exhibited peak activity at panel release. A burst of neural activity occurred just prior to panel release in warming, noxious and light trials and therefore was independent of the intensity or modality of the relevant cue. The burst was absent if the monkey failed to detect the cue and released the panel inappropriately. The neural discharge occurred following experimenter-presented trials, when monkey hand movements were not present at panel release. The neural activity also could be temporally separated from the lip perch in preparation for reinforcement intake. The latter was monitored by EMG recordings from the lip musculature. Thus, the burst of neural activity was temporally related to panel release, but it was not reliably correlated with any of the motor behaviors accompanying panel release. Finally, the burst of discharges was reduced in amplitude and eventually disappeared when reinforcement was withheld during experimenter-presented trials. These findings indicate that some neurons in DHM exhibit maximum neural activity when the monkey detects the relevant cues that reliably predict reinforcement. The neural response is the same irrespective of stimulus modality or stimulus intensity and occurs in the absence of appropriate motor responses. We conclude that neural activity at an early stage of central nervous system processing may be associated with attentional processes involved in organizing goal-directed behaviors.

2077 NOCICEPTIVE AND NON-NOCICEPTIVE TRIGEMINAL NEURONS: BRAINSTEM INTRANUCLEAR PROJECTIONS AND MODULATION BY PERIAQUEDUCTAL GRAY AND NUCLEUS RAPHE MAGNUS. J.W. Hu and B.J. Sessle. Fac. of Dentistry, Univ. of Toronto, Canada M5G 1G6.

In addition to its role as a major nociceptive relay site in the brainstem, trigeminal nucleus caudalis has also been implicated in ascending tonic modulatory influences on sensory transmission through neurons in more rostral trigeminal brainstem nuclei (e.g. nucleus oralis). Such a projection from caudalis has in fact been indicated in anatomical studies, but direct physiological evidence of the projection is lacking and no evidence is available of the functional properties of the caudalis neurons involved in the projection. We have tested for such a projection in nucleus caudalis and examined the functional properties of projection neurons.

In anesthetized adult cats, the activity of single caudalis neurons was recorded and their orthodromic responsiveness noted to noxious or innocuous orofacial stimuli. A neuron projecting to the ipsilateral oralis and/or contralateral posterior thalamus was identified by its antidromic responses to stimulation at these sites. Effects of conditioning stimulation of PGM and NRM were tested on both orthodromically evoked and antidromically evoked activity. A decrease in orthodromic responsiveness reflected inhibition of the caudalis neuron;an increase in antidromic excitability was an indirect measure of presynaptic depolarization of the axonal endings in oralis of the caudalis neuron.

Of 83 caudalis neurons tested, 45 could be antidromically excited from oralis; 3 of these 45 neurons also had a contralateral thalamic projection. Both non-nociceptive and nociceptive caudalis neurons located in marginal and deeper layers of nucleus caudalis were found to project to oralis, and the axonal conduction velocity of the former (mean 5.7 m/sec) was significantly faster than that of the nociceptive neurons (mean 1.7 m/sec); both values are significantly slower than trigeminothalamic velocities. Conditioning stimulation of PGM and NRM produced inhibition of the orthodromically evoked responses of both types of caudalis neurons. Moreover, conditioning stimulation also induced presynaptic depolarization lasting more than 400 msec in the axonal endings in oralis of both nociceptive and non-nociceptive neurons.

These studies have provided direct physiological evidence of an axonal projection from nucleus caudalis to more rostral trigeminal nuclei. Both nociceptive and non-nociceptive caudalis neurons are involved in the projection, and PGM and NRM exert inhibitory influences within caudalis as well as presynaptic modulatory influences on the axonal endings within oralis of both types of caudalis neurons.

2078 RELEASE OF SUBSTANCE P AND SOMATOSTATIN, IN VIVO, FROM PRIMARY AFFERENT TERMINALS IN MAMMALIAN SPINAL CORD. T.M.Jessell, A.W.Mudge*, S.E.Leeman and T.L.Yaksh. Depts. of Pharmacology and Physiology, Harvard Medical School, Boston, Mass. and Dept. of Neurologic Surgery, The Mayo Clinic, Rochester, Minn.

Substance P and somatostatin are contained within small diameter primary sensory neurons which terminate in the dorsal horn of the spinal cord and are associated with the transmission of noxious peripheral stimuli. However there is no direct evidence for the release of either peptide after activation of nociceptive afferents. By superfusing the mammalian spinal cord we have demonstrated the release of substance P and somatostatin in vivo following chemical and physiological stimulation of sensory afferents involved in pain transmission.

Rats and cats were anesthetized and a polyethylene (PE-10) cannula inserted into the sub-arachnoid space to the caudal margin of the lumbar spinal cord. Outflow was collected from a cannula in the cisterna magna (rats) or by a concentric cannula opening at the level of T-12 (cats). The spinal cord was then superfused with CSF containing serum albumin and bacitracin at a rate of 0.1 ml/min and samples were collected every 10 min (rat) or 30 min (cat) and divided for measurement of released peptide by radioimmunoassay. In rats, the release of substance P detectable in control periods was 1-2 fmol/min and release of somatostatin was 4-5 fmol/min. Superfusion of the rat spinal cord with CSF containing 40 mM potassium for 10 min evoked a 4-fold increase in the release of substance P, a 2.8-fold increase in somatostatin release and a 1.8-fold increase in neurotensin release. Chronic administration of capsaicin to rats has been shown to deplete substance P from sensory neurons. In the present experiments, superfusion of the rat spinal cord with capsaicin ($3x10^{-4}$M) produced a 9-fold increase in substance P release and a 4-fold increase in somatostatin release. The release of neurotensin,a peptide found in substantia gelatinosa interneurons but not in sensory neurons,was not increased by superfusion with capsaicin. Release of substance P evoked by potassium and capsaicin was greatly reduced by superfusion of the spinal cord with 2 mM cobalt.

In cats, bilateral stimulation of the sciatic nerve at intensities sufficient to activate only rapidly conducting, low threshold afferents did not increase the release of substance P. Increasing the stimulus intensity to recruit A delta and C fibre afferents produced a 2.6-fold increase in the release of substance P. In preliminary experiments, the release of substance P by high intensity sciatic nerve stimulation was reduced by the systemic administration of morphine (2mg/kg) and restored by systemic naloxone (1mg/kg).

2079 SUCCESSFUL PREDICTION BY THE AFFECT CODE OF UNIT RESPONSES TO REWARDING MEDIAL FOREBRAIN BUNDLE STIMULATION. James J. Keene. Dept. Physiol., Sch. Med., Univ. Puerto Rico, San Juan, PR 00936.

This report deals with verification of the proposed affect code (see Fed. Proc., 37: 2246-2250) which describes how certain anatomically localized neurons may represent information pertaining to an affective dimension (reward-pain) in their firing rates. Such "affect coding" neurons have been found in the medial thalamus where decreased and increased firing rates are associated with reward and pain respectively.

If the affect code is correct, one should be able to predict how an arbitrary input A with reward value would interact at the unit level with an aversive input, on the basis of knowledge of how reward input B interacts with the aversive input.

Four chronically implanted cats were trained to self-stimulate for medial forebrain bundle stimulation (MFB) (reward input A), and to escape from midbrain reticular (RET) stimulation (0.5 sec, 50 Hz trains). During these behavioral tests, 335 units were recorded from medial thalamus and overlaying cortex, with stereotaxically guided, moveable tungsten microelectrodes. Each unit was also tested with random presentations of the MFB and RET trains and of both simultaneously so that interaction of the effects could be examined. Separate tones distinguished self-stimulation and escape periods. The animal's enclosure was suspended so that a tension transducer provided continuous data on gross movements (slight turning of the head, positioning, etc.). Unit, movement, and bar pressing data were tabulated over real time by a PDP 11/20 computer system.

In the above model, escape was considered to be "reward input B". Since the RET stimulation was demonstrated to be aversive, and the majority of the units showed increasing excitation up to the bar press which initiated a 10 sec escape period, it was reasoned that decreased firing may code for reward in a unit (Type I) if it's activity in the escape period was less than the typical excitation after a randomly given RET train in a comparable post-stimulus period. In Type II units, post-escape activity was greater than that following a single RET train (temporal summation of pre-escape RET trains). If inhibition codes reward in Type I units, then MFB trains would be predicted, according to the affect code, to decrease unit activity and/or block RET-elicited excitation, as was found. On the other hand, MFB trains did not have this effect on Type II units. Indeed, they summated with the excitatory RET effects on Type II units producing an arousal coding pattern (see ref.) characterized by similar responses to the motivationally opposite MFB and RET stimuli.

These results showthat the affect code can be used to predict unit responses to motivational stimuli. NIH Grant RR-08102

2080 RESPONSES OF VPL_c NEURONS IN THE PRIMATE THALAMUS TO NOXIOUS THERMAL STIMULI. D.R. Kenshalo, Jr., G.J. Giesler, Jr., R.B. Leonard and W.D. Willis. Marine Biomed. Inst., and Depts. of Anat. and of Phys. & Biophys., UTMB, Galveston, TX 77550.

The primate spinothalamic tract terminates in several thalamic nuclei, including the caudal part of the ventral posterior lateral nucleus (VPL_c). To study the possibility that neurons in this nucleus are capable of transmitting nociceptive information, we have recorded from single thalamic units in monkeys (Macaca fascicularis). The animals were anesthetized with α-chloralose and an infusion of sodium pentobarbital (4 mg/kg/hr). Recordings were made with tungsten microelectrodes, and the position of units marked with electrolytic lesions. Noxious stimuli applied to the cutaneous receptive fields included intense mechanical stimuli and graded noxious heat pulses from 35°C adapting temperature to 43°, 45°, 47° and 50°C. Innocuous stimuli included hair movement, touch, rotation of joints, tapping and vibration. Many of the thalamic units were activated antidromically from the SI cortex using either a platinum ball electrode for surface stimulation or a steel microelectrode for stimulation within the cortex.

To date we have recorded from 94 thalamic neurons. Of these, 41 were excited by noxious heat pulses. The remaining neurons responded best to innocuous stimuli, and, of the five tested, none responded to noxious heat pulses. Reconstructions from electrolytic lesions indicated the cells responsive to noxious stimuli were somatotopically organized, those from the hindlimb being placed more laterally and those from the forelimb more medially in VPL_c. An ascending series of noxious heat pulses produced stimulus-response functions similar to those of spinothalamic tract neurons. In two experiments, lesions of the dorsolateral fasciculus on the side of the receptive field produced no observable changes in the responses of thalamic cells to noxious heat pulses, whereas in one case a lesion of the contralateral ventrolateral quadrant completely abolished the response.

We were able to activate antidromically 25 thalamic cells that responded to noxious heat pulses by surface stimulation of the SI cortex. Of these 13 were also antidromically activated by microstimulation within or subjacent to the SI cortex.

Based on these findings, we speculate that neurons located in the VPL_c nucleus of the primate receive input from the spinothalamic tract and transmit nociceptive information to the somatosensory cortex.

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2081 PAIN ADAPTATION AND HYPERALGESIA DURING THERMAL STIMULATION OF HUMAN SKIN. R.H. LaMotte, C.J. Robinson and J.G. Thalhammer*. Dept. of Anesthesiology, Yale University, New Haven, Conn. 06510.

In these studies we measured the time course of changes in human sensitivity to pain during and following intense heating of the skin. Human subjects, each of whom gave informed consent to an approved protocol, made continuous category judgments of the magnitude of warmth and pain during constant-temperature, localized heating of the hand or forearm with stimuli of 38 to 47°C (10 min duration) or 50° (1 min). Stimuli greater than 45° were perceived as painful throughout the presentation, with ratings of magnitude remaining constant or increasing slightly. Pain evoked by stimuli of less than 45° was transient and adapted (disappeared) within 1 min. In some experiments, the capacity to detect and to rate brief (3-5s) increments in temperature (0.05-1.6°) superimposed on these sustained temperatures was also determined. Thresholds near the beginning and the end of each stimulation were not appreciably different regardless of base temperature.

In other experiments we obtained magnitude ratings for test stimuli (0.1-8.0° above a base of 38°) delivered before and at varying intervals of time following a conditioning stimulus (CS) of 50°. Durations of the CS varied from 5 to 100s. For a CS of less than 30s, magnitude ratings of warmth and pain were typically lower (suppression) by an amount equivalent to 3-6° immediately following the CS and recovered to normal over the next 30 min. For a CS of greater than 30s, suppression also occurred; but within 5 min after the CS, pain threshold was lower than normal and magnitude ratings of all stimuli were 2-6° elevated over those obtained for the same stimuli prior to the CS (hyperalgesia). Ratings remained elevated for 2 or more hours. Also present during this time was a mildly unpleasant sensation evoked by lightly rubbing the skin with gauze (dysesthesia). The development of suppression and hyperalgesia following a CS of 50° for 60s corresponded, in time and in magnitude, to the sequence of fatigue and sensitization observed in C-fiber mechanoheat nociceptive afferents (CMHs) in the monkey (Thalhammer and LaMotte, this vol.). The adaptation of pain during sustained stimulations at lower temperatures may be accounted for by fatigue of CMHs while the failure to adapt at higher temperatures may result, in part, from a combined input from CMHs and an increasingly active population of sensitized A-fiber nociceptive afferents.
(Supported by NIH grant NS 14624)

2082 QUANTAL OPIATE ANALGESIA IN POST-OP PAIN. Jon D. Levine*, Newton C. Gordon*, Richard Smith* and Howard L. Fields. Dept. Neurol., Physiol., & Oral Surgery, UCSF, San Francisco, CA 94143.

The change in response to increasing drug dose may be discrete (quantal) or continuous (graded). Most researchers have assumed that opiate analgesia is a graded process. However, D'Amour and Smith (J. Pharmacol., 1941, 72, 74) and others, using the rat, have demonstrated a quantal suppression of tail flick by opiates. We present evidence that morphine analgesia in humans with clinical pain may also be quantal.

Twenty-one female and 18 male patients between the ages of 18 and 34 were studied. All patients underwent extraction of impacted third molars, without narcotics. Details of the procedure have been published (Levine et al, Lancet, 1978, ii, 654). At two hours following surgery all patients received a placebo (an equal volume of intravenous saline) and at three hours either a placebo again or a hidden intravenous injection of 4, 6, or 8 mg. of morphine. Pain was evaluated using a visual analog scale (10 cm. blank line; ibid.).

Blind administration of morphine produced a dose response curve which was monotonic over the range studied. However, individual patient's pain reports 50 minutes following morphine injection were clustered around two separate mean levels. The mean pain level of one of these clusters was above and the other below the pre-treatment mean. These clusters were defined as morphine responders and non-responders, respectively.

The mean pain level for responders (and non-responders) is independent of morphine dose. We found that for increasing dose, the decrease in mean pain is due to a larger percentage of morphine responders. Thus morphine analgesia is quantal. We have observed a similar division into two groups following placebo administration (Levine et al, PNAS in press). The mean pain level for morphine responders and consistent placebo responders (and non-responders) are not significantly different.

We conclude that opiate analgesia is a quantal process and that it may share a common neural circuit with placebo analgesia.

2083 EFFECT OF MORPHINE, PIMOZIDE AND NALOXONE ON RESPONDING BY RATS IN A SIGNAL DECISION PARADIGM: 23 DAY EFFECT OF PIMOZIDE. V. A. Lewis and I. Cathy Liles*, Department of Pharmacology, Dental Branch, University of Texas, Houston, 77025.

Signal detection theory has been employed in the evaluation of human response to noxious stimulation to differentiate the sensory components from the decision making or psychological components of the response. In this study, a signal decision paradigm has been adapted to evaluate the effect of analgesic and antipsychotic drugs on a foot shock escape response in rats. Four groups of 10 male Sprague-Dawley rats were trained to escape a 1.2 ma. foot shock by climbing onto a platform (15 x 9 x 3 cm.) After training, the effect of the narcotic-analgesic morphine (1,3,10 mg/kg); the selective dopamine-antagonist pimozide (.08,.20,.64 mg/kg); the narcotic-antagonist naloxone (1,3,10 mg/kg) and vehicle control were assessed on escape from tone and shock (HIT) and tone and no shock (False alarm, FA) contingencies. The frequency of escape and latency to escape were recorded for HITs and FAs. During any session only one drug and one shock probability was presented and 5 day rests were scheduled between sessions to minimize residual drug effects and tolerance. Sessions of 20 escape trials (7 s. trial and 15 s. ITI) with shock presented on 45% or 75% of the trials were run. The order of shock presentations, drug treatments and shock probabilities were randomized. Analysis of variance revealed significant effects for morphine and pimozide but not for naloxone, vehicle control or the different probabilities of shock. Morphine at 3 and 10 mg/kg significantly reduced responding to both HITS and FAs and increased the latency for response to shock. Morphine primarily increased the withdrawal criterion. Pimozide significantly lowered HIT and FA probabilities and prolonged escape latencies when compared to the vehicle control group, but not when compared to its within group vehicle control. These data suggested that pimozide had a prolonged behavioral effect lasting longer than 5 days. A pimozide time course study (0.64 mg/kg S.C in .1N acetic acid) demonstrated that pimozide maximally depresses responding between 8-24 hours post injection. HIT rates returned to control by day 5 but FAs were significantly depressed for 23 days and returned to control levels after 36 days. These studies demonstrate that for noxious stimuli, the response to HITs and FAs can be modified by pharmacologically distinct mechanisms and that dopamine is important in the generation of FAs. In addition, it has been shown pimozide may be a suitable agent for studying the long term effects of dopamine receptor blockade.

2084 STIMULUS-DEPENDENT CHANGES IN FOOTSHOCK SENSITIVITY FOLLOWING MEDIAL FOREBRAIN BUNDLE LESIONS IN THE RAT. Carlton E. Lints, Leonard H. Nenja*, and John F. Miller*. Dept. of Psychol., No. Illinois Univ., DeKalb, IL 60115.

Forebrain serotonin (5-HT) depletion has been reported to produce an increased sensitivity to footshock in rats, whereas forebrain norepinephrine (NE) depletion produces hyperalgesia as measured by the hot-plate technique. Thus, there appears to be stimulus specificity with respect to the type of hyperalgesia produced by forebrain monoamine depletion (e.g., Simansky & Harvey, ASFN, 1978, 4, 283). Medial forebrain bundle (MFB) lesions deplete the forebrain levels of both of these amines and have been reported to produce both types of hyperalgesia. However, difficulty in replicating the effects of MFB lesions on footshock sensitivity in this laboratory suggested that there might be considerable shock stimulus specificity within the forebrain 5-HT system mediating this effect. To test this possibility normal (N), sham-operated (SHM) and MFB-lesioned rats (N=8/gp.) were tested for sensitivity to footshock using the flinch-jump technique and two different constant current shockers that differed in terms of the nature of the current delivered to the grid floor of the test chambers (ac or dc). The animals were then retested with the ac shocker using three different shock durations (.05, .1 and .2 sec) with each of two different shock series (REG & Half). The REG series was the same series of shocks used in the initial tests and the Half series used current intensity increments 50% smaller than those of the REG series. The animals were always retested in the same chamber. Following behavioral testing the forebrains were assayed for 5-HT and NE levels and the brainstems were used for histological verification of the locus and extent of the lesions.

The lesions were well localized and produced 47% and 40% depletions of forebrain 5-HT and NE respectively. Jump thresholds of the MFB group were significantly lower than those of the SHM group with both shockers using the REG series, and there were no significant effects of the lesion on flinch thresholds. With the dc shocker the jump thresholds of the MFB group were also significantly lower than those of the N group, but this was not the case for the ac shocker. With both series on the ac shocker the jump threshold of all three groups decreased significantly as shock duration increased. However, only the .1 sec shocks in the REG series produced a significant lowering of the MFB jump threshold. Jump thresholds of the MFB rats were never significantly lower than those of the control groups with the Half series. Flinch thresholds of the lesioned rats were significantly lowered by the .05 and .1 sec shocks in the Half series and by the .05 sec shocks in the REG series.

2085 SENSATIONS AND MASSETERIC INHIBITORY PERIODS PRODUCED BY ELECTRICAL TOOTH PULP STIMULATION. Patricia A. McGrath*, Yair Sharav*, Richard H. Gracely* and Ronald Dubner (SPON: W.M. Falls). Neurobiology & Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20205.

Electrical tooth pulp stimulation in humans evokes inhibitory periods in the ongoing masseteric activity, that are usually regarded as indices of pain sensation. This study investigates both the sensations produced and the masseteric inhibitory periods evoked by electrical tooth pulp stimulation in 30 subjects. Stimuli consisted of a train of 30 monophasic, monopolar, 1 msec duration, cathodal pulses delivered at 2 sec intervals. EMG masseter activity, from surface electrodes, was rectified and averaged for the 30 pulses at each stimulus intensity. Three configurations of inhibitory periods were produced: Single, (S), a single inhibitory period with a latency of 10-15 msec and a duration of 10-20 msec; Double, (D), two inhibitory periods separated by a burst of muscle activity, the first having a latency and duration similar to S and the second having a latency of 40-50 msec and a duration of 10-20 msec; and Merged, (M), an elongated period in which the inhibitory periods of D seemed to merge. These inhibitory periods were correlated with stimulus strength, not magnitude of sensation; S and D periods were evoked at low and moderate stimulus intensities while M periods were evoked at high intensities. Non-pain and pain sensations were dissociated from inhibitory periods by the administration of a narcotic, fentanyl (.08 mg/45 kg body weight), in 15 subjects. Fentanyl significantly increased non-pain and pain thresholds and significantly reduced the magnitude of sensations in all subjects, but there were no changes in the latency, duration or configuration of the inhibitory periods. Non-pain and pain sensations were also dissociated from inhibitory periods by the application of an electrical conditioning stimulus (CS) to the central incisor in 15 subjects. CS, a 30 pulse train at a high stimulus intensity (70 uA ± 25), produced a significant elevation in non-pain and pain thresholds and a significant reduction in magnitude of sensation in the adjacent incisor, but produced no change in the masseteric inhibitory periods. The administration of an opiate antagonist, naloxone, 10 mg, partially reversed the suppression of sensation in 5 subjects tested. These data suggest that: 1) the masseteric inhibitory periods can occur independent of any detectable tooth pulp sensation; 2) narcotic manipulations reduce tooth pulp pain sensation, but do not affect the inhibitory periods; 3) the inhibitory periods involves trigeminal brain stem pathways distinct from those that mediate pain sensations; and 4) the suppression of tooth pulp non-pain and pain sensations by conditioning stimulation may be mediated in part by endogenous opiate pathways.

2086 PAG MORPHINE AND STIMULATION AND MORPHINE MICROIONTOPHORESIS EFFECTS ON BRAIN STEM NEURONS IN THE RAT. J. Scott Mohrland* and G. F. Gebhart, Dept. of Pharmacol., Univ. of Iowa, Iowa City, IA 52242.

Focal electrical stimulation or microinjection of morphine into the periaqueductal gray region (PAG) of the mesencephalon is known to produce analgesia. The purpose of the present investigation was to evaluate the involvement of neurons of the brain stem nucleus reticularis gigantocellularis (NGC), nucleus reticularis paragigantocellularis (NPGC), and nucleus raphe magnus (NRM) in the analgesia produced by these manipulations in PAG. Cannula electrode assemblies were implanted (chronically) into the PAG of 54 rats. Hot plate and tail flick tests were used to establish analgetic efficacy after either PAG stimulation or morphine administration (17.5 nmoles in 0.5 µl). Single unit activity in NGC, NPGC and NRM was recorded employing standard methods and the effects of PAG morphine and stimulation on both spontaneous and noxious stimulation evoked activity was examined in these brain stem areas. Multibarrel micropipettes were employed to locally apply morphine at the recording locus and compare effects to morphine applied at a distant, analgesia-producing locus (i.e., PAG). Stimulation of the PAG produced both excitation and inhibition of spontaneous activity in NGC and NPGC whereas only inhibition was observed in NRM. PAG stimulation inhibited the noxious evoked activity in greater than 80% of the cells in all three brain stem nuclei. Microinjection of morphine into the PAG produced an increase in spontaneous activity in 53%, 45% and 38% of the neurons in NGC, NPGC and NRM, respectively. Microiontophoresis of morphine at the recording site also produced excitation in these nuclei (42% in NGC, 54% in NPGC, and 20% in NRM) which could be reversed by microiontophoretic naloxone. Morphine administration in PAG inhibited noxious evoked activity in a high percentage of neurons in each of the three nuclei; inhibition of noxious evoked activity was rarely observed after morphine administered microiontophoretically. Morphine or stimulation at PAG cannula/electrode placements which were not analgesia-producing were without effect on noxious evoked activity in NGC, NPGC or NRM. Supported by NIH grants NS 12114, NS 06043 and GM 22026.

2087 RESPONSES OF SQUIRREL MONKEYS TO NOXIOUS AND INNOCUOUS THERMAL STIMULI. Thomas J. Morrow and Kenneth L. Casey. Depts. of Physiol. & Neurol., Univ. of Mich., Ann Arbor, MI 48109.

Pain assessment in animals is typically carried out using qualitative tests (pinch, pin prick, etc.), however, more quantitative measures would often provide more useful information. In response to this need, we have developed a humane, quantitative method of measuring the unlearned responses of squirrel monkeys to controlled natural stimuli. Subjects are trained to pull and hold one of two heated response levers (thermodes) to receive a liquid banana reward. Food availability is signaled by a light turning on over the respective thermode. Thermal pulse stimuli of 43, 47, 52 and 55°C (7 sec duration, 18°C/sec rise from a 38°C baseline) are delivered either to the hands or shaved tail during feeding or to the tail between feedings. The appropriate responses (thermode release or tail movement) terminate food delivery and/or the thermal pulse. A minicomputer controls the feeding schedule, stimulus parameters, timing and acquisition of response data. Each monkey has received an average of 309 ± 158 trials at each stimulus intensity.

When food availability is signaled (light on), the monkeys show a marked reduction in spontaneous movement, resulting in a lower number of responses to blank (38°C) trials as compared with the number of blank trial responses emitted between feedings. Consequently, all 3 monkeys show significant (χ^2 $p << .01$) responses to stimuli of 47°C delivered during feeding (prob. = 0.16),but show significant responses only to stimuli of 52°C or higher (prob. > 0.5ϕ) delivered between feedings. These differences are attributable to the smaller number of blank trial responses during feeding. Response latencies average 2.47 ± 0.55 (s.e.m) seconds from stimulus onset and are nearly the same during and between feedings; no changes in latency were noted as stimulus intensities increased. These data provide the necessary background for future neurophysiological and behavioral studies of pain mechanisms in this species and emphasize the importance of behavioral control (behavior clamping) during tests of responses to noxious stimuli.

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2088 INHIBITION OF SPINAL NEURONES BY STIMULATION OF RAPHÉ MAGNUS IN THE RAT. J.A. Pearson, M.C. Green* and Carolyn Watson*. Dept. of Physiology, Univ. of British Columbia, Vancouver, B.C., Canada, V6T 1W5

Stimulation of the mesencephalic periaqueductal grey (PAG) results in an elevation of pain threshold which is dependent upon the integrity of serotonin-containing neurones. In view of the fact that PAG stimulation causes inhibition of spinal interneurones it is possible that stimulation-produced analgesia might be a consequence of the activation of descending inhibitory systems. The axons of serotonin-containing neurones of the raphé magnus (RM), unlike those located within the mesencephalic dorsal raphé, project to the spinal cord. Stimulation of the RM has been shown to cause analgesia in the rat and cat. The RM might therefore be an essential relay mediating the inhibitory influence of PAG stimulation upon spinal neurones.

Experiments were carried out to determine the effect of RM stimulation upon the activity of spinal neurones in urethane-anaesthetized rats. Two populations of cells were studied: a) spinothalamic tract neurones, identified by antidromic activation by stimuli applied to the contralateral ventral thalamus, and b) unidentified spinal neurones. Most of these cells responded to cutaneous stimulation. They could be activated by both weak, tactile stimuli and by intense, noxious inputs. The response pattern to electrical cutaneous stimulation consists of a short latency (5-10 msec) burst of action potentials to weak stimuli and a late (>50 msec) discharge to high stimulus intensities. Trains of stimuli applied to RM (20-200μA; 200Hz; 0.2 msec; train duration 45 msec, starting 50 msec prior to the cutaneous stimulus) inhibited the responses to weak cutaneous stimuli in 86% of cells tested and those to intense stimuli in 67% of neurones. The mean, threshold, RM stimulus intensities required to inhibit responses to "non-noxious" and "noxious" inputs were 102 ± SE 11.0μA and 153 ± SE 16.0μA respectively. The difference between these values is significant ($p<0.02$; t-test). In contrast to the findings obtained from experiments in monkey and cat, RM stimulation was more effective in inhibiting responses to weak cutaneous stimuli than those to more intense inputs. RM stimulation was shown to block the antidromically evoked action potential in 53% of the spinothalamic tract cells. This implies that the inhibitory effect of RM upon responses to cutaneous inputs may be a consequence of a direct post-synaptic influence on these cells.

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2089 SYNAPTIC ACTIVATION AND INHIBITION OF NUCLEUS RAPHE MAGNUS (NRM) NEURONS FOLLOWING ELECTRICAL STIMULATION OF PERIAQUEDUCTAL GRAY (PAG) IN THE RAT. Scott Pomeroy and Michael M. Behbehani, Dept. Physiol., Coll. Med. U. of Cincinnati, Cincinnati, Ohio 45267.

Recent evidence has shown that electrical stimulation of PAG can produce analgesia by activating a system that projects to the spinal cord via the dorsolateral funiculus (DLF) to inhibit pain-transmitting neurons. Since PAG has no substantial projection to the spinal cord via the DLF, nuclei with direct spinal projections have been examined to determine whether they contribute to this analgesic system. It is now evident that the NRM has a critical role in the production of analgesia by PAG stimulation.

To examine the interaction of PAG and NRM, post-stimulus time histograms were recorded of NRM unit activity while electrically stimulating PAG. Single barreled glass electrodes filled with 2% pontamine sky blue in .5 M sodium acetate were made suitable for extracellular recording of single units and were stereotaxically positioned in NRM. Bipolar tungsten stimulating electrodes were positioned into ventrolateral PAG. Stimulus parameters: frequency = 1 Hz, duration = 30-50 microsec., amplitude = .5-2 ma. In approximately one-half of the experiments, NRM neurons were tested for spinal projection by electrically stimulating the lumbar DLF. NRM neurons were designated as raphe-spinal if DLF stimulation produced antidromic responses which were abolished by collision with orthodromic spikes. A total of 67 neurons were studied in all experiments. The distribution of responses was found to be: 54% facilitation, 15% inhibition, 12% facilitation and inhibition, and 19% no response. The mean latency to onset of facilitated response was 12.0 ± 1.6 ms (mean ± S.E.M.) and the mean latency to onset of inhibition was 2.8 ± .3 ms. Of 34 neurons tested, 26% were shown to have spinal projections (mean conduction velocity was calculated to be 14.6 m/s). No overt differences were seen in comparing distribution or latency of facilitated response between raphe-spinal and non-raphe-spinal neurons. For seven cells, the extent of current spread at the stimulating electrode was measured by systematically moving the PAG electrode 1,2, or 3mm dorsally or ventrally and recording additional post-stimulus time histograms. In all cases, moving the electrode 2mm dorsally abolished the response. Three neurons showed an attenuated response at 2mm ventral to reference but no response at 3mm ventral to reference.

On the whole, these data show that electrical stimulation of PAG most often leads to synaptic activation of NRM neurons. This lends further support to the hypothesis that activation of raphe-spinal neurons mediates spinal anti-nociceptive responses to PAG stimulation.

2090 EVIDENCE FOR SITE SPECIFICITY IN NALOXONE'S ANTAGONISM OF STIMULATION-PRODUCED ANALGESIA IN THE RAT. G. J. Prieto*, G. J. Giesler, Jr., and J. T. Cannon (SPON: J. C. Liebeskind). Dept. Psychol., UCLA, Los Angeles, CA 90024.

The phenomenon of stimulation-produced analgesia (SPA) has been characterized by inter-subject variability both in post-stimulation duration of SPA and in naloxone's ability to block it. We sought to determine if some of this variability could be due to differences in stimulation loci. Rats were implanted with bipolar stimulating electrodes along the midline of caudal periaqueductal gray (PAG) and the median raphe n. Stimulation consisted of constant current, biphasic pulse pairs (20/sec). Each member of the pair was 50 μsec in duration, with a 100 μsec separation. Analgesia was assessed with the tail-flick test using a modified ascending method of limits to determine currents necessary to inhibit this reflex (cut-off latency = 7 sec). In each session, separate determinations were made for thresholds of "during-stimulation" analgesia (DSA) and "post-stimulation" analgesia (PSA). Stimulation was delivered either for 10 sec before and continuing throughout tail-flick testing (DSA) or for 15 sec immediately prior to tail-flick testing (PSA). After DSA and PSA threshold determinations on the second and third test days, rats were injected with either naloxone (10 mg/kg, s.c.) or saline according to a counterbalanced order, and thresholds were reassessed. Most animals later received lesions of the n. raphe magnus (NRM) and were tested again at least 1 week after surgery.

Several differences of interest were found between placements in or below dorsal raphe n. versus those in PAG above this structure. For more dorsal placements, currents necessary to produce DSA (mean = 2.3 ma) and PSA (mean = 5.1 ma) were highly correlated ($r = .91$, $p < .01$). For deeper placements, however, this correlation was weaker ($r = .52$, n.s.), and significantly higher currents were required for PSA (mean = 10.8 ma) than was true for the dorsal placement group ($p < .01$). DSA thresholds for deeper electrode placements (mean = 2.7 ma) did not significantly differ from those of the dorsal group. Several animals with ventral placements were exceptional in that they failed to exhibit PSA within the current limitation of the stimulator (18 ma), even though their DSA thresholds were in the normal range. Whereas naloxone failed to antagonize SPA in any of the 16 animals with dorsal placements, SPA thresholds were elevated by this drug in 6 of 12 animals with ventral placements. To date, NRM lesions have not been seen to cause reliable changes in SPA thresholds even when adjacent reticular structures were invaded.

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2091 EFFECTS OF MEDULLARY RAPHE LESIONS ON MORPHINE-INDUCED ANALGESIA AND NOCICEPTIVE THRESHOLD: DEPENDENCE ON THE POST-LESION TEST INTERVAL. Herbert K. Proudfit, Dept. of Pharmacol., Univ. of Ill. at the Med. Ctr., Chicago, IL., 60612.

Electrolytic lesions of the nucleus raphe magnus (NRM) result in hyperalgesia and attenuation of morphine-induced analgesia (MIA) when tested between ten days and two weeks following surgery (Proudfit and Anderson, 1975). However, in subsequent studies animals tested at seven days or less following the placement of lesions, showed no alteration in MIA. Therefore, we examined the time-course of lesion effects on both nociceptive threshold and MIA. Following electrolytic lesions of either the NRM or raphe pallidus, rats were tested at seven day intervals for 35 days using the tail flick test. Unoperated control rats were similarly tested. Seven days after lesioning the nociceptive threshold was reduced by 33%, and by 14 days by 42% of control. However, testing at subsequent seven day intervals showed a gradual recovery and return to control values by day 35. By contrast, the effect of lesions on MIA (5 mg/kg) was quite different. No effect was seen at seven days, but testing at subsequent seven day intervals revealed a gradually increasing attenuation of MIA throughout the 35 days of testing.

The effect of reversible lesions of the NRM produced by the microinjection of the local anesthetic tetracaine (TET; 5 ug in 0.5 ul of saline) was also examined. Rats were implanted with chronic indwelling microinjection guide tubes (25 ga) and seven days after surgery the nociceptive threshold was determined and morphine sulfate (5 mg/kg, sc) was injected. At the peak of MIA (30 min) TET was slowly infused through a 28 ga injection cannula into the NRM. Tail flick latencies determined 5, 10, 15, 25 and 45 minutes after TET injection were not significantly different from those determined before TET. However, when TET was injected locally in the absence of morphine pretreatment, the animals exhibited hyper-reactivity. This effect was evident immediately after the injection, but declined to near normal reactivity by 45 minutes.

These results suggest that raphe lesion-induced attenuation of MIA and hyperalgesia are mediated by the destruction of separate neuronal systems. In addition, the failure of acute raphe lesions or TET-induced inhibition of the NRM to alter MIA indicates that the NRM is not involved in mediating the anti-nociceptive actions of opiates. It is likely that an effect secondary to destruction of the NRM is responsible for the attenuation of MIA observed following chronic NRM lesions.
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2092 INTRACEREBRAL SUBSTANCE P IN MICE: BEHAVIORAL EFFECTS AND NARCOTIC AGENTS. Anita Rackham and Norman N. Share*. Dept. Pharmacol., Merck Frosst Laboratories, Kirkland, Quebec, Canada.

In preliminary studies with mice, acute intracerebral injection of substance P was found to induce a unique behavioral phenomenon characterized by reciprocal hindlimb scratching movements. In mice weighing 18 to 22g, an injection site approximately midline at the level of the external auditory meatus, using a 27 gauge hypodermic needle of 3.5 mm in length, yielded the most dramatic and consistent responses. Dye marker experiments indicated injection directly into the IIIrd ventricle with the solution (2 µl) spreading to all cerebral ventricles.

Acute intracerebral injections of substance P induced distinctive behavioral changes whose intensity appeared dose-related. Within 60 to 90 seconds, the mice seemed agitated, preened excessively, and engaged in reciprocal hindlimb scratching movements directed toward the sides of the upper body and jaw areas. These latter responses were paroxysmal, being interspersed with periods of exaggerated preening and biting at the abdomen and hindquarters. The substance P-induced reciprocal hindlimb scratching response was the most consistent and reproducible of the behavioral responses observed, generally occurring within 2 minutes and always within 5 minutes after intracerebral injection. Considering a single reciprocal hindlimb scratching episode observed within 5 minutes after intracerebral injection as a positive response, the ED_{50} was for substance P ≃ 0.06 µg/mouse. Similar intracerebral dose-responses were obtained by the related undecapeptides physalaemin (ED_{50} ≃ 0.025 µg/mouse) and eledoisin (0.004 µg/mouse), but not by several unrelated peptides (TRH, neurotensin, bradykinin, somatostatin).

Analgesic narcotic agents with predominant agonist activity administered intraperitoneally prevented the reciprocal hindlimb scratching response induced by intracerebral substance P (0.625 µg/mouse = ED_{95}). In this in vivo assay their order of potency (etorphine > etonitazine > phenazocine = levorphanol > dihydromorphine ≥ methadone ≥ morphine > meperidine ≥ d-propoxyphene ≥ codeine) was similar to that reported for binding to rat opiate receptors in vitro. Narcotic agents with predominant antagonist activity (nalorphine, pentazocine) were inactive while the narcotic antagonist naloxone completely reversed the action of morphine.

Evidence suggests that the undecapeptide substance P may serve as an important transmitter or modulator of sensory processes, and its action may be associated with algesia or analgesia. (Fredrickson et al, Science, 199: 1359, 1978). The above findings suggest that acute intracerebral substance P-induced reciprocal hindlimb scratching responses in mice involves algesic systems within the central nervous system.

2093 COMPARISON OF ALTERNATING VERSUS DIRECT CURRENT ELECTROANALGESIA APPLIED THROUGH INTACT TOOTH ENAMEL. Patrick J. Reynolds, Ann Kloka*, Richard B. Tacke* and R. Wayne Fields. School of Dentistry, University of Oregon Health Sciences Center, Portland, Oregon 97201.

We have shown that trains of rectangular pulses, of 10% duty cycle at 1000Hz, capacitively coupled to effect alternating polarity, can block afferent activity from cat tooth pulp when applied to exposed dentin at levels of 70-100µA as effectively as direct anodal current of similar strength (Fields et al., Abstr. Soc. Neurosci. 4-459, 1978). For clinical use for electroanalgesia (EA), low duty cycle alternating pulse trains are conceptually superior to direct current (Fields et al., Oral Surg. 34:694-703, 1972) because of lower power dissipation (small duty cycle) and reduced monodirectional iontophoresis (alternating polarity). In unpublished observations, we have also found that neither alternating nor direct currents ranging to 1000µA cause histologically detectable pulpal aberrations when applied to intact teeth (through the enamel). Such current application simulates a practical clinical EA protocol, but it is not known if it truly induces afferent unit hypoexcitability.

This question was addressed in the present studies in which excitability of single pulp-driven units in cat Gasserian ganglion was observed during and following application of 0-100µA ac (1000Hz, 10% duty cycle rectangular pulses, capacitively coupled) and dc EA current to intact maxillary canine teeth. The excitability index was in terms of threshold to electrical stimulation via a pair of dentinal electrodes (e.g., Fields, et al., Exp. Neurol. 53:386-398, 1976). For both ac and dc waveforms a progressive rise in pulp-driven unit threshold, compared to pre-EA control, was seen with increasing current over the 0-100µA range. Threshold elevation of at least 500% was seen for both waveforms at 100µA after as little as 1 minute duration. Recovery to control threshold following 1 minute of dc at 100µA required as much as 10 minutes; for the majority of units examined, recovery periods following ac were shorter than following dc of similar intensity.

These results demonstrate that a) both ac and dc EA currents applied to intact enamel of cat maxillary canine teeth are capable of attenuating activity of pulp-driven units in the Gasserian ganglion, and b) the ac EA employed exhibited adequate efficacy but superior recovery characteristics when compared to dc of similar intensity.
(supported by NIH Grant DE 04281)

2094 PROFOUND POTENTIATION OF MORPHINE'S ANALGETIC POTENCY BY CONCURRENT INTRATHECAL AND INTRAVENTRICULAR ADMINISTRATION. Thomas A. Rudy and Joseph C. Yeung*. School of Pharmacy, Univ. of Wisconsin, Madison, WI 53706.

To examine the mode of interaction between the spinal and supraspinal narcotic-sensitive structures in the mediation of analgesia, we have measured the antinociceptive effect [hotplate (HP) and tailflick (TF) methods] produced by concurrent intrathecal (i.t.) and intraventricular (i.vt.) injections of morphine sulfate. Rats in which the fourth ventricular exits had been acutely occluded received an i.t. injection of morphine (lumbar spinal subarachnoid space; 0-10 ug in 4 ul) followed immediately by an i.vt. injection (ventral third ventricle; 0-50 ug in 5 ul). Each rat was used in only one experiment. That there was no significant transfer of morphine between the spinal and supraspinal compartments at the time of peak analgesia was shown in diffusion studies using radiolabeled morphine.

It was found that the analgetic potency of morphine injected i.vt. was profoundly potentiated by concurrent administration of morphine i.t., and vice-versa. Dose-response curves (minimum of 50 rats/curve) for i.vt. morphine were shifted progressively to the left as the spinal dose of morphine was increased. At the optimal balance of spinal and supraspinal dosage, the ED50's for i.vt. morphine for the HP and TF tests were reduced by factors of 45 and 33, respectively. A similar, but less profound, shifting of the i.t. dose-response curves was seen when the i.vt. dosage was progressively increased. Isobolographic analysis of the data revealed that the isobols were hyperboloids having extreme negative curvature at all effect levels. Inspection of the isobols indicated that, at all ratios of spinal to supraspinal agonism which could conceivably be obtained when morphine is given systemically, the spinal-supraspinal interaction is purely multiplicative. In a multiplicative interaction, removal of either input reduces output to zero. Thus, it seems likely that narcotic agonism at both the spinal and supraspinal morphine-sensitive structures is essential to the production of analgesia by systemically administered morphine and that neither action locus can be considered the "primary" site of action.

2095 MODULATION OF SENSORY EXCITABILITY IN THE CAT TOOTH BY EXTRACELLULAR POTASSIUM AND SODIUM. Donald Scott, Jr. and W.I.R. Davies* Dept. Physiol. University of Penna. Phila.,Penn.19174

The effect of thermal, mechanical and other forms of tooth stimulation encountered by normal mammals have been extensively studied and shown to evoke characteristic impulse frequency patterns which can be recorded by electrodes in dentinal cavities. Whereas the response to cold stimulation was similar to that observed when cooling an axon, the effect of heat showed marked differences which could be attributed to the specialized receptor. Davies (1969) examined the excitability of dentinal receptors in the cat tooth in response to heat and found an increase of excitability when extracellular potassium was decreased and an inhibition when it was increased. These observations have been confirmed and extended. However, increases in extracellular sodium concentration have only resulted in comparable increases in excitability. Since the sensory response to heat is only slightly affected by topical application of tetrodotoxin (TTX), sodium may not be a major contributor to the transducer process. This finds support in the report by Rockert (19) that intracellular potassium in the odontoblast process may be significantly above the level in dentinal fluid. A scheme for transducer function consistant with these observations is proposed.

2096 ENDOGENOUS OPIATE-RELATED INFLUENCES FROM PERIAQUEDUCTAL GRAY AND NUCLEUS RAPHE MAGNUS ON SOLITARY TRACT NEURONS AND RESPIRATORY-RELATED FUNCTIONS. B.J. Sessle, G.J. Ball and G.E. Lucier (SPON: A.T. Storey). Fac. of Dentistry, Univ. of Toronto, Toronto, Canada, M5G 1G6.

Much emphasis has recently been placed on the role of the periaqueductal gray matter (PGM) and nucleus raphe magnus (NRM) in endogenous opiate-related mechanisms of analgesia. Little consideration has been given to possible influences from these two sites on functions other than those involved in pain and its control. Yet projections from one or both these sites, as well as opiate receptors, have been found concentrated in central regions concerned with functions other than nociception, e.g. the solitary tract nucleus (STN), the site of respiratory reflex interneurons and respiratory neurons. We wished to determine if the activity of such STN neurons, and respiration and related reflex functions, are influenced by PGM and NRM stimulation and if any observed influences could be reversed by the administration of the opiate antagonist naloxone.

In anesthetized cats, we first determined if some of the functions that are subserved by STN neurons (respiration, swallowing, coughing) could be influenced by PGM and NRM stimulation. Only a transient depression of respiration was observed with PGM and NRM stimuli, but a marked decrease occurred in the incidence of coughing and swallowing elicited by stimulation of the superior laryngeal nerve or upper respiratory tract; naloxone (0.4 mg/Kg, I.V.) could reverse these PGM and NRM induced depressive effects. The effects were reflected in changes in the activity of functionally identified single neurons recorded during microelectrode penetrations of STN. These neurons were characterized by their rhythmic respiratory-related activity, or their short-latency reflex responsiveness to low-threshold superior laryngeal or vagal nerve stimuli. The rhythmic activity of respiratory neurons was generally retained during PGM and NRM stimulation, although a decrease in the peak firing frequency of each rhythmic burst was regularly observed. In contrast, the reflex responses of STN neurons could be powerfully suppressed by PGM and NRM stimulation, and the suppression could be reversed by naloxone.

These studies indicate that respiration and in particularly the associated reflex activities of coughing and swallowing are depressed by endogenous opiate-related influences derived from PGM and NRM. Such effects may be associated with the depression of respiration and coughing that can result from opiate overdose. The depressive effects appear to be reflections of the susceptibility of STN neurons that are involved in respiration and associated reflex activities to inhibitory influences from PGM and NRM, and indicate that PGM and NRM may be involved in functions other than analgesia. (Supported by NIH).

2097 LOCAL BRAIN GLUCOSE UTILIZATION EVOKED BY DENTAL PULP STIMULATION IN THE RAT AND THE CAT: A COMPARATIVE STUDY. Andrew G. Shetter and Carol Kreinick.* Neurosurgical Research Laboratory, Barrow Neurological Institute, Phoenix, AZ 85013

The pattern of local brain glucose uptake produced by dental pulp stimulation in cats and rats was investigated using the 2-deoxyglucose method of Sokoloff. Bipolar electrodes were inserted into the pulp cavity of either a mandibular or a maxillary incisor tooth under pentobarbital anesthesia. Constant current 10Hz electrical pulses were delivered at an intensity sufficient to produce a visible jaw jerk reflex. An intravenous injection of ^{14}C-2-deoxyglucose (10μCi/100gm) was given and continuous stimulation was performed for 45 minutes. Autoradiographs were prepared from unfixed sections of the brainstem and upper cervical spinal cord, and the resultant optical density patterns were analyzed with the aid of a microdensitometer. Control groups included animals who had electrodes implanted but not stimulated, and those in whom maximal stimulation was performed after surgical exoneration of the pulp cavity.

Stimulus-related increases in glucose uptake were seen throughout the entire trigeminal sensory system in both species, extending from nucleus caudalis at C1-2 to nucleus oralis - main sensory nucleus in the rostral midbrain. Enhanced glucose metabolism was less pronounced in nucleus interpolaris, and no changes were apparent in the trigeminal mesencephalic nucleus or the motor nucleus. The increases in metabolic activity produced by dental pulp activation in the rat were exclusively ipsilateral to the site of stimulation. Alterations in the cat were present bilaterally but were maximal on the ipsilateral side. Afferent input from mandibular pulp stimulation evoked increased glucose utilization in the most dorsal portions of the trigeminal sensory nuclei. Maxillary pulp input was represented more ventrally and covered a larger area. This somatopic distribution was apparent in both species, but was more discrete in the rat than in the cat. No alterations in glucose uptake were detected in extra-lemniseal or reticular pathways for any of the animals studied. The 2-deoxyglucose technique appears to be a useful means of investigating trigeminal nociceptive systems.

2098 TRIGEMINAL PRIMARY AFFERENT DEPOLARIZATION AND REFLEX INHIBITION PRODUCED BY PERIAQUEDUCTAL GRAY AND RAPHE MAGNUS STIMULI: INFLUENCE OF NUCLEUS CAUDALIS AND EFFECTIVENESS OF OTHER BRAINSTEM STIMULATION SITES. R. Sumino, J.W. Hu, J.O. Dostrovsky and B.J. Sessle. Fac. of Dentistry and Dept. of Physiology (J.O.D.), Univ. of Toronto, Canada M5G 1G6.

We have recently found that electrical stimulation of the periaqueductal gray matter (PAG) or nucleus raphe magnus (NRM) can cause a powerful suppression of the digastric (jaw-opening) reflex and primary afferent depolarization (PAD) of tooth pulp afferent endings in trigeminal (V) brainstem nuclei oralis or caudalis. Many recent studies have implicated nucleus caudalis in the relay of orofacial pain, and PAG and NRM in endogenous opiate-related mechanisms of analgesia. Moreover, caudalis receives a direct projection from NRM and is also a major site of opiate receptors. Thus it might be expected that the reflex inhibition and PAD in oralis induced by PAG and NRM stimulation would be abolished by procedures that disrupt nucleus caudalis. Accordingly, we have examined the effects of such procedures on these PAG and NRM induced effects. We have also tested to see if the reflex inhibition and PAD effects are specifically induced from PAG and NRM or whether they can be produced by stimuli delivered to brainstem regions outside PAG and NRM.

Utilizing methods previously described (Brain Res. 117: 211, 1976; Nature 276: 283, 1978), we verified in anesthetized or decerebrate cats that PAG or NRM stimulation could induce suppression of the digastric reflex evoked by pulp or low-intensity infraorbital nerve stimuli, and PAD of pulp afferent endings in oralis. A V tractotomy procedure or cold block of synaptic transmission in nucleus caudalis was then used to assess the influence of caudalis in these effects. The cold block procedure was used most often since it has the advantage that it is reversible and tests could be repeated numerous times in the same animal. Although tractotomy or cold block abolished synaptic transmission in caudalis, we consistently found no change in the threshold inhibition of the digastric reflex evoked by pulp or low-intensity infraorbital nerve stimuli or in the PAD of pulp afferent endings in oralis. This indicates that these effects of PAG and NRM are not dependent on relays through nucleus caudalis for their production. Furthermore, brainstem regions other than PAG and NRM may be similarly involved in the reflex inhibition and PAD in view of results obtained by carefully mapping the effects of stimuli applied in a series of electrode penetrations within and outside PAG and NRM in 6 cats. Reflex inhibition and PAD could be induced from reticular formation areas outside PAG and NRM at stimulation intensities that were comparable to those effective within PAG and NRM.

2099 FATIGUE AND SENSITIZATION OF NOCICEPTIVE AFFERENTS IN THE MONKEY. J.G.Thalhammer* and R.H. LaMotte. Dept. ofAnesthesiology Yale University, New Haven, Conn. 06510. (SPON.:J.G.Collins)

In these studies we measured the time course of changes in responsiveness of nociceptive afferents in monkeys during and following intense local heating of the skin. Twenty-three C-fibers (CMHs) and 25 A-fibers (AMHs) were classified as sensitive to noxious mechanical and noxious heat stimuli. Initial heat thresholds of all AMHs were greater than 48°C while those of CMHs averaged 45°. A conditioning stimulus (CS) of 50° (60s duration) was delivered to the receptive fields of 14 CMHs and 17 AMHs and 9 warm (C) fibers innervating the hand or foot. During the CS, CMHs responded with a relatively high rate of discharge at onset of the stimulus and then adapted,while most AMHs (n = 12) responded only after a long latency with a rate of discharge that increased gradually. The warm fibers responded only during the first few seconds of the stimulus. After termination of the CS, brief stimuli of 39-47° or 39-51° were delivered over a period of 0.5-30 min. Initially, CMHs were fatigued and their thresholds increased to 2-8° above corresponding values obtained prior to the CS; but, by 5-10 min after the CS,the thresholds were 2-6° below prior values and their responses to suprathreshold stimuli greater than normal (sensitized) and remained so up to the end of testing. At this time, some CMHs had decreased thresholds to mechanical stimuli and some could be activated by lightly rubbing the skin with gauze.

All the warm fibers were suppressed without sensitization following the CS. Six of those 12 AMHs responsive to the CS became sensitized within 10 min after the CS. Neither AMHs nor CMHs developed spontaneous activity. Many of those AMHs that did not become sensitized following the 50° CS did so following multiple stimulations of higher temperatures (e.g. 53°, 5-20s) and some of these developed spontaneous activity as well as lowered mechanical thresholds and a sensitivity to rubbing the skin with gauze.

Fatigue and sensitization of CMHs following a stimulus of 50° for 60s may account, in part, for the sensory phenomena of suppression and hyperalgesia (LaMotte et.al., this vol.). The sensitization of AMHs is likely to contribute to the hyperalgesia induced by stimuli of higher intensities or longer durations.

(Supported by NIH grant NS 14624)

2100 A NEUROANATOMICAL STUDY OF ANALGESIA AND CATATONIA INDUCED BY ETORPHINE IN THE RAT. B. E. Thorn, R. A. Levitt, J. T. Hill and K. Ward. Dept. Psychol. and Sch. Med., Southern Ill. Univ., Carbondale, IL 62901; Dept. Psychol., Univ. of Ala. in Birmingham, Birmingham, AL 35294.

Etorphine hydrochloride is a fast-acting narcotic analgesic, several thousand times more potent than morphine. We studied the analgesic and catatonic properties of etorphine when microinjected into one of 11 neuroanatomical sites: periaqueductal gray; midbrain reticular formation, cerebral aqueduct; cerebellum; caudate putamen; basolateral amygdala; cortico-medial amygdala, globus pallidus, medial thalamus, hippocampus. Each site was represented by a minimum of 6 subjects.

The flinch-jump technique was used to assess pain sensitivity and the bar test was used to study catatonia. Etorphine was administered in a 1 µg dose. Each animal was used only once in one experiment and was administered etorphine only one time. The animals received a baseline bar test followed by a flinch-jump test followed by a second bar test (taking 10 min). They were then injected with drug (either water or etorphine in a 1 µl solution) into one neuroanatomical location. All animals received both water and etorphine, presented in a counterbalanced order and separated by a four-day interval.

Neuroanatomical location ranged from AP+3.0 to AP-9.0. Significant analgesia and catatonia was elicited with 1 µg injections of etorphine into the periaqueductal gray (PAG), the midbrain reticular formation (MRF) and the cerebral aqueduct (AQ). Degree of analgesia or catatonia was not significantly different between these three sites. Injection of 1 µg etorphine into the medial thalamus (MT) resulted in analgesia and catatonia that approached significance. Injections into the cerebellum (CB), basolateral amygdala (BLA), cortico-medial amygdala (CMA), caudate putamen (CPU), globus pallidus (GP) or hippocampus (HP) failed to elicit significant elevation of the nociceptive threshold, although catatonia was elicited in the CMA and GP.

Although in most cases a high correlation was found between analgesia and catatonia, this and previous studies (Thorn and Levitt, 1978) suggest that at some sites these behavioral effects may be separable. Interesting differences between results of this and other studies will be discussed. Results suggest a possibility for separate neurological substrates for analgesia and catatonia. Additionally, results show at least some site-specificity for analgesia and catatonia after etorphine injection, suggesting that the effect is not just a result of gross diffusion following microinjection of a lipophilic substance in the brain.

2101 EFFECTS OF 6-HYDROXYDOPAMINE AND 5,7-DIHYDROXYTRYPTAMINE BRAINSTEM LESIONS ON MORPHINE ANALGESIA. Yousef Tizabi, V. John Massari, Thomas L. O'Donohue*, David M. Jacobowitz and Agu Pert. Dept. Pharmacol., Howard Univ., Washington DC 20059 and Lab. Clin. Sci. and Biol. Psychiat. Br., NIMH, Bethesda, MD 20205

Opiate analgesia, at least in part, appears to be determined by the activation of descending pathways from the brainstem to the spinal cord. Considerable evidence has been presented to implicate both descending serotonergic (5-HT) pathways originating from the nuclei raphe magnus, pallidus and obscurus and noradrenergic (NE) pathways originating from the A-1, A-2 and A-6 catecholamine nuclei in morphine analgesia. To decide which pathways are predominantly involved, morphine analgesia was assessed in rats that had been lesioned in the brainstem raphe nuclei with 9 µg of 5,7-dihydroxytryptamine or in the A-1 catecholamine nuclei with 6 µg of 6-hydroxydopamine injected bilaterally. At the termination of the study, the spinal cord was removed and sectioned into 500 µm slices. The dorsal and ventral horns were microdissected from these slices, and monoamines were assayed using sensitive radioenzymatic methods. The lesions were confirmed by measuring the monoamines at the injected sites. Destruction of the raphe nuclei resulted in a 50% depletion of 5-HT in the dorsal horn. NE in the ventral horn was unaffected. Destruction of the A-1 catecholamine nuclei resulted in a 64% depletion of NE in the dorsal horn. Analgesia was assessed 30 min after injections of either 5 or 10 mg/kg of morphine in the tail-flick, hot-plate and finch-jump tests. The effects of the lesions were relatively subtle and varied in modifying morphine analgesia across the various tests. Raphe lesions had little effect in the tail-flick test, whereas morphine analgesia in the hot-plate and flinch-jump tests was slightly attenuated. A-1 lesions appeared to attenuate morphine analgesia in the hot-plate tests and at 5 mg/kg in the tail-flick test. Combined lesions of A-1 and raphe nuclei were not significantly more effective in attentuating morphine analgesia. Thus, neither system appears to be critical for mediating systemically induced opiate analgesia. This suggests either the presence of other descending systems that are activated by morphine, the ability of the remaining descending catecholamine pathways to mediate opiate analgesia or the predominance of spinal actions. Studies are underway to ascertain whether these monoaminergic lesions would be more effective in attenuating morphine analgesia induced by direct injections either into the periaqueductal gray matter or the n. gigantocellularis. Additional studies are directed at ascertaining the importance of other catecholaminergic brainstem nuclei (e.g., A-2, A-5 and A-6) in morphine analgesia following either single or combined lesions.

2102 ECTOPIC GENERATION OF IMPULSES IN PERIPHERAL SENSORY NERVE FIBRES IN MAN. Erik Torebjörk* and José Ochoa. University Hospital, Uppsala, Sweden, and Dartmouth Medical School, Hanover, NH, USA.

Although much is known about pathological peripheral nerve fibres as abnormal conductors of impulses, little is known about them as abnormal generators. There are some insights into abnormal spontaneous activity of motor nerve fibres, but knowledge about similar activity in sensory nerve fibres in man is virtually nonexistent. This paper reports abnormal activity in single peripheral sensory fibres and correlates such activity with abnormal sensation, in volunteers experiencing a variety of paresthesiae.

Methods: Paresthesiae were induced after release of a sphygmomanometer cuff inflated above systolic blood pressure round the arm for about 30 minutes. Single unit impulses were recorded from sensory nerve fibres in the median and ulnar nerves, proximal and distal to the cuff, using the microneurographic technique of Vallbo and Hagbarth (1969).

Results: "Buzzing" paresthesiae of high frequency, referred predominantly to finger tips, peaked during the first 90 seconds after deflation of the cuff. High frequency unitary discharges (up to 220 impulses/sec) lasting for 1-7 seconds were prominent during this stage of paresthesiae. "Buzzing" was progressively substituted by sharp painless "pins and needles" and intermitent "tingling"; now the neural bursts recorded were shorter and often appeared in a rhythmical fashion.

Conclusions: Post-ischemic paresthesiae in man are due to abnormal impulse generation within the sensory unit. Recognizable changes in perception of paresthesiae follow modulations of frequency, duration, and rhythm of the abnormal discharges. It is possible, but not yet proven,that a simple change in the rhythm of discharge might determine a fundamental change in the quality of sensation under these abnormal circumstances.

Although the contribution to paresthesiae from different fibre types has not yet been investigated, the sustained, very high frequency of discharge recorded in some fibres endorses participation of myelinated fibres.

2103 INTRACARDIAC BRADYKININ INJECTIONS AND THEIR EFFECT ON SPINAL NEURONS HAVING VISCEROSOMATIC CONVERGENCE. R. Neal Weber* and Robert D. Foreman, Dept. Physiology & Biophysics, Univ. of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma 73190.

Previous studies from this laboratory have shown that the neural discharge rate of spinal neurons either increased or decreased during occlusion of the left coronary artery or the pulmonary artery. The purpose of this study was to determine whether an algesic substance such as bradykinin would influence the discharge rate of these cells when the substance was injected into the heart. The activity of single cells was recorded extracellularly from the left gray matter of the T_2 and T_3 spinal cord segments in 12 chloralose anesthetized cats. All the cells responded both to mechanical manipulation of the somatic receptive field and to electrical stimulation of the left T_2 to T_3 sympathetic chain. These cells responded to noxious pinch when it was applied to the receptive field of the left forelimb and chest. After the cells were tested for viscerosomatic convergence, bradykinin was injected into the chamber of either the left atrial appendage or left ventricle. The injected concentration of bradykinin varied between 100 µg/ml and 1 mg/ml in doses of .2 to .3 ml giving a total of 20 to 300 µg per injection. A total of 31 cells responded to both somatic manipulation and sympathetic stimulation. Fourteen of the 31 cells responded to the chemical injection while the remainder showed no change in their discharge pattern. Of the 14 cells, 13 were excited and one was inhibited following the chemical injections. The latency from the injection to onset of the response ranged from 4 to 20 seconds and had an average latency of 11 $\pm$ 0.4 (S.E.)s. Following the intracardiac injections, the same volumes and concentrations of bradykinin were injected into the femoral artery. Five of the 11 cells tested were responsive but they had a lesser increase in discharge rate and the onset of the response averaged 20 s. In two cells the excitatory responses to bradykinin were reduced or abolished following transection of the left sympathetic chain. These results lead to the suggestion that injections of bradykinin caused the excitation of cardiac receptors whose afferents made synaptic connections with cells of the thoracic spinal cord. Since these cells received input from nociceptors located in somatic receptive fields and from visceral receptors activated by algesic chemicals, they may be involved in transmitting information that is important for referred pain. Supported by NIH Grant 22732 and the Oklahoma Affiliate of the American Heart Association.

2104 LONG TERM THERMAL ANALGESIA AND SPINAL SUBSTANCE P (sP) DEPLETION BY INTRATHECAL CAPSAICIN (C). T.L. Yaksh, D.H. Farb, S.E. Leeman, G.M. Tyce*and T.M. Jessell. Mayo Fdn., Rochester, MN 55901; Dept. of Pharmacology, Harvard Med. Sch., Boston, MA 02115.

Following the rationale that sP may be a nociceptive transmitter in primary afferents, its depletion in the spinal cord might result in analgesia. C given systemically can deplete sP in the cord. We therefore sought to examine the effect of this drug administered intrathecally on the nociceptive threshold and sP levels in the rat. To carry out these experiments, rats were chronically implanted with intrathecal catheters inserted through the cisterna magna into the lumbar subarachnoid space. Following a 7 day recovery, animals received C (3 or 30 µg/15 µl of 50% saline-dimethysulfoxide vehicle) or vehicle. The injection of C, but not vehicle produced a 2-phased dose-dependent response: the first, lasting 1-3 min was characterized by muscular contractures; in the second phase the animal regained voluntary control and exhibited intense gnawing and scratching of the caudal regions of the body (15-30 min). After these phases the animals displayed no unusual motor signs and exhibited normal reflex function. Subsequent nociceptive testing revealed that 60-70% of the animals receiving 30 µg C showed no response to otherwise noxious thermal stimuli as measured with the 55°C hot plate or tail flick. These animals showing significant blockade continued to show no response for periods up to 3 months after C treatment (until natural death or sacrifice). The aversive behavior produced by subcutaneous injections of formalin (0.1 ml/2%) was substantially reduced but not blocked in C-treated animals as compared to non-treated controls. In contrast to the elevated thermal and chemical threshold, these C-treated rats displayed a normal response to mechanical pinch applied to the paws. Intrathecal C produced a dose-dependent depletion up to 50% in the spinal but not hind brain levels of sP. Comparison of degree of thermal analgesia with degree of depletion of sP indicated a highly significant correlation. I.V. administration of 30 µg of C produced no change in either sP levels or in the nociceptive threshold. Intrathecal 5,6-dihydroxytryptamine (20 µg in 15 µl ascorbic acid vehicle) produces a 70-90% decline in spinal 5-HT and a 40% decline in spinal sP. This neurotoxin in contrast to C, produced a significant reduction in the nociceptive threshold. These results suggest that there are two populations of sP containing spinal terminals, a portion is associated with the descending serotonergic system and a second portion is located in primary afferent terminals, the latter of which is depleted intrathecally. With regard to nociceptive transmission this data suggests that sP may be associated with primary afferents which carry information related to heat and peripherally applied algesic chemicals. This work supported by NINCDS 14629.

2105 TAIL-FLICK NEURONS OF THE NUCLEUS RAPHE MAGNUS. M.D. Zaretsky, J.D. Levine* and H.L. Fields. Depts. of Neurology and Physiology, Univ. of California, San Francisco, CA 94143.

We have recorded from individual neurons of the nucleus raphe magnus (NRM) in rats performing tail-flicks in response to noxious heating of the tail. The tail-flick test has been used to demonstrate both stimulus-produced (Mayer. D.J. et al, SCIENCE 174: 1351, 1971) and opiate (Akil, H. et al, SCIENCE 191:961, 1976) analgesia. Histological studies and electrical stimulation of the NRM suggest that the NRM is a significant region of a descending analgesia pathway (Basbaum, A.J. et al, PNAS 73:4685, 1977). We now report that the NRM contains two classes of neurons with discharge patterns that are strongly correlated with tail-flicks in response to noxious heating. In the first class of neurons, tail-flick triggers, strong excitation occurs when noxious heating results in a tail-flick. Excitation of these units does not occur when noxious heating fails to produce a tail-flick. Increase of the firing rate of these neurons begins just prior to the onset of the tail-flick. Sone neurons of this class discharge briefly, others continue to discharge after the tail-flick occurs. The second class of NRM neurons, off-units, firing spontaneously at a moderated rate, are inhibited from firing by noxious heating of the tail.

PLASTICITY

2106 UPTAKE AND RETENTION OF ^{45}Ca IN HIPPOCAMPAL PYRAMIDAL NEURONES DURING LONG TERM POTENTIATION. K.G. Baimbridge* & J.J. Miller (SPON: T.W. Calvert). Dept. Physiology, Univ. British Columbia, Vancouver, Canada V6T 1W5.

Previous studies have demonstrated that the presence of extracellular Ca during tetanic stimulation of hippocampal afferents is necessary for the development of long term potentiation (LTP) of synaptically evoked responses in this region. Inasmuch as Ca plays a significant role in transmitter release, and an augmented transmitter output may underly the phenomenon of LTP, the present experiments were undertaken to determine whether there is a significant uptake and retention of Ca following brief tetanic stimulation of the Schaeffer-collateral input to Ca_1 pyramidal neurones which induces LTP. Transverse slices of the rat hippocampus were prepared for electrophysiological recording and incubated in a modified Ringer's solution containing ^{45}Ca for 2 hrs prior to stimulation. Paired control and tetanized (50-100 Hz, 2-5 sec.) slices were removed at 5 and 30 min. following stimulation and washed in Tris-buffer (pH 7.4) containing 10 mM $LaCl_3$ in order to remove surface bound ^{45}Ca and to prevent release of intracellular Ca. Slices were freeze dried and the soluble protein dissolved in buffer and aliquots taken for total protein determination and ^{45}Ca content. In all pairs and at both time intervals, tetanic stimulation resulted in an increased ^{45}Ca content compared to controls. No loss of ^{45}Ca was observed 30 min. after tetanization compared to 5 min. post-tetany. The large influx of Ca which occurs as a result of tetanic stimulation and the fact that this Ca is retained for at least 30 min. suggests that an augmented transmitter release, dependent in part upon uptake and retention of intracellular Ca, may underly the phenomenon of LTP.

(Supported by MRC of Canada.)

2107 EFFECTS OF UNILATERAL HORIZONTAL SEMICIRCULAR CANAL PLUGS AND THE INTRAVENOUS ADMINISTRATION OF DIAZEPAM ON THE DEVELOPMENT AND COMPENSATION OF ASYMMETRY OF THE HORIZONTAL VESTIBULO-OCULAR REFLEX OF THE RABBIT. Neal H. Barmack and Vito E. Pettorossi*. Department of Ophthalmology, Neurological Sciences Institute, Good Samaritan Hosp. & Med. Cntr., Portland, OR.

The activity originating from the ampulla of a horizontal semicircular canal (HSC) is increased by ipsilateral angular acceleration of the head and decreased by contralateral angular acceleration. This activity directly excites the ipsilateral medial vestibular nucleus (MVN) and indirectly inhibits the contralateral MVN via a GABA-ergic commissural pathway. This reciprocal organization can be altered by plugging one HSC. The postplug activity of the MVN contralateral to the plugged HSC would be modulated only by an excitatory signal originating from the intact HSC. The activity of the MVN ipsilateral to the plug would be modulated only by an inhibitory signal relayed through the commissural pathway. We have studied the effects of unilateral plugs of the HSC on the horizontal vestibuloocular reflex (HVOR) of the rabbit, and examined the influence of intravenously administered diazepam (0.4mg/kg), a GABA agonist, on the HVOR following unilateral plugs of the HSC. An HSC was plugged by inserting a small silver spindle into the bony canal. The HVOR was evoked by sinusoidal oscillation of rabbits on a rate table (0.01-0.8Hz ±10 degrees) and measured with an infrared technique. Immediately following a unilateral HSC plug, the gain (eye velocity/head velocity) of the HVOR is reduced. If "postplug" vestibular stimulation is maintained, an asymmetry in the HVOR slowly develops (3-15hr). This asymmetry reflects a relative increase in the gain of the HVOR when the eyes move toward the side of the plugged canal. Maximum asymmetry occurs in 24-48hr. The onset of the asymmetry can be delayed by postponing the initiation of postoperative vestibular stimulation. Although the average gain never recovers, the relative asymmetry of the HVOR is gradually compensated in a frequency-dependent manner. After 5-10 days this compensation is even reversed, causing a relatively higher gain of the HVOR when the eyes move toward the intact side. Intravenous injections of diazepam in normal rabbits reduce the gain of the HVOR. Diazepam injections after unilateral HSC plugs greatly increase the asymmetry of the HVOR. The time course of this diazepam-induced effect corresponds to that of the development and compensation of the asymmetry. The efficacy of the diazepam might be attributed to the relatively increased postsynaptic sensitivity of the MVN on the plugged side to GABA-ergic agents. This increased sensitivity may be stimulated by the near-exclusive modulation of the MVN on the plugged side by the GABA-ergic commissural system. (Supported by PHS Grant EY-00848 and The Oregon Lions Sight & Hearing Foundation.)

2108 THE CRITICAL PERIOD FOR STRABISMUS-INDUCED LOSS OF BINOCULARITY IN CAT VISUAL CORTEX. Nancy Berman and E. Hazel Murphy. Departments of Physiology/Biochemistry and Anatomy, The Medical College of Pennsylvania, Philadelphia, PA 19129.

We produced a surgically induced strabismus in 11 kittens by cutting the right medial or lateral rectus muscle at different ages. Following at least one year of visual experience, we recorded from single neurons from area 17, and for each unit we determined the eye dominance group, receptive field type (simple or complex), receptive field size and position in the visual field.

We found a gradual increase in the percentage of binocularly driven units with age at surgery. In kittens made strabismic between postnatal days 10 and 18, only 10% of the units could be driven by both eyes. Surgery performed on days 26, 36-38, and 60 gave percentages of binocular cells of 25%, 30% and 50% respectively. Following surgery on day 80, the percentage of binocularly activated units was not significantly different from that found in normal adult cats. We found no abrupt change which might signify the end of the critical period.

In kittens operated before day 43, a high proportion of those cells which were binocularly driven had complex receptive fields, which is in agreement with observations that complex cells are more resistant than simple cells to other manipulations which reduce binocularity (Payne et al. 1979).

We found some cells with abnormal receptive field properties in these kittens. In the earlier operates, we commonly found visually unresponsive cells at the borders between the eye dominance columns. In the later operates, we found binocularly driven cells with abnormally large receptive fields (up to 11x15°).

The critical period for changes in binocularity resulting from surgically induced strabismus differs somewhat for the critical period for the effects of monocular suture. Following monocular suture, all units will be driven only by the experienced eye even if the deprivation is begun as late as day 30 (Hubel and Wiesel, 1970) whereas kittens made strabismic on day 26 retain 25% binocularly driven units. Our findings suggest: (i) some complex cells can retain their input from both eyes despite a severe eye misalignment; (ii) abnormal receptive fields can be produced in kittens without deprivation of visual experience of form or movement; (iii) the critical period for squint ends earlier and more gradually than the critical period for monocular deprivation.

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2109 CYCLIC DENDRITIC DEGENERATION AND REGENERATION OF RAT MOTONEURONS AFTER VENTRAL ROOT SECTION. Jerald J. Bernstein and Nancy Standler.* Dept. Neurosci. Univ. Fla. Coll. Med., Gainesville, Fla. 32610.

Somatic bouton rejection and dendritic branch loss can be induced by axotomy. The following experiment studies the dendritic profile of rat motoneurons in the segment under the T_1-T_2 vertebrae after ventral root section (Normals, 14, 30, 60, 90 days postoperative). Six animals per group were utilized and the segment of spinal cord of the crushed root impregnated by the tungstate modificating the Golgi technique. Neurons were selected by size and location. Six independent samples were generated per postoperative day. Dendrites were analyzed for total number of branches, branches that were terminal and serpentine length (total length of entire dendrite). (Statistics: ANOVA a priori; Neuman-Keuls a posteriori). There was a significant, cyclic, loss and gain of numbers of dendritic branches (Low, 14 and 60 days, high 30 and 90 days) and numbers of branches that were terminal (Low, 14 and 60 days, high 30 and 90 days). The serpentine length of the dendrites showed the same significant cycle. These data show that following axotomy motoneurons show cyclic growth and branching.

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2110 SPROUTING OF PERIPHERAL SYMPATHETIC NEURONS IN THE ABSENCE OF AFFERENT NEURONAL INPUT. Leslie Brothers*, Keith A. Crutcher and James N. Davis. (Spon: E. W. Busse), VA Medical Center and Duke University Medical Center, Durham, N. C.

Although it is well established that neuronal rearrangements occur after brain injury, relatively little is known of the factors that regulate such plasticity. One particular rearrangement, the appearance of peripheral noradrenergic neurons in the dentate gyrus several weeks after medial septal lesions, provides a useful model for the study of the regulation of reactive synaptogenesis or sprouting.

We took advantage of the accessibility of the superior cervical ganglion to surgical manipulation and cut the preganglionic input to the ganglion (decentralization) in some animals, removed the ganglion in others and carried out a sham neck operation on the remainder. The decentralized and sham-operated controls had a bilateral medial septal lesion placed the day following neck surgery and were sacrificed four weeks later. The ganglionectomized animals were also sacrificed four weeks after septal lesioning, but their superior cervical ganglia were removed 3 weeks after lesioning (one week before sacrifice). All decentralized animals developed an ipsilateral Horner's syndrome which could be reversed by parahydroxyamphetamine eye drops. Furthermore peripheral noradrenergic fibers in the pineal and on pial blood vessels appeared unaffected by the decentralization. The septal region was sectioned and examined histologically to verify lesion placements in each animal. The hippocampal formations were studied with a glyoxylic acid method for visualizing catecholamine fibers.

All sham-operated controls demonstrated obvious bilateral noradrenergic sprouting identical to non-operated animals. Ganglionectomized animals had no peripheral noradrenergic fibers ipsilateral to the removed ganglion, but sprouting in the contralateral hippocampal formation appeared similar to controls. Decentralization did not prevent sprouting, since all but one decentralized animal had peripheral noradrenergic fibers in the hippocampal formation ipsilateral to the decentralization.

Our data show that afferent input and neuronal activity is not necessary for peripheral noradrenergic neurons to sprout. These data further show that some factor in the target tissue initiates the sprouting response. The attenuation of sprouting in the decentralized animals is harder to interpret. It may be that, like the developing animal, nerve impulse flow plays an important role in the extention or maintenance of sprouted fibers.

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2111 NEURONAL CONNECTIVITY IN ISOLATED GANGLIA OF ADULT HELISOMA: STABILITY AND LABILITY DURING CULTURE. Andrew G. M. Bulloch, A. Don Murphy and Stanley B. Kater. Dept. Zoo., Univ. of Iowa, Iowa City, Iowa 52242.

Previously we described a system for study of neuronal regeneration in the snail Helisoma trivolvis which involves culture, in the hemocoel of host snails, of the buccal mass with attached buccal ganglia and salivary glands (Murphy and Kater, Brain Res, 156, 322-328, 1978). Under these conditions synaptic connections remain stable for many months. Recently we have cultured isolated, i.e. completely axotomised ganglia, and in contrast to ganglia with intact target organs, found both physiological and morphological changes. Of particular interest are apparent changes in connectivity, indicated by the diminished efficacy of certain chemical synapses, whereas electrical synapses remain stable.

To date we have concentrated on the Protractor Motoneurons (PMN's), a group of cells that receive IPSP's from a coupled network of premotor neurons, the Cyberchrons. In ganglia cultured for one week, spontaneous IPSP's are no longer apparent in most PMN's and have been replaced by EPSP's. In one pair of PMN's the IPSP is present, but is diminished both in amplitude and duration.

In normal ganglia the IPSP may be assayed by Esophageal Trunk (ET) stimulation, owing to the presence of Cyberchron axons in this nerve. On blocking chemical synapses with high Mg^{2+} 0 Ca^{2+} saline, ET stimulation evokes a barrage of electrical EPSP's in PMN's. These are normally shunted by the chemical IPSP.

ET stimulation of ganglia cultured for one week produces an EPSP barrage in PMN's. These EPSP's are voltage insensitive, are not accompanied by any apparent change of membrane conductance, and are therefore thought to be electrical in origin. High Mg^{2+} 0 Ca^{2+} saline causes the amplitude of the EPSP's to increase, presumably by removal of a shunt due to remaining IPSP's.

After two or more weeks of in vivo culture the IPSP of some PMN's was apparently restored to normal. However, such ganglia often become wrapped in a dense neuroma which makes intracellular recording progressively more difficult.

The temporary loss of efficacy at some chemical synapses is presumably a response to axotomy, while the neuritic growth and apparent restoration of normal connectivity indicate a considerable degree of regulative ability in the adult buccal ganglia.

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2112 INFLUENCE OF EARLY MONOCULAR DEPRIVATION ON [^{14}C]-2-DEOXYGLUCOSE LABELING PATTERN IN PIGEON VISUAL STRUCTURES. Andreas Burkhalter* and Peter Streit* (SPON: Michel Cuénod). Brain Research Institute, CH-8029 Zurich.

The visual behavior of pigeons, monocularly deprived for the first 8-11 months, was found to be deficient in respect to pattern discrimination and interocular transfer. Since the [^{14}C]-2-deoxyglucose method provides an instrument to visualize functional activity of cerebral structures, this method was used to study patterns of glucose consumption after long-term deprivation in the pigeon.

Six pigeons were raised for 244 to 341 days with one eye occluded by an opaque plastic cap from the day of hatching. At the end of the deprivation period 50µCi [^{14}C]-2-deoxyglucose were injected i.v. in 3 groups of 2 awake animals: During 45 min, both eyes were free (DBE) or one eye was covered, either the deprived one (DDE) or the nondeprived one (DEE). The autoradiographic labeling pattern of (DBE), (DDE) and (DEE) was compared with the pattern of normal adult binocularly (BE) and monocularly (ME) exposed animals. During the experiment the animals were freely moving in an aviary.

In groups DDE, DEE and ME labeling of all known visual structures was asymmetrical and heavier on the side contralateral to the open eye. However the asymmetry, predominantly in the hyperstriatal areas IHA and HA, was most pronounced in DDE. In DBE, with both eyes free an asymmetry was observed only in IHA and HA where the labeling was stronger on the side contralateral to the deprived eye. After binocular exposure of normal adult animals (BE) however the hyperstriatum, receiving input from both eyes, was always symmetrically labeled.

Present results suggest, that the asymmetry of hyperstriatal labeling in experiments with both eyes free (DBE), seems to be more closely related to the system having its origin in the contralateral, deprived eye.

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2113 EFFECT OF EARLY GUSTATORY NEOCORTICAL LESIONS ON TASTE AVERSION LEARNING IN RATS. Rebecca J. Cabral, Stephen W. Kiefer and John Garcia*. Dept. Psychol., Mental Retardation Research Center, UCLA, Los Angeles, CA 90024.

In adult rats, lesions of the gustatory neocortex severely disrupt the ability to associate a taste with illness. However, quite often cortical lesions produced before maturity result in less detrimental effects on some learning tasks.

To investigate plasticity in taste aversion learning rats received bilateral gustatory neocortical lesions (GNC) as neonates (10 days), weanlings (20 days) or adults (70 days). They were allowed to recover and were tested on acquisition and extinction of a saccharin aversion at 120 days of age. For training, the rats were habituated to a drinking schedule in the home cage. They received a preexposure to a 0.1% saccharin solution (day 117), three saccharin-lithium chloride (0.15M, 2% bwt., i.p.) acquisition trials (days 120, 123, 126) and five saccharin extinction trials (days 129, 132, 135, 138, 141). After testing, histological verification of the lesions was performed.

The results showed that rats receiving GNC lesions as neonates (n=10), weanlings (n=17) or adults (n=10) demonstrated significant deficits in acquisition and extinction when compared with control-lesioned, sham-operated or normal control rats. After three acquisition trials GNC rats, regardless of age at lesion, reduced saccharin consumption to 50% of water baseline compared to 100% reduction by control rats.

Thus, sparing of taste aversion learning does not occur when GNC lesions are sustained as early as ten days of age. These results contrast with other reports of recovery of function after early cortical lesions.

2114 RAPID KINDLING INDUCED BY LOW-FREQUENCY STIMULATION OF THE AMYGDALA. Donald P. Cain and Michael E. Corcoran, Dept. Psychology, U. Western Ont., London, Canada N6A 5C2 and Dept. Psychology, U. Victoria, Victoria, Canada V8W 2V2.

Previous research in kindling has suggested that low-frequency stimulation (below approximately 5.0 Hz) is ineffective in kindling amygdaloid seizures. The present experiment reassessed this question using biphasic square wave pulses of 1.0 msec duration delivered at 2.0 or 3.0 Hz through bipolar electrodes chronically implanted into the amygdala of 24 male hooded rats. Animals were stimulated once daily with a 60-sec train of pulses. All animals developed fully generalized seizures after a mean of 3.1 evoked afterdischarges. A number of animals displayed a generalized seizure upon the first application of the stimulation. Rekindling with a conventional 60 Hz 1-sec train of pulses confirmed that all animals were fully kindled. The threshold current intensity necessary was approximately 300 μA (peak pulse intensity measured baseline-to-peak). Intensities much below this were completely ineffective even when administered repeatedly. Pulses of 0.1 msec duration were also completely ineffective. Frequencies of 2.0 and 3.0 Hz were about equally effective in kindling seizures. The results indicate that amygdaloid kindling can proceed very rapidly using low-frequency pulses providing the peak pulse intensity exceeds a certain threshold level. This suggests that it is necessary to activate a 'critical mass' of neurons for kindling to proceed, and that low-frequency activation is more effective than activation with conventional 60 Hz currents in kindling.

2115 VERY LONG AND SHORT-TERM CHANGES IN EXCITABILITY OF RAPHE-EVOKED THALAMIC POTENTIALS. K. L. Casey and T. J. Morrow. Depts. of Physiol. & Neurol., Med.Sch., Univ. Mich., Ann Arbor, MI 48109.

Electrical stimulation within the nucleus centralis superior (nCS) evokes two different field potentials in the thalamus of awake squirrel monkey and cat. A 2 msec peak latency potential is recorded bilaterally from the pulvinar-posterior nucleus complex of the posterolateral thalamus (P-PO). Repetitive nCS stimulation (1 Hz for 10 min or 10 Hz for 30 sec) produces a 2 to 4 fold enhancement (to nearly 800 μV) of response amplitude which gradually decays over a period of at least 2 hours. Stimulation of adjacent pontine reticular formation is ineffective. During the course of 8 experimental sessions over a 3 week period in one monkey, the duration of this enhancement was extended to at least 5 days. Once enhanced, the P-PO potential is not affected by general anesthesia with ketamine, thiamylal or fluothane, but the effect of repetitive stimulation is reduced. Morphine (1 mg/kg I.M.) markedly reduces the amplitude of an enhanced P-PO potential, reduces the enhancing effect of repetitive stimulation, and accelerates the decay of enhancement to about 30 min. Naloxone (0.1 mg/kg I.M.) reverses the effect of morphine and unmasks the stimulus-induced enhancement blocked by morphine.

A 4 msec peak latency potential is recorded bilaterally with maximum amplitude in the centre-median parafascicular complex (CM-Pf) of the medial thalamus. This potential is unaffected by repetitive stimulation but shows a second to second variation in amplitude (from 100 μV to 2000 μV) in the awake animal. Repeated testing and observation show that maximum response amplitude is not simply related to "arousal" for continuous tail pinch is ineffective. Rather, the excitability of this nCS-elicited response appears greatest when the animal is actively exploring and attending to environmental events. The CM-Pf potential is eliminated by general anesthesia with fluothane or chloralose and markedly reduced by morphine (1 mg/kg I.M.). The effect of morphine is reversed by naloxone (0.1 mg/kg I.M.).

These findings demonstrate the existence of opiate and anesthetic-sensitive raphe-mediated influences which differentially affect different thalamic regions and are likely to play an important role in the selective acquisition and storage of information. The results further emphasize the importance of using unanesthetized animals in neurophysiological investigations of reticulo-thalamic systems.

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2116 ANALYSIS OF CORTICOPONTINE SPROUTING AFTER CORTICAL LESIONS IN NEWBORN RATS. Anthony J. Castro. Dept. Anat., Stritch Sch. Med., Loyola University, Maywood, Il 60153

A somatotopic organization of corticopontine projections has been described in several animals. In general terms, although more specific details are available, fronto- and occipitopontine fibers demonstrate a medial to lateral projection pattern within the basilar pontine gray. The present study was undertaken in search of alterations of these projection patterns that might ocurr in response to neonatal cortical lesions.

Under hypothermic anesthesia, newborn (2-3 day old) Long-Evans black-hooded rats sustained unilateral frontal or occipital cortex ablations. At maturity, animals with neonatal frontal cortex lesions sustained ablations under sodium pentobarbital anesthesia of either the contralateral frontal cortex or of the occipital cortex ipsilateral to the neonatal lesions; and animals with neonatal occipital cortex lesions sustained ablations of either the contralateral occipital cortex or of the frontal cortex ipsilateral to the neonatal lesion. Animals were sacrificed by anesthetic overdose and vascular perfusion 4-7 days after adult lesions. Corticopontine projections were examined using the Fink-Heimer method and were compared to control animals that only received adult frontal or occipital cortex lesions.

Control frontopontine projections are mostly ipsilateral but with a small crossed projection to the opposite medial pontine gray. After neonatal frontal cortex lesions, an increase of the crossed frontopontine projection from the neonatally unablated hemisphere was observed. Anatomical remodelling of corticopontine projections was not observed in any of the other experimental groups.

The observed apparent specificity of corticopontine plasticity is attributed to two possible factors: (1) the small normally occurring crossed frontopontine projections would seemingly provide a substrate of fibers capable of sprouting to innervate cells deafferented by the neonatal lesions, and (2) the ultrastructural differences between fronto- and occipitopontine terminals (Mihailoff, 1978, Neurosci. Let. 8:219) suggest a compatibility for homotypic (frontal-frontal) remodelling but not heterotypic (frontal-occipital) remodelling.

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2117 QUANTITATIVE STUDY OF SYNAPTIC REMODELING IN THE COCHLEAR NUCLEUS FOLLOWING DEAFFERENTATION. Stephen D. Collins and Robert L. Gulley Dept. of Anatomy, Case Western Reserve Univ., Cleveland,OH 44106, and Univ.of Texas Health Science Cntr. San Antonio, Tx 78284.

Reorganization of non-primary neuronal input following removal of primary afferents has been quantitatively studied in a discrete neuronal population of defined morphology. The rostral pole of the guinea pig anteroventral cochlear nucleus is homogeneously composed of large spherical cells,each receiving a single large primary terminal from a spiral ganglion cell in the cochlea. This calyceal terminal covers about 46% of the spherical cell perimeter. The terminals of the spiral ganglion cells degenerate after cochlear ablation, thus removing all primary sensory innervation to the large spherical cells. Three morphological types of boutons (O-boutons,F-boutons,and SR-boutons) are apposed to approximately 13,5, and 4% of the normal spherical cell perimeter (Schwartz & Gulley,1978). All primary terminals have degenerated by four days after cochlear ablation. Up to six days after cochlear ablation, significant remodeling of non-primary inputs to the spherical cell has not occurred (Wenthold and Gulley,1977). In the present study, perimeters from 250 spherical cells from 15 animals were examined at 7,14,21,28 and 180 days following cochlear ablation. Following bouton identification,cell perimeter and bouton appositional length were measured on a digital plotter in conjunction with a PDP-11/45 computer. At day 7,the amount of spherical cell perimeter covered by O-boutons increases from 13% to approximately 33%, an increase of 250%. By day 7, the amount of F-bouton apposition has nearly quintupled,now covering aproximately 25% of the spherical cell. The SR-boutons remain nearly the same in their appositional length. The O and F-bouton appositional length remains increased throughout the time period studied. In the external membrane leaflet of the spherical cell following deafferentation,three responses are observed in freeze-fracture studies (Gulley et al, 77): Non-aggregate particles increased seven fold; perisynaptic aggregates are removed from the membrane and the number of particles in junctional aggregates remains the same. In the present study 7 days following cochlear ablation the increase in non-aggregate particles reverses towards normal levels, perisynaptic aggregates return to the membrane and the number of particles in junctional aggregates decrease.

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2118 CEREBRAL PROTECTION IN SHRSP RAT. Peter Coyle. Dept. Anat., Univ. Mich. Med. Sch., Ann Arbor, MI 48109.

Occlusion or transection of the middle cerebral artery (MCA) in the Wistar rat is followed by expansion of the collateral arteriole supply vessels and MCA field tissue survival (Neurosci. Abstr. 4: 469, 1978). In contrast, for spontaneously hypertensive stroke-prone rat (SHRSP) the procedure results in tight endothelial cell junctions opening, Evans blue-albumin extravasation, reduced arteriole filling, tissue necrosis and atrophy extending from the occlusion site to the anastomotic zone (Anat. Rec. 193: 740, 1979). Objectives were to evaluate the response in SHRSP following ipsilateral carotid artery occlusions and/or cervical sympathectomies. Twenty-one SHRSPs ranging in age from 33 to 330 days and of either sex were anesthetized intramuscularly with ketamine hydrochloride, 125-200 mg/kg body wt. In 6 animals the right superior cervical ganglion was excised or the cervical sympathetic trunk was transected 6 to 20 days prior to the MCA experiment. In the remaining rats, the nerves were carefully dissected from the common carotid artery then the artery and its external ramus were each double ligated and cut 0-15 days prior to transection of the right MCA just dorsal to the rhinal fissure. To mark opened endothelial cell tight junctions and tissue necrosis, 1 ml of 2% Evans blue in physiologic saline was injected intraperitoneally immediately after the MCA operation. Six rats died 3-5 days later, 8 had Horner's Syndrome. Lesion size was graded 1-4 with 4 extending from the transection site to the zone of anastomoses. Young (33-46 day old) and old (268-330 day) rats showed grade 3 and 4 lesions whereas 6 intermediate age SHRSPs (66-86 day) had lesions graded 1 or 2. Lesion grade was not related to the sympathectomy. MCA vessels in rats with grade 1-2 lesions were filled more extensively with Vultex or Rockland liquid photographic emulsion than those in grade 3-4 rats. The data indicate carotid occlusions 5-6 days prior to the MCA experiment facilitate cerebral collateral expansions, possibly due to a lowered blood pressure.

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2119 THE EFFECTS OF BILATERAL SERIATIM VERSUS SINGLE-STAGE HIPPOCAMPECTOMY ON THE ACQUISITION OF DRL 20 IN JUVENILE, ADULT AND AGED RATS. S.D. Curtis* and A.J. Nonneman. Dept. Psychology, Univ. of Kentucky, Lexington, KY 40506

Previous research has shown that performance on a low rate operant schedule (DRL 20) after prior high rate operant experience (CRF) is severely impaired after bilateral single-stage lesions of the hippocampus in adult rats. In contrast, DRL 20 performance is much more efficient if the hippocampus is removed in two stages separated by interoperative DRL training. This study attempted to determine whether this serial lesion effect would also occur in juvenile or aged rats.

Rats from each of 3 age groups (35, 160, or 570 days) received training on CRF prior to either single-stage hippocampectomy, sham operative treatment, no surgical treatment or bilateral lesion of of either anterodorsal or posteroventral hippocampus. After forty days of DRL 20 training, the seriatim groups received bilateral lesions of remaining hippocampus. All animals then received thirty final days of DRL training. Relative to the single-stage hippocampal operates, serially lesioned animals of all ages demonstrated substantial recovery of DRL 20.

Also addressed in this study were 1) the nature of the "recovered" behavior and 2) the generality of the "recovery" across tasks. The former was examined by periodic mapping of the movements of each animal while performing DRL and the latter by a final shift back to CRF. Both of these measures indicated that the serially lesioned "recovered" animals were still very much like the single-stage operates (i.e. they tesnded to show less variety of operant box movements than controls and they shifted to CRF more slowly than controls).

The facts that 1) all three ages of serially lesioned animals demonstrated similar DRL capacities and 2) the "recovery" was apparently task spedific, both serve to predict a recovery mechanism that does not involve substantial rearrangement of brain connectivity (a capacity that is apparently age dependent). Rather a learning mechanism is proposed that is in essence an application of Kamin's multiple cue blocking theory. Specifically, it is proposed that during a serial lesion sequence, the subtotal disruption of a neural system that exists during the interoperative period allows only subasymptotic performance and thereby forces a recognition and recruitment of redundant, normally nonprefered cues. Following the second lesion these now 'primed', nonprefered cues serve to mediate the behavior.

2120 STUDIES OF THE MODIFIABILITY OF THE VISUAL CALLOSAL PATHWAY IN RATS. C. Cusick* and R. D. Lund. Depts. Biol. Struct. and Neurosurg., Univ. Wash. Sch. Med., Seattle, WA 98195.

The configuration of callosal degeneration in the occipital region following total section of callosal fibers has been described previously for normal adult albino and pigmented rats (Cusick, 1978, Neurosci. Abstr.). We have examined the consequences of the following experimental manipulations on the developing visual callosal pathway: 1) dark rearing, 2) unilateral enucleation, 3) bilateral enucleation and 4) unilateral section of an optic tract.

Animals were reared in the dark from birth or were operated on the day of birth. At 4-8 weeks of age the fibers of the corpus callosum were sectioned by a paramedian approach. Lesions were performed ipsilateral to unilateral enucleation and contralateral to the tract lesions. After a 2-4-day survival, the animals were perfused and the brains processed by the Fink-Heimer method. Brains were sectioned coronally, or the unlesioned hemisphere was flattened during fixation, sectioned tangentially and prepared by a bleached Fink-Heimer technique.

Dark rearing and unilateral enucleation have little appreciable effect on the configuration of the callosal projection, its density, or the breadth of its distribution. However, bilateral enucleation and optic tract section produce very pronounced changes. After bilateral enucleation, the normal callosal pattern is recognizable, but additional bands and rings of callosal input lie within normally acallosal regions of areas 17 and 18a. The spread of degeneration into area 17 is prominent laterally and posteriorly. Anteriorly, it virtually fills in area 17. In contrast, the callosal projection to the 17/18a border in the hemisphere ipsilateral to the neonatal tract lesion is severely decreased in width relative to normal. This effect is specific to the visual callosal pathway, since the callosal input to nonvisual areas of the same brains appears normal in distribution and density.

Comparison of the results from albino and pigmented rats and the findings in dark reared and unilaterally enucleated animals shows that in cases in which an intact but abnormal visual input is present, the callosal pathway appears normal. This contrasts with the results from similar experiments in cats. However, after neonatal bilateral enucleation, optic tract section, or thalamectomy (Cusick, 1978, Neurosci. Abstr.), cases in which the cortext is totally deprived of a retinal input via the ipsilateral lateral geniculate nucleus, the callosal pathway is abnormal. (Supported by USPHS Grants EY-00596 and GM-07108 from the National Institutes of Health.)

2121 *RECOVERY FROM UNILATERAL NEGLECT: BEHAVIORAL AND FUNCTIONAL ANATOMIC CORRELATIONS IN MONKEY.* *Ruthmary K. Deuel, Robert C. Collins, Nancy Dunlop* and Torris V. Caston*. Departments of Pediatrics & Neurology, Washington University, St. Louis MO 63110.*

Unilateral damage to frontal cortex in the periarcuate region (FPC) in monkeys results in a syndrome of contralateral visual and tactile neglect and an absolute preference for the ipsilateral hand. Spontaneous, complete recovery occurs by unknown mechanisms. We have used the ^{14}C-deoxyglucose autoradiographic technique (DG) to study functional cerebral anatomy (glucose utilization) during three phases of recovery. Five adolescent Macaca fascicularis were tested with a standard neurological exam and taught a conditional motor response to criterion before operation. FPC was removed by subpial resection from the right hemisphere, testing was repeated postoperatively, and animals were sacrificed following DG infusion at 4, 14 or 23 days. Brains were perfused with formalin, frozen in freon at -50° C, then cut at -30° C. Four adjacent 30 µm sections were taken at 600 µm intervals through the entire brain (three for densitometric analysis, the other for histologic study).

Animals examined within two weeks after injury showed severe contralateral neglect, including hemianopsia and absolute preference for the hand ipsilateral to the lesion, and no retention of the conditional motor response. DG studies showed a 10-40% depression of DG uptake in ipsilateral (Right) subcortical structures, primarily thalamus and basal ganglia. Compared to contralateral (Left) homotopic sites, the greatest depression of DG uptake occurred in n. medialis dorsalis (Right = 58% of Left), n. reticularis (R = 70% L), n. ventralis anterior (R = 74% L), n. X of Olszewski (R = 75% L), caudate (R = 75% L), putamen (R = 77% L), and pulvinar (R = 80% L). Animals showed substantial recovery from neglect by four weeks. DG studies at the time revealed only a slight depression of glucose uptake (<10%) in ipsilateral subcortical sites, principally in caudate (R = 93% L) and putamen (R = 92% L). In none of these three phases was depression of ipsilateral cortical DG marked.

The findings lead us to postulate that the multimodal neglect is related to a widespread metabolic dysfunction in subcortical grey masses. The monkeys' recovery from neglect occurs in parallel with reappearance of symmetrical metabolic activity in these structures.

2122 GOLGI ARCHITECTONICS OF A REGION INVOLVED IN SONG PRODUCTION IN THE CANARY. T. J. DeVoogd and F. Nottebohm. Rockefeller University, New York, N. Y. 10021.

Nucleus robustus archistriatalis (RA) is a large telencephalic nucleus in the canary. When RA is lesioned in singing male canaries, song is disrupted (Nottebohm et al., J. Comp. Neur., 165:457, 1976). This effect is much more pronounced on the left side than on the right: song production appears lateralized to the left side. RA is substantially smaller in female canaries which do not normally sing. RA has been shown to receive input from hyperstriatum ventrale, pars caudale (HVc) another song integration center. However, the cell types and connectivity pattern within RA have not yet been described.

In the present experiment, six pairs of one year old male and female canaries were sacrificed when in full reproductive condition. Brains were stained with several rapid Golgi variants. All sections were retained, allowing reconstruction of nuclei and of the total dendritic fields of the cells observed. RA was clearly delineated in the Golgi stained tissue. The nucleus was 39% as large in the females as in the males, in accord with previous Nissl studies. Input from HVc was observed to course to RA in large fiber bundles which arborized in a dense fiber band at the dorsal surface of RA. Fiber bundles exited RA caudally and medially. Four distinct cell types common to both males and females were associated with RA. The first, with a large soma (13-17u) and fine, highly branched, spiny dendrites, is embedded in the initial arborizations of incoming axons just dorsal to RA proper. The second is a small aspinous cell (8-12u) with a highly branched compact dendritic field. The third has a large cell body (15-20u) and many fine, sparsely spined dendrites. The fourth cell type was the most frequently observed. These have a large soma (18-23u) and have thick, extremely spiny dendrites. Four or five primary dendrites give rise to a dendritic tree covering a radius of about 100u. The axons project anteriorly and send out a complex network of local collaterals. Camera lucida tracings were made of a sample of these cells. Preliminary Sholl and branch segment analyses indicate that male and female cells do not differ in number of primary branches or in the amount of dendrite arising from each primary. However, they do appear to differ in the distribution of that dendritic material. Male cells have 20% less dendrite than females within 50u of the soma but have 32% more dendrite beyond 50u. No consistent left-right differences were seen in these measures.

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2123 LONG-LASTING POTENTIATION IN THE PERFORANT PATHWAY TO CA1 NEURONS IN THE HIPPOCAMPUS, IN VITRO. Herbert J. Doller* and Forrest F. Weight (SPON: J. Y. Summy-Long). Laboratory of Preclinical Studies, National Institute on Alcohol Abuse & Alcoholism, Rockville, MD. 20852.

In the hippocampus several pathways have been shown to exhibit a long-lasting potentiation following a brief tetanic stimulation of the pathway investigated. This potentiation of evoked responses lasts for minutes to hours in in vitro preparations (Brain Res. 89:107, 1975) and has been reported to last for days in whole animal preparations (J. Physiol. 232:357, 1973). Anatomically, the perforant pathway terminates, in part, on the apical dendrites of the CA1 pyrmidal neurons. We recently reported electrophysiological evidence for this pathway in the hippocampal slice preparation (Soc. Neurosci. Abst. #679, 1978). We report here a long-lasting potentiation of this perforant to CA1 pathway in the hippocampal slice.

Male Hartley guinea pigs weighing 300 to 400 gm were sacrificed. The hippocampus was quickly removed and placed in ice cooled medium. Thin (350 to 400 um) tranverse sections of the hippocampus were cut using a Sorvall tissue sectioner. Using small knives, a section (1 to 2 mm wide) in the CA1 region of the slice was removed. This section started at the ventricular surface and included regions down to but not including perforant pathway. Removal of this section insured that CA1 activation was not the result of stimulating other layers of the CA1 region. Slices of the hippocampus were then placed in a trough-like chamber and superfused at rate of 5 ml/min with medium saturated with 95% O2 & 5% CO2. The medium contained 124 mM NaCl, 5 mM KCl, 2.4 mM CaCl2, 1.3 mM MgSO4, 1.24 mM NaH2PO4, 10 mM glucose, and 26 mM NaH2CO2. A bipolar stimulating electrode was placed in the perforant pathway on the entorhinal side of the removed CA1 section. The pathway was stimulated at a frequency of 1/min with pluses of 0.02 msec duration and amplitudes of 10 - 100 V. To produce potentiation, a tetanus of 15 Hz for 15 sec was used. A glass recording microelectrode (filled with 3 N NaCl) was placed in the region of CA1 cell bodies on the CA3 side of the removed CA1 section. The electrode resistances were 2 - 8 megohms. The amplitude of the field potential was determined by measuring from the maximum negative peak of the population spike to the peak of the positive wave following the population peak. Following a tetanus, the amplitude of the field potential increased 150% to 300% and remained elevated for at least 15 min. These data indicate that CA1 neurons exhibit long-lasting potentiation in response to perforant pathway stimulation.

2124 PERMEABLE AND IMPERMEABLE TECTAL BARRIERS IN GOLDFISH: DIFFERENT EFFECTS ON ROSTRAL COMPRESSION AND CAUDAL ESCAPE BY OPTIC FIBERS. M. A. Edwards* and M. Jacobson. Depts. of Physiology & Anatomy, Univ. of Utah School of Medicine, Salt Lake City, UT 84108.

Electrophysiological mapping and anatomical methods were used to evaluate changes in the retinotectal projection of goldfish 20-200 days after insertion of various barriers into an incision which bisected the tectum into rostral and caudal halves. Yoon's report (Exp. Neur. 35: 565, 1972) that Gelfilm barriers (300 Å m thick) induce an orderly, near-complete compression of the visual representation in rostral tectum was replicated. The time course of decrease in the rostrocaudal retinotectal magnification factor (MF) was similar to that seen in half-tectal control fish, both reaching about 60% of normal control values 2-3 mos postoperatively. Barriers constructed of Nucleopore filter material with no holes (10 Å m thick) caused a similar, but slower, compression, whereas equivalent barriers with either .1 Å m or 8 Å m holes did not cause significant compression.

Surprisingly, the caudal tectum became reinnervated among all groups, such that the visual representation was exclusively partitioned on the two sides of the barriers. In contrast to the normal projection usually reformed behind permeable barriers, the regenerated projection caudal to Gelfilm and no-hole Nucleopore barriers consisted of an expanded representation of about 20° of the extreme temporal visual field. Rostral and caudal MF's were inversely related. Reinnervation was well in progress by 1 mo postoperatively, which was in advance of the onset of compression. Escape took the form of huge fiber bundles that dived under the barriers no matter how deeply they were placed, according to H^3-proline autoradiography and the orthograde horseradish peroxidase technique (cut nerve application; DAB; 30-50 hrs). The former method was more effective in revealing the optic fibers in caudal tectum indicated by the mapping data. Regeneration under the barriers was not merely a mechanical response to obstruction, for when interrupted Gelfilm barriers were implanted, optic fibers funnelled through the gap staying within their appropriate layers.

The evidence suggests that diffusible factors released from the deafferented caudal tectum and passing through the pores of permeable barriers, somehow facilitate successful regenerative escape by optic fibers; impermeable Nucleopore and Gelfilm barriers block these signals more effectively, reducing the amount of escape, and the disconnected fibers are forced to take an aberrant accommodation in front of the barrier, displacing the intact projection rostrally in the process. The possibility of a regulative respecification of the tectum appears unlikely.

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2125 SPECIFICITY OF REINNERVATION OF FROG SYMPATHETIC GANGLIA. Daniel H. Feldman* (SPON: K.T. Brown). Dept. Physiology, Sch. Med. UCSF, San Francisco, CA 94143

In frog lumbar sympathetic ganglia, B-cells and C-cells can be distinguished by the propagation rate of antidromic spikes elicited in the postganglionic nerve trunk. The two types of ganglion cells are selectively innervated by separate populations of preganglionic axons; B-fibers innervate B-cells, and enter the sympathetic trunk at levels anterior to the sixth segmental ganglion, while C-fibers innervate C-cells and enter the trunk at the seventh and eighth segmental levels. Thus, fast excitatory synaptic inputs to B-cell or C-cell populations may be driven separately by stimulating the appropriate preganglionic nerve roots. This simple anatomical arrangement of selective inputs to two cell populations allows examination of the specificity of innervation of both normal and reinnervated ganglia using intracellular recording techniques.

In normal ganglia of Rana pipiens, C-fiber input to B-cells (n=59) has never been observed, and B-fiber input has been observed in approximately 4% of C-cells (n=48). When examined 6-22 weeks after crush of both B- and C-fibers, most ganglion cells were reinnervated. From a total of 188 B-cells examined, only 2 were reinnervated by C-fibers, while most were appropriately reinnervated by B-fibers. Approximately 16% of reinnervated C-cells, or 12% of the total C-cell population (n=146), were driven by B-fibers, while most were appropriately reinnervated by C-fibers. Thus, while regenerating preganglionic fibers occasionally form synapses with inappropriate target cells, reinnervation is on the whole highly specific when assessed at relatively long times after denervation. Preliminary observations 1½-6 weeks postoperatively suggest that during early reinnervation, there is a higher incidence of inappropriate connections: 75% of reinnervated C-cells (46% of all C-cells) in 4 preparations had been reinnervated by B-fibers. No B-cells innervated by C-fibers were observed.

These results indicate that the selective innervation of B- and C-cells which exists in normal adult frogs is restored upon reinnervation. This selectivity appears to be generated by a combination of accuracy in restoration of contacts and elimination of inappropriate connections which do arise.

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2126 TWO FORMS OF SPROUTING ARE PRESENT IN THE NEONATALLY DEAFFERENTED DENTATE GYRUS OF THE RAT. Christine Gall and Gary Lynch. Dept. of Psychobiology, UCI, Irvine, Ca. 92717.

The commissural afferents of the dentate gyrus of the rat, normally restricted to the inner one third of the dendritic field, sprout into the more distal region following deafferentation of that territory by removal of the ipsilateral entorhinal cortex. Previous autoradiographic and electron microscopic studies have demonstrated that following such lesion placement in the 14 day old rat the commissural projections rapidly expand to occupy the full depth of the dendritic field and ultimately establish an even density of synapses throughout. In the present study this sprouting response was reexamined using Holmes stain for normal fibers.

In those pups sacrificed 4 days after the lesion, the dentate's inner molecular layer fiber plexus (known in the adult rat to correspond with the commissural and associational afferent systems) was not found to be expanded; a surprising observation in light of autoradiographic data demonstrating that the commissural system has in fact sprouted by this postlesion interval. However, by 8 days postlesion the plexus was enlarged to approximately the same extent as seen after entorhinal lesions in the adult. With longer postlesion survival this plexus expands no further relative to controls leaving the most distal aspects of the deafferented dendritic field remarkable fiber-poor. In addition to this delayed and limited plexus expansion the appearance of a few unusually large caliber axons in the most distal deafferented field was noted, being particularly evident at the longer survival intervals.

The present data, considered in light of the previous autoradiographic and electron microscopic work on sprouting of these afferents, suggest that two forms of sprouting are present in the neonatal hippocampus: one being rapid and extensive as described by the autoradiographic and electron microscopic data and the second being more limited and exhibiting a several day postlesion delay to onset. This latter form observes the same spatial and temporal parameters exhibited by the commissural sprouting induced by entorhinal cortex removal in the adult rat. It is proposed that the former more extensive form of growth is exhibited by only the more immature axons in the field and that this form of sprouting is lost to unmask with development the more limited pattern of adjustment. This second more restricted form of sprouting is then retained in the adult.

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2127 VISUAL FIELD DEFICITS IN CATS WITH ONE EYE ROTATED. Barbara Gordon, Jeffrey Moran* and Joelle Presson. Dept of Psychology, University of Oregon, Eugene, OR 97403.

We have performed visual perimetry on cats reared with one eye rotated in order to determine (1) whether the animals can localize objects accurately using the rotated eye and (2) whether the rotated eye has a normal visual field. Four groups of animals were used: (1) right eye intorted 67-90°; (2) right eye intorted about 65-75°, left eye sutured; (3) right eye extorted about 90°; (4) right eye, all extraocular muscles cut, eye in normal position (muscle cut control). When the animals were about 4 months of age or older, they were trained on a modification of the visual perimetry task described by Sprague and Meikle and by Sherman. For the normal eye, the horizontal visual field extended from about 80° in the nasal retina to about 30-45° in the temporal retina. The vertical visual field extended from about 45° in the inferior retina to about 30° in the superior retina. Perimetry with the rotated eye showed that the cats are quite good at localizing visual stimuli with the rotated eye. Only 5% of the responses were to an incorrect location. For the normal eye the comparable figure is 1%. The rotated eye did, however, have a considerably smaller visual field than the normal eye. The visual field deficits appear to depend upon the orbital position occupied by each portion of the retina. The retina that occupied the temporal position appeared virtually blind, whether that retina was anatomic inferior retina (animals with right eye intorted) or anatomic superior retina (animals with right eye extorted). The retina that occupied the nasal position showed no detectable deficit. The retina that occupied the superior position had a reduced field regardless of whether that retina was anatomic temporal retina or anatomic nasal retina. The retina that occupied the inferior position had little or no deficit.

These results are specific to rotation and do not result from mere ocular immobility; the muscle cut control animals had normal visual fields. The deficits do not result from damage to the optic nerve during surgical rotation; the animals with their right eye intorted and their left eye sutured had a normal visual field using their intorted eye. We conclude that the visual field deficits result from changes in the central nervous system that depend on the animal's failure to use visual information from the rotated eye.

2128 ACOUSTIC EXPERIENCE MODIFIES DENDRITIC FORM IN NUCLEUS LAMINARIS Lincoln Gray*, Daniel J. Smith and Edwin W Rubel, Department of Otolaryngology, University of Virginia Medical Center, Charlottesville, Virginia 22908

The brain stem auditory system of chickens provides an opportunity to predict specific cellular changes in central neurons that may result from differences in sensory experience. The dorsal and ventral dendritic arborizations of nucleus laminaris (NL), a third-order brain stem auditory nucleus, receive segregated excitatory inputs from the ipsilateral and contralateral auditory nerves respectively, through one intervening excitatory synapse in nucleus magnocellularis (NM). Neuronal activity in the 8th nerve, NM and NL monotonically increases with increasing acoustic stimulation.

Silicone plastic ear plugs that provide a 40 db broadband conductive hearing loss were placed in one ear of embryonic chickens before they entered the air space of their eggs (day E 18) and were maintained in place for 28 days thereafter. At 25 days post-hatch, the brains were coded and then impregnated by a Golgi-Kopsch method. Six to ten clearly impregnated NL cells from each side of each brain were paired by position in the nucleus and drawn under camera-lucida. Blind procedures were used to prevent observer bias. The number of primary dendrites and the total projected length of the dendrites were measured on the dorsal and ventral side of every cell that had been drawn. In each bilateral pairing of cells, the dorsal and ventral dendrites that received input from the normal ear were compared with the dorsal and ventral dendrites from those same cells that received input from the deprived ear.

Analysis revealed a statistically reliable decrease ($p < .01$) in the number of primary dendrites on the sides of the NL cells receiving input from the deprived ear. An average loss of 14% of the primary dendrites on the deprived sides of the cells was observed. A small decrease in the projected length of the deprived dendrites, that was not statistically significant, was also observed.

In conclusion, a unilateral change in sensory experience differentially affected the form of specific dendritic surfaces of central neurons. This change was predictable from the structure and pattern of innervation of those neurons. These results are consistent with the hypothesis that local synaptic activity can influence the form of postsynaptic elements. (Supported by NSF grant #BNS 78-04074, funds from the Deafness Research Foundation and the Sloan Foundation, and NIH grant #MH-05949)

2129 THE EFFECTS OF EXTENDED PERIODS IN A COMPLEX OR ISOLATED ENVIRONMENT ON DENDRITIC BRANCHING IN THE VISUAL CORTEX OF ADULT RATS. William T. Greenough, Janice M. Juraska, Cynthia Elliot*, Richard Berkowitz* and Kenneth Mack*. Dept. Psychol. and Neural & Behav. Biol. Prog., Univ. of Ill., Champaign, IL 61820.

Placing adult rats in complex environments in adulthood results in heavier and thicker visual cortices (Riege, Dev. Psychobiol. 4:151,1971; Rosenzweig et al., Macromolecules and Behavior, J. Gaito (ed.), 1972) and longer terminal basilar branches in occipital layer III pyamidal cells (Uylings et al., Exper. Neurol. 62:658, 1978) than are seen in littermate controls in standard laboratory cages. These effects are not as pronounced as those following differential housing imposed immediately after weaning. Also, we have reported that after daily Hebb-Williams maze training adult rats exhibit more oblique branches in the upper apical dendrite of layer IV and V pyramidal neurons in occipital cortex, compared to handled controls (Greenough et al., Beh. Neural Biol., in press). In the present experiment male hooded rats were housed in social littermate groups from weaning to 145 days of age. They were then placed in either a complex environment (EC) with other rats and toys changed daily or in isolated, standard laboratory cages (IC) for 12 weeks, a more prolonged period of exposure than in the post-weaning studies. At approximately 230 days of age the rats were sacrificed and their brains Golgi-Cox stained. One hundred micron coronal sections were taken from the occipital cortex. Fifteen layer III pyramidal neurons per animal were traced with the aid of a camera lucida from six littermate pairs. Each neuron was scored for number and length of basilar dendritic branches at each order. There were no significant changes in the number of branches at any order. However, neurons from EC rats exhibited greater overall dendritic length ($p < .005$) greater length of terminal branches ($p < .05$) and fifth order branches ($p < .05$). This is a pattern of change in the length of distal basilar dendrites similar to that found by Uylings et al. after only 30 days of environmental exposure. We are further analyzing this cell population, as well as extending the results to other neuron populations in the visual cortex.

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2130 CHARACTERIZATION OF IDENTIFIED NEURONS OF HELISOMA MAINTAINED IN IN VITRO ORGAN CULTURE FOR THE STUDY OF REGENERATION. Robert D. Hadley and Stanley B. Kater. Dept. Zoology, Univ. Iowa, Iowa City, IA 52242.

Previous studies using an in vivo organ culture have shown that identified neurons of the snail Helisoma can functionally regenerate and specifically reinnervate postsynaptic target organs subsequent to axon interruption (Murphy and Kater, 1978). We are developing an in vitro organ culture method for Helisoma tissues using media based on commercial M199 (GIBCO) medium with salts adjusted to Helisoma hemolymph values.

Rhythmic, coordinated muscular contractions characteristic of feeding behavior in vivo are observed for at least two weeks in culture. Intracellular recording and staining techniques show that the physiology and morphology of identified neurons differ little from normal preparations. Overshooting action potentials and synaptic inputs to specific neurons in vitro have the same characteristics as neurons in non-cultured preparations.

Earlier studies have shown (with in vivo culture) that, following a nerve crush, axons of salivary secretoeffector neurons extend many sprouts through the crush site and reach the salivary glands in less than one week. Present in vitro studies show that these neurons, when axotomized, extend sprouts beyond the crush site in a similar length of time, though the exact time course of growth is variable. Furthermore, sprouting in vitro occurs by exactly the same mechanism as regeneration. That is, growth occurs by the extension of numerous small sprouts from the injured axon and these sprouts extend through and beyond the site of nerve trunk injury. Finally, in agreement with previous findings, the presence of a viable postsynaptic target organ is not necessary for initiation of sprouting.

Additionally, we have found that sprouting of neurons in the buccal ganglia is not dependent on trophic influences from other nervous tissue such as the central ganglionic ring. Sprouting of injured neurons can occur in vitro in a preparation consisting of only the buccal ganglia and buccal musculature. Observations on growing neurons in vitro are quite comparable to regeneration in vivo and allow us to employ in vitro organ culture as a higher resolution approach to questions of neuronal pathfinding.

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2131 TWO-DIMENSIONAL MAPPING OF PROTEINS FROM RAT SYMPATHETIC GANGLIA FOLLOWING AXOTOMY AND NERVE GROWTH FACTOR TREATMENT. Michael E. Hall and David L. Wilson. Dept. Physiology and Biophysics, Sch. Med., Univ. Miami, Miami, FL 33101.

Axotomy induces a number of changes in the sympathetic ganglion, including neuronal chromatolysis, cessation of ganglionic transmission due to synaptic disjunction, and changes in the two-dimensional pattern of newly-synthesized proteins (Hall, et al, 1978). It has recently been shown that, when axotomy is followed by administration of nerve growth factor (NGF), chromatolysis and the synaptic disjunction characteristic of axotomized neurons are at least partially prevented (West and Bunge, 1976; Nja and Purves, 1976). We have now examined the pattern of newly-synthesized proteins in rat superior cervical sympathetic ganglia (SCSG) following axotomy and NGF administration. The postganglionic axons exiting the SCSG in the internal carotid nerve were sectioned, under ether anesthesia, and small cubes of gelfoam containing approx. 100 µg of NGF (Mobley, Schenker, and Shooter, 1976) in a buffered salt solution, or gelfoam cubes containing only the salt solution, were implanted adjacent to the severed nerve ending. Other rats were implanted with NGF-containing gelfoam cubes without prior axotomy. Upon recovery from anesthesia, the presence of ptosis was used to confirm that the postganglionic nerve had been severed. Three days later, all SCSG were removed and the extent of ganglionic synaptic transmission (an index of synaptic disjunction) was assessed electrophysiologically. Suction electrodes were used to stimulate the preganglionic fibers and to record population spikes from the postganglionic nerve stump. Following this brief electrophysiological examination all ganglia were incubated for 1 hr in media containing ^{14}C-leucine, then homogenized and subjected to two-dimensional gel electrophoresis as previously described (Wilson, et al, 1977). The proteins were thereby separated according to both isoelectric point and molecular weight. Results indicated that, while NGF at least partially maintained ganglionic transmission, as previously reported, it did not consistently prevent the qualitative changes in the pattern of newly synthesized proteins typically induced by axotomy. NGF treatment did selectively enhance the relative rates of synthesis of some protein species. Quantitative analysis of changes in the pattern of newly-synthesized proteins following axotomy and NGF treatment will be presented.

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2132 NEURONS IN HOST RAT BRAINS WHICH PROJECT TO TECTAL TRANSPLANTS. A. R. Harvey* and R. D. Lund. Dept. Biol. Struct., Univ. Wash. Sch. Med., Seattle, WA 98195.

It has been shown previously that tectal tissue transplanted from fetal to newborn rat brains not only survives but receives afferents from the host retina and visual cortex (Lund et al., Science 193 [1976]). More recently we have used horseradish peroxidase (HRP) and ^{3}(H) proline to examine in more detail both the afferent and efferent connections of tectal transplants. The work presented here is confined to a description of the host cells projecting into the transplants.

Whole or half tectae from 15- or 16-day fetal rats were transplanted onto the dorsal surface of the superior colliculus of newborn rats. After 4 to 6 weeks the skull of the host animal was opened and the transplant injected with HRP dissolved in ^{3}(H) proline. After 24 hours the animals were sacrificed and the brains processed with either tetramethyl benzidine or diaminobenzidine as well as for autoradiography. The majority of transplants in this series were found to lie over the inferior colliculus and rostral part of the cerebellum. They were usually connected unilaterally with the host superior colliculus by one or more fiber trunks.

The most consistent input to tectal transplants arises from pyramidal cells in lamina V of the cortex ipsilateral to the side with which the transplant connects. These cells are often located in area 17 and adjacent visual areas but are also frequently found in other regions including auditory and sensorimotor cortex. Some cells have been found in cortex contralateral to the transplant. Labelled cells have also been identified in the ipsilateral and contralateral superior colliculus and occasionally in some visual and nonvisual tegmental nuclei.

The density of the host cortical projection to tectal transplants stands in contrast to that found for cortical transplants, even though the two types of tissue are commonly placed in the same region of the host brain. Experiments are currently in progress to examine the development of afferent connections to the superior colliculus in normal rats in order to compare these projections with those found to tectal transplants.

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2133 NEONATAL RATS: EFFECTS OF SUBSTANTIA NIGRA DESTRUCTION AND THE ONTOGENY OF SENSORIMOTOR AND REGULATORY BEHAVIORS. Mark Henault* and C. Robert Almli. (SPON: M. M. Patterson). Psychol. Dept., Ohio Univ., Athens, Ohio 45701.

Newborn albino rats (males and females) sustained bilateral electrolytic lesions of the substantia nigra-ventral tegmental area (SN) at one day of age (24 ± 12 hours postpartum). From one through 35 days of age, the SN and sham-operated control rats were subjected to a sensorimotor testing battery which included evaluation of orientation to multimodal sensory stimulation (e.g., vision, olfaction, somatic sensation) and simple and complex motor reflexes, as well as observations of reactivity level, activity level, posture and gait. After 40 days of age, the SN and control rats were tested for feeding and drinking behaviors under a variety of conditions, e.g., hypertonic saline injections, food deprivation, amphetamine injections. Body weight was measured daily from one to 35 days of age, and every fifth day through 100 days of age.

Following SN destruction the rats displayed attenuated growth as evidenced by reduced body weight. The tests of feeding and drinking behaviors revealed that the SN rats responded at control levels on most tests (e.g., hypertonic saline injection, amphetamine injection); however, the SN rats differed from control rats on tests involving taste and preference. For example, in a simultaneous choice situation (.05% quinine hydrochloride, demineralized water, .01% saccharine), the SN rats differed from control rats by displaying a reduction in the amount of saccharine solution consumed.

Evaluation of sensorimotor behaviors revealed an essentially normal ontogenetic development of orientation to multimodal sensory stimulation and performance of simple and complex motor reflexes. However, the SN rats tended to be hyperreactive to tactile stimulation.

The present results for SN destruction in newborn rats are in strict contrast to the severe and persistent sensorimotor and regulatory deficits which occur following SN destruction and/or catecholamine depletion in older infants and adult rats. For example, destruction of the SN in rats at 10 days of age produces widespread sensorimotor deficits, and the rats die unless maintained by hand and/or tube feeding. However, damage to this system only nine days earlier in life results in remarkable sparing or escape from sensorimotor or regulatory deficits. These results suggest that this neural system is undergoing significant maturation during the first week of life in rats.

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2134 QUANTIFICATION OF SYNAPSES AND BOUTON SIZES IN CENTRAL NOREPINEPHRINE FIBERS DURING POST-NEUROTOXIN REMODELLING. B.-H. Hwang*, J. Jew and T. H. Williams. Dept. Anat., Univ. of Iowa, Iowa City, Iowa 52242

This is part of a quantitative study of turnover of central catecholaminergic (CA) elements under experimental conditions. The tendency of boutons to reaccumulate within the paraventricular hypothalamic nucleus (PAR) subsequent to neurotoxin lesions is noteworthy and has been demonstrated by fluorescence microscopy and electronmicroscopy. CA terminals in the PAR are further characterized by a high proportion of synaptic contacts. The paraventricular nucleus therefore provides an opportunity to assess the impact and limits of the sprouting responses, the presumption being that the synapses are in a functional state. Data about bouton sizes and numbers of synapses will be dealt with separately.

Young adult male rats were perfused and the hypothalamus processed for fluorescence microscopic (FM) and electron microscopic (EM) examination at 4, 21, 56 and 180 day intervals after administration of 200 µg 6-OH-DA into the right lateral cerebral ventricle. CA varicosities in the PAR were counted. Norepinephrine is the major catecholamine present in this nucleus.

FM data showed CA varicosities at 45% of control numbers 4 days after 6-OH-DA, with partial restoration to 79% of controls by 180 days. EM data for CA terminals (identified by the marker 5-OH-DA) showed a more severe reduction (to 12% of control numbers) after 4 days, with partial restoration to 54% by 180 days. Average bouton diameter (for terminals identified as CA by EM marker) was strikingly similar (1.0 µ) for controls and all survival intervals, although boutons larger than 2.3 µ in diameter were encountered with greater frequency in all experimental groups than in controls.

Specializations that meet accepted criteria for synapses were counted around the contours of CA terminals. Synapses were recognized around profiles of 33% of CA terminals in control preparations. Synapses were evident around 31% of boutons 4 days after 6-OH-DA; 42% of boutons 21 and 56 days after treatment; and 36% of boutons 180 days after the neurotoxin. These results suggest that the new CA sprouts (seen 21, 56 and 180 days post-lesion) are associated with synaptic specialization. (HL21914 to JJ; NS11650 to THW).

2135 IS SENSORY NERVE ACTIVITY NECESSARY FOR COLLATERAL SPROUTING IN THE SKIN OF ADULT RATS? Patrick C. Jackson and Jack Diamond. Dept. Neurosciences, McMaster Univ. Med. Ctr., Hamilton, Ontario, Canada L8S 4J9.

We found previously in rats that intact low-threshold mechanosensory nerves appeared to sprout into adjacent denervated skin only prior to about 20 days of age; in older animals this "denervation sprouting" did not occur (Jackson and Diamond, Abstr. 1515, Soc. Neurosci.; 1978). We have new evidence that high-threshold mechanosensory nerves can be induced to sprout in denervated adult rat skin. In these experiments the presence of high-threshold innervation is defined behaviourally in quiet unanaesthetized rats, by the ability of brief forceps-pinches of back skin to elicit a reflex contraction of the underlying cutaneous trunci muscle. In three groups of adult animals clearly defined areas of back skin lacking sensibility were produced by section of selected dorsal cutaneous nerves. The first group was behaviourally mapped one day after operation. The second group was mapped 24 days after operation. The animals of the third group were lightly anaesthetized at 4 day intervals, and pinches (somewhat stronger than those used for the behavioural testing) were applied to both innervated and denervated skin regions; at 24 days after the denervation behavioural mapping was done.

The results from the first two groups showed that in the 24 days following the denervation there was no significant shrinkage of the insensitive regions (675 $\pm$ 140 (mean $\pm$ S.D.) mm^2, and 625 $\pm$ 150 mm^2 respectively); i.e. there was no evidence of high-threshold nerve sprouting. In the third group however the insensitive skin regions shrank (to 215 $\pm$ 150 mm^2). It would appear then that stimuli normally adequate to excite high-threshold endings provoked nerve sprouting into denervated skin in the adult animal; in the absence of such stimulation sprouting did not occur. Significantly, the post-operative pinching did not cause an extension of the low-threshold "touch" fields, suggesting that regeneration after damage to nerve endings may not have been responsible for the decrease of the insensitive area. Among the possibilities we are now examining is that the combination of nerve-free target tissue plus activity in remaining nerves causes collateral sprouting in the adult animal.

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2136 CONNECTIONS BETWEEN CEREBRAL CORTEX TRANSPLANTS AND RAT HOST BRAIN. Christine B. Jaeger and Raymond D. Lund. Depts. Biol. Struct. and Neurosurg., Univ. Wash. Sch. Med., Seattle, WA 98195.

Cerebral cortex from rat embryos survives and matures after transplantation to newborn rat brains. We studied the question whether transplants form connections with the host brain, and if so, whether these connections were specific for the type of brain region transplanted or whether they were influenced by implant position within the host brain.

Cerebral cortex from rat embryos of gestational age 16 to 17 days was transplanted to the tectal region of newborn rats and allowed to mature for periods of 4 to 7 weeks. Subsequently, efferent fibers were demonstrated using anterograde transport of tritiated amino acids and the Fink-Heimer degeneration technique. Afferent fibers to the transplant could be shown by retrograde transport of horseradish peroxidase. Animals with transplants that were either embedded in the host tectum or connected to it by a recognizable fiber trunk were selected for this study.

Small bundles of efferent fibers could be demonstrated most often in the central gray and superior colliculus of the host. In some cases distribution of transplant efferents was noted in the pretectum and midbrain tegmentum.

Afferent connections to cortex transplants were identified from layer V pyramids in various regions of the host cortex, pretectal neurons, and occasional neurons from deep layers of the host superior colliculus. The afferent fibers to cortex transplants are relatively sparse compared to afferent connections that are formed if embryonic tectum is transplanted to the same region, as shown in other experiments. In no case tested could retinal afferents be demonstrated within the cortex transplant.

The results show that cortex transplants develop fiber connections with the host brain. Except for the lack of retinal afferents the pattern of connections formed more closely resemble those expected of superior colliculus rather than cerebral cortex. This suggests that location is a significant factor in determining fiber connections which form between the transplant and host. However, qualitative and quantitative differences in transplant-host fiber connections could be observed in cortex transplants, compared to tectal or retinal transplants placed in a similar position of the host brain. Consequently, the influence of neuron specific qualities on pathway formation cannot be disregarded. (Supported by USPHS Grant EY-01950 from the NIH.)

2137 EFFECTS OF DIFFERENTIAL POSTWEANING ENVIRONMENTS ON DENDRITIC FIELDS OF MALE AND FEMALE RATS. Janice M. Juraska and William T. Greenough. Dept. Psychol. and Neural & Behav. Biol. Prog., Univ. of Ill., Champaign, IL 61820.

There is behavioral evidence that females are less susceptible to the effects of the environment during development than males (e.g., Sackett, Sex Differences in Behavior, R. C. Friedman et al. (eds.), 1974). There is also neuroanatomical evidence that female rats, when exposed to a complex environment from 60 to 116 days of age, show a smaller change in visual cortex thickness than do male rats (Diamond et al., Intern. J. Neurosci. 2:171,1971). In the present experiment dendritic fields were examined after male and female rats had been exposed to differential environments at weaning. Twelve littermate sets of two male and two female hooded rats were assigned to either a complex environment (EC) with other same sex rats and daily toy changes or to an isolated standard laboratory cage (IC) at weaning (23-25 days of age). After 30 days of environmental exposure, the brains of all animals were stained with the Golgi-Cox method and 100 nm coronal sections were taken from the visual cortex area. Fifteen layer IV stellate neurons were traced with the aid of a camera lucida from each member of seven littermate sets. Each neuron was scored for number and length of dendritic branches and the number of intersections of branches with a overlay of concentric rings at 20 micron intervals. Although there were no differences in the number of dendritic branches, the concentric ring analysis revealed that the EC animals had significantly more intersections in the middle rings in both sexes. In the outermost rings there were significant environment by sex interactions where male EC rats had more outer dendritic material than female EC rats while there were no differences between male and female IC rats. Dendritic length measures followed the same pattern of greater length for EC and male rats. Another cell population is currently being collected. The results thus far show that the dendritic processes of both sexes may respond to differential environments but that the male response is somewhat greater.

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2138 REDIRECTION OF PYRAMIDAL TRACT AXONS IN NEONATAL HAMSTERS: SOME MECHANISMS OF AXONAL GUIDANCE DURING DEVELOPMENT.
K. Kalil and T. Reh. Dept. of Anatomy and Neurosciences Training Program, Univ. of Wis., Madison, WI 53706.

In previous experiments we have shown that when pyramidal tract axons are cut in infant hamsters there is massive regrowth of the severed axons via a new brainstem pathway which decussates in an aberrant position, descends through the brainstem in an abnormal course, but nevertheless terminates appropriately in the dorsal column nuclei and spinal cord on the correct side of the brain (Kalil and Reh, '78). Regrowth of the cut fibers is maximal in animals 4-8 days of age.

In the present experiments, animals younger than 48 hours received a unilateral lesion of the medullary pyramid several mm rostral to the decussation of the tract. At this age a substantial portion of the fibers are still growing into the medullary pyramid. Thus when (H^3) proline was injected into the ipsilateral sensorimotor cortex of animals surviving to adulthood, we observed not only labeled regrowing fibers as previously described, but also many apparently later arriving fibers which were redirected rather than damaged by the lesion. Many of the fibers were deflected across the midline and descended through the intact contralateral pyramid. Surprisingly, when these fibers reached the decussation they recrossed to the "wrong" side of the brain. Other fibers which were deflected completely away from both pyramids descended through the brainstem to form a completely anomalous pathway in the lateral margin of the spinal cord. These findings suggest that (1) Growing axons deflected across the midline to the opposite side of the brain appear to be pulled along to the incorrect side by the decussating normal fibers from the intact pyramid, (2) Axons deflected far from the decussation continue on that side of the brain rather than crossing, (3) Growing axons appear able to descend through channels or pathways which under normal circumstances they never follow. These results indicate that mechanical factors in the substrate rather than intrinsic factors in the axon itself may play a more important role in determining specifically how axons cross and more generally how they are guided to their destinations in the developing brain.

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2139 **NEUROGENESIS IN THE ADULT RAT VISUAL CORTEX**
Michael S. Kaplan* (SPON. J.W. HINDS). Dept. Anatomy, Boston University School of Medicine, Boston, MA. 02118

The existence of newly formed neurons in the adult mammalian neocortex has been reported by several investigators using the light microscope. Unfortunately, none of these studies could confirm that the cells labeled with H^3-thymidine were indeed neurons because in light micrographs labeled neurons cannot be distinguished with certainty from neuroglial cells. In the present study it has been confirmed that new neurons are indeed forming the adult visual cortex, substantiated by a method that allows serial thin sectioning of 1μm, plastic embedded sections previously prepared for light microscopic radioautography.

Four normal male rats (Charles River) were injected once at 90 days of age with H^3-thymidine (4.3 μc/gm body weight), and after a 30 day survival the animals were perfused. Very rarely, labeled cells were found in 1 μm plastic sections that resembled small neurons. Analysis of electron micrographs of selected examples of these labeled cells clearly demonstrated stellate neurons in layer IV with synapses along their cell bodies and dendrites. In order to quantify the relative frequency of labeled neurons, the number of heavily labeled cells seen in 1μm sections was expressed as a percentage of the total number of neurons identified in sections through the entire visual cortical thickness; the percentage was 0.0037%.

The labeled neurons are not considered to result from polyploidy since several recent investigations discredit earlier reports of polyploid DNA in large neurons, and small neurons have never been observed to be greater than the diploid amount. Furthermore, all of the labeled stellate neurons have been found in layer IV, which makes the possibility that the labeling results from non-specific DNA repair unlikely. The results of the present study are in agreement with evidence of neurogenesis of granular type neurons in the adult rat olfactory bulb and dentate gyrus (Kaplan & Hinds 1977). Thus it has now been confirmed that relatively small neurons and their synapses are found in at least 3 brain regions (olfactory bulb, dentate gyrus and visual cortex) in a normal adult mammal.

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2140 SPREAD OF CATECHOLAMINES PERFUSED INTO VISUAL CORTEX: EFFECTIVE CONCENTRATION FOR MODIFICATION OF CORTICAL PLASTICITY.
Takuji Kasamatsu, Toru Itakura*, and Gösta Jonsson*. Div. Biology, Calif. Inst. Tech., Pasadena CA 91125, and Dept. Histology, Karolinska Institute, Stockholm, Sweden.

We have been studying the role of catecholamine (CA)-containing terminals in the modulation of synaptic plasticity in kitten visual cortex. In the previous physiological assay, 4 mM 6-hydroxydopamine (6-OHDA) seemed to reach a region about 6 mm away from the perfusion site after a week-long, continuous microperfusion (Kasamatsu et al., J. Comp. Neur. 185, 1979). In the present study, we have further examined the extent of intracortical diffusion 6-OHDA and norepinephrine (NE) delivered through the continuous perfusion technique. Three methods have been used.

1) CA histofluorescence

The kitten visual cortex was perfused locally with 4 mM 6-OHDA for a week, and then prepared for CA histofluorescence using a modified glyoxylic acid-cryostat method. We found an area close to the perfusion site in which no greenish fluorescent fibers and terminals remained. The radius of this primary lesion area was 4-5 mm from the center of perfusion.

2) Endogenous CA

An electrochemical assay was performed for NE and dopamine (DA) (Keller et al., Life Sci. 19, 1976) in kitten visual cortex after the microperfusion of 6-OHDA. Endogenous NE was less than 10% of the control 3-4 mm away from the perfusion site, and then it increased to 80-90% of the control at 7-8 mm. The DA content was less affected by 6-OHDA treatment.

3) Intracortical distribution of tritiated NE

The spatial distribution of tritium in the neocortex was studied following 1, 3, or 7 days of continuous perfusion with 50 μM nonradioactive NE mixed with a tracer quantity of tritiated NE. As reported previously, counts were maximal at the site of perfusion and decreased exponentially in the neighboring area (Kasamatsu et al., J. Comp. Neur. 185, 1979). This distribution pattern was attained as early as 1 day after the start of perfusion. Although the radioactivity at a given site in the cortex increased with time, its maximum detectable extent was about the same (10 mm) for the three different perfusion methods.

Combined with the dose-response relation obtained in other physiological experiments, the above information has enabled us to estimate that the threshold concentration for NE's effect on synaptic plasticity is around 10^{-7} M.

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2141 FAILURE TO FIND SPARING OF SPECIES TYPICAL BEHAVIORS FOLLOWING NEONATAL PREFRONTAL CORTEX LESIONS.
Bryan Kolb and Ian Q. Whishaw. Dept. of Psych., U. of Lethbridge, Canada.

Rats with lesions of the prefrontal cortex (PFC) in infancy exhibit remarkable sparing of function compared to adults with similar removals. Lesions of the medial frontal (MF) cortex in infancy fail to produce deficits on tests of cognitive behavior such as spatial reversals, delayed response and active avoidance which are seriously disrupted by MF lesions in adulthood. Further, unlike adult lesions these early lesions fail to produce the degeneration in the dorsomedial nucleus of the thalamus that typically accompanies adult lesions. (Kolb & Nonneman, Br. Res., 1978, 151, 135).

The findings that early lesions can spare certain functions led to two questions that we addressed in the current project. 1) Was the sparing of function being mediated by other frontal cortical zones? This was tested by removing the entire frontal lobe at 7, 25 or 100 days of age. 2) Would there be sparing on such tests of natural behaviors as food hoarding or maternal behavior? This was studied by observing the same animals on tests of spatial reversals, active avoidance, hoarding, and maternal behavior as well as by videotaping each rat's home cage behavior for 24 h.

The results showed that sparing following PFC lesions is task specific as species typical behavior shows little sparing as compared to tests of cognitive behavior. Removal of the PFC at 7 or 25 days of age allowed normal performance on spatial reversals and active avoidance but abolished hoarding and maternal behavior. In the 24 hour home cage study the 7 day Ss were virtually identical to controls whereas the 25 and 100 day Ss had difficulties in drinking from a water spout and were excessively active.

Anatomically, the lesions in 7 day operates were smaller to visual inspection but the brains of these animals were significantly smaller overall than those of older animals, weighing about 85% of the 25 and 100 day Ss even though there was no difference in body weight. We again failed to find degeneration in the dorsomedial nucleus of the thalamus of the 7 day Ss as compared to the 25 and 100 day animals.

These results imply that species typical behaviors are more sensitive to neonatal brain injury than are tests of cognitive behavior. It is likely that other regions of the brain can assume general functions required for acquiring problems such as spatial reversals but the coordination of the details of complex praxic behaviors such as are observed in many species-typical behaviors can only be performed by the prefrontal cortex.

2142 PLASTICITY OF A SOMATOSENSORY CORTICAL COLUMN: A COMPARISON OF THE EFFECTS OF NEONATAL AND ADULT RECEPTOR ABLATIONS IN THE RAT USING THE (^{14}C)-2-DEOXYGLUCOSE TECHNIQUE. M. Kossut*, P. Hand, J. Greenberg*, A. Sylvestro*, C. Goochee*, and M. Reivich. Departments of Animal Biology and Neurology, Schools of Veterinary Medicine and Medicine and Institute of Neurological Sciences, University of Pennsylvania, Philadelphia, Pennsylvania 19104; Department of Neurophysiology, Nencki Institute of Experimental Biology, Warsaw, Poland; and Laboratory of Cerebral Metabolism, NIMH, Bethesda, Maryland, 20014.

Plasticity of cortical representation of mystacial vibrissae of rats (barrel columns in SI cortex) after receptor ablation was studied by the (^{14}C)-2-deoxyglucose (2DG) method in unanesthetized, restrained rats. The local cerebral metabolic rate of glucose (LCMRG) of layers I-VI of the SI barrel column responding to stimulation of C3 vibrissa was determined. The stimulation was brush stroking for 45 minutes preceeded by an intravenous injection of 50 uCi of 2DG as described by Hand et al. (Neurosci. Abst., 3,4:1977,78). They demonstrated that stimulation of one whisker increases the LCMRG of one cortical column, which extends from layer I to VI in a candle-pin shape. Columnar plasticity was investigated 3 months after unilateral ablation of all but the C3 vibrissal follicle, done either in adulthood or 2 to 4 days postnatally. With adult receptor damage the appearance of the column related to single vibrissa stimulation resembles the control, being easily discernable in all layers. However, LCMRG of layer IV of the column is decreased by 7%, while that of the lower part of lamina V is higher (18%) than in the control column. The diameter of layer IV activation is 20% larger than on the control side, which is in agreement with the barrel measurements on thionin sections. In addition, the activated region surrounding the columnar profile is more extensive in layers I-IV. With neonatal follicle ablations, the cortical area labeled by single whisker stimulation is enlarged and, with the exception of layer IV, the typical columnar profile is lost. The diffuse label within SI cortex is patchy, especially in supragranular layers, unlike the normal column in which the pericolumnar activity gradually diminishes. The LCMRG in layer IV is reduced by 15%, but is elevated in supragranular layers. The tangential spread of pericolumnar activity is much greater in rats with neonatal damage. In conclusion, these results are in agreement with the findings of electrophysiological alterations observed after neonatal vibrissal removals (Killackey et al., Neurosci. Abst., 3, 1977) and stress the role of supragranular and to a lesser extent, infragranular layers in cortical plasticity. (Supported by grants NS-06716 of USPHS and 76-10-9 of Sloan Foundation).

2143 GROWTH OF AXONAL PROCESSES FROM IN VIVO TRANSPLANTS OF RAT LOCUS COERULEUS. Richard M. Kostrzewa and Hideki Fukushima*. Dept. of Pharmacology, College of Medicine, East Tennessee State University, Johnson City, Tennessee 37601.

As a means of determining mechanisms associated with neuronal growth and development, we have transplanted a discrete central noradrenergic nucleus of newborn rats to other neonatal rat brains. Sterile technique was used to remove the locus coeruleus which was then immediately (< 2min) placed into a number of different recipient brain sites. Using a glyoxylic acid staining procedure we were able to observe fluorescent cell bodies of the coeruleus, when the latter was placed either within the cerebellum or neocortex. Cell bodies of the transplant retained their fluorescence, and axonal processes began to emerge from the transplant into recipient brain tissue within 2 days. Using a trihydroxyindole fluorimetric assay, we have also found that the norepinephrine content of the cerebellum is elevated at 2 months after transplantation into this region. These studies indicate that selective noradrenergic cell nuclei remain viable after transplantation, and that such nuclei can innervate recipient brain tissue. (This study was supported by NEUA grant no. NS-14797).

2144 ULTRASTRUCTURAL CHANGE IN THE HIPPOCAMPAL SLICE FOLLOWING REPETITIVE ACTIVATION. Kevin S. Lee, Mike Oliver*, Frank Schottler* Bob Creager* and Gary Lynch. Dept. of Psychobiology, Univ. of Calif.,Irvine, Irvine, Calif.,92717

Recently we reported that changes in synaptic structure occur in conjunction with the induction of long-term potentiation (Anat.Rec.193,3:601,1979). Specifically, an increased incidence of synapses onto dendritic shafts was observed in the stratum radiatum of CA-1 in the rat hippocampus following high frequency stimulation of the combined Schaffer collateral and commissural afferents. In the present study we sought to replicate this finding utilizing the in vitro hippocampal slice preparation. This technique has been extensively employed for electrophysiological and biochemical studies, however, little information exists as to its utility for anatomical investigations. Initially parametric analyses were performed to optimize the preservation of neuronal elements. Once conditions for achieving replicable tissue quality were ascertained, experiments were undertaken to examine the effects of repetitive afferent activation on neuronal ultrastructure. Evoked potentials recorded in the stratum radiatum were tested prior to and following either high (200 sec^{-1}) or low frequency (0.2 sec^{-1}) repetitive stimulation of the Schaffer collateral and commissural axons. High frequency stimulation consistently resulted in a non-decremental (over 15 min) increase in the size of the evoked response while low frequency stimulation had little or no effect on the potential. Ultrastructural analyses of the synaptic region which was activated showed no difference between the high and low frequency stimulated slices in the following measures: 1)the incidence of synapses onto dendritic spines, 2)the number of multiple synaptic boutons, 3)spine area, 4)spine stalk width, 5)psd length for synapses onto dendritic spines and 6)psd length for synapses onto dendritic shafts. However, in the potentiated slices, the incidence of synapses onto dendritic shafts was increased by 50% ($p < 0.01$, on a two-tailed test). These results are consistent with our previous study in which the only structural modification observed was an increase in the number of dendritic shaft synapses. The primary difference between this and the previous study, aside from the in vitro versus in vivo preparations, was the time interval allowed between afferent stimulation and tissue fixation. The slices were tested for only 15 minutes following stimulation, after which time they were immediately immersed in fixative. It thus appears that the structural change observed can occur at a very rapid rate.

2145 UNUSUAL REORGANIZATION OF THE INTACT MOUSE OLFACTORY BULB AFTER LONG-TERM SURVIVAL OF NEONATAL UNILATERAL BULBECTOMY. Richard R. Levine* and Pasquale P. C. Graziadei (SPON: Karen K. Glendenning). Dept. Biol. Sci., Unit I, Florida St. Univ., Tallahassee, FLA 32306.

In previous work we have shown that by 30 days following unilateral bulbectomy in neonatal mice, olfactory fibers regenerate and penetrate various regions of the ipsilateral telencephalon. Here they form glomerular structures and synapse with neurons within the host forebrain.

Recently, however, we have encountered a number of preparations with long-term survivals displaying a heretofore unobserved degree of neuronal reorganization. Each of these animals was bulbectomized at 5 days postnatally, sacrificed 180-365 days later, and prepared routinely for light microscopy. Our histological results, in general, indicate the typical regenerative phenomena previously noted, i.e., innervation of the ipsilateral telencephalon and glomerular formation in the forebrain by the reconstituted olfactory axon terminals. However, in some animals, we have also observed a medial expansion of the intact, unoperated bulb into the space vacated by the operated bulb. Furthermore, many of the regenerated axons on the operated side could be seen entering the fiber plexus innervating the intact bulb. This outgrowth of the intact olfactory bulb may be characteristic of all its cellular components, although it is most strikingly displayed by the granule cells. In one case, the expansion and reorganization of the intact bulb was rather extensive, so that the bulb appeared to be continuous with both the ipsilateral and contralateral telencephalic hemispheres. The role of possible injury to the intact bulb and/or neighboring bone and the pattern of bulbar reorganization is presently under study.

Both the observed bulbar outgrowth and the subsequent innervation of regenerated fibers may result in non-specific connections within the olfactory system and illustrate a potential capacity for neuronal plasticity after surgical insult to this system. Supported by NIH Grants 5/T/32 NS07010 (R.R.L.) and NS 08943 (P.P.C.G.) and NSF Grant BNS 77/16737 (P.P.C.G.).

2146 CORTICAL ORGANIZATION IN PRENATAL ENCEPHALOCLASTIC PORENCEPHALY. A GOLGI STUDY. M. Marin-Padilla and M.T. Marin-Padilla*. Dept. Pathology, Dartmouth Medical School, N.H. 03755.

Prenatal encephaloclastic porencephalies are characterized by focal destruction of the cerebral wall which may be reduced to a thin membrane composed of ependymal and arachnoidal elements, by hydrocephalus and by glial and vascular scar formation. It is belived that this type of congenital malformation is caused by an accident of undetermine nature (vascular,ischemic or infeccious) which resulted in cortical destruction, secondary hydrocephalus and eventual formation of porencephalic cysts. The fundamental plan of neuronal migration and cortical organization are considered to be primarily unaltered in this disorder. Therefore, the cerebral cortex away from the cystic lesions may appear to be histologically 'normal' or without obvious cytoarchitectural abnormalities. On the other hand, areas closer to the defects depict glial and vascular scars, prominent cytologic anomalies and various degrees of cortical atrophy. Intermediate cortical areas depict, however, more subtle changes including: a tendency of neurons to form columnal aggregates in upper layers which alternate with cell-free spaces, arachnoidal glial and vascular scars and the presence of abnormaly large neurons in layer II and III.

Rapid Golgi preparations of the cerebral cortex of a premature infant with this type of disorder has permitted, for the first time, a morphological investigation of changes described above which has resulted in a better understanding of their nature. This study has also demonstrated that many of those changes could be the result of an active reorganization of cortical structures which has followed the prenatal injury and reflect the degree of fibrillar-neuronal plasticity still present. Of special interest is the bizarre dendritic morphology of the giant neurons of the upper cortical layers. Detailed morphological analysis of the features of these giant abnormal neurons and of the development of the glial and vascular scars will be presented. The case illustrates the capacity and degree of the human cerebral cortex to undergo structural reorganization following a prenatal injury. It should be pointed out, that infants with this disorder may survive and become mentally retarder and epileptics. the interrelationships between a prenatal injury, the degree of cortical reorganization and the subsequent appearance of epilepsy should be explored in this type of congenital disorder.
(Supported by NICH-HD grant # 09274).

2147 CHRONIC INTRAVENTRICULAR ADMINISTRATION OF LSD AFFECTS THE SENSITIVITY OF CORTICAL CELLS TO MONOCULAR DEPRIVATION. Maureen A. McCall*, David G. Tieman*, and Helmut V.B. Hirsch. Neurobiology Research Center, State University of New York at Albany, Albany NY 12222.

In kittens, but not in adult cats, depriving one eye of vision (MD) for a week reduces the proportion of units in the visual cortex that can be activated by both eyes. The sensitivity of cortical units in adult cats to MD can be altered by changes in levels of monoamines in the brain (Kasamatsu, Pettigrew and Ary, 1979). Since LSD interacts with monoamines, we have examimed the effects of chronic administration of LSD on the sensitivity to MD of cortical cells in adult cats. Cats were assigned randomly to one of four conditions: MD/LSD; MD/no-LSD; no-MD/LSD; no-MD/no-LSD. An osmotic minipump (Alza Corp) was placed subcutaneously, and attached to a cannula made from a 25-gauge needle which was implanted in one lateral ventricle. The pump delivered either LSD tartate (0.88 µg/kg/hr dissolved in normal saline at pH 5.5) or the vehicle solution alone for a period of one week. In addition, at the time of implantation, the lids of one eye of the MD animals were sutured shut. The behavior of the animals was observed during administration of the drug, but no obvious anomalies were detected. One week after the implantation of the cannula/minipump assembly the response properties of single units in area 17 of the visual cortex were studied. The experimenters did not know the contents of the individual minipumps during the course of the recordings.

With the exception of ocular dominance, the response properties of units recorded in all animals did not differ from those of cells present in normal cats. In the three control conditions (MD/no-LSD, no-MD/LSD, no-MD/no-LSD) the proportion of monocular cells was less than 20%. This percentage does not differ from that observed for normal adult cats. However, if LSD was administered during the period of monocular deprivation, some 40% of the cells were monocular. Comparable results were obtained from three animals given LSD during MD in a pilot study. Our results suggest that chronic intraventricular administration of LSD affects either directly or indirectly the sensitivity of cortical neurons to monocular deprivation (supported by USPHS Grant RO1DA01684 and by Alfred P. Sloan Foundation Fellowship BR1677).

2148 DEVELOPMENT OF RETINA TRANSPLANTED TO THE TECTUM OF NEONATAL RATS. Steven C. McLoon and Raymond D. Lund. Dept. Biol. Struct., Univ. Wash. Sch. Med., Seattle, WA 98195.

We have attempted to dissociate some of the developmental patterns which are innate to the developing retina from those imposed upon the tissue by the context in which it develops. Retinas were excised from rat embryos and injected adjacent to the left superior colliculus of neonatal rats from which the right eye was removed. Four weeks later lesions were made in the transplanted retinas. After an appropriate survival time they were perfused with paraformaldehyde and sections of the brains were processed with neurofibrillar, degeneration and Nissl stains.

Embryonic retina transplanted to the superior colliculus survived and frequently fused with the host tissue. Large retinal transplants were often embedded in the tectum. Histological examinations revealed the retinal transplants to be well differentiated. They developed the three cell layers characteristic of normal retina. Frequently, these layers were arranged in rosettes with the photoreceptor cells towards the center. The photoreceptor cells did not have outer segments, but they did show a ciliated border. The ganglion cell layer was sparsely populated with large well differentiated neurons, presumably ganglion cells.

The neurofibrillar stain revealed fibers coursing between the transplants and the host brains. The greatest number of fibers appeared to run between the ganglion cell layer and the superficial layers of the host's tectum. It was common for transplants to be embedded in the anterior cerebellum with grossly discernible tracts connecting it with the tectum. This facilitated lesioning the transplant without damaging the tectum. Degeneration analysis after these lesions showed projections only to visual nuclei. The heaviest projection was to the superficial layers of the tectum, the stratum opticum and stratum griseum superficialis. There were also projections to the pretectum, dorsal terminal nucleus and the dorsal lateral geniculate nucleus. The discrete efferent projections of retinal transplants contrast with the broader distribution of connections from cortex and tectum transplanted to the same region.

These results suggest that retinal efferents may have a special affinity for denervated visual nuclei; furthermore, this appears to be a useful preparation for studies on neuronal specificity. (Supported by USPHS Grant EY-01950 from the NIH.)

2149 COOPERATIVITY IN THE GENERATION OF POST-TETANIC SYNAPTIC ENHANCEMENT APPEARS TO REQUIRE NEAR SIMULTANEITY OF AFFERENT INPUT. B. L. McNaughton and C. A. Barnes. Dept. Psych., Dalhousie Univ., Halifax, Nova Scotia, CANADA, B3H 4J1.

Following brief episodes of high frequency activity, synapses of the perforant pathway may undergo a prolonged enhancement of their efficacy (Bliss & Lømo, J. Physiol., 1973, 232, 331-356). This enhancement has been shown to involve a cooperativity among coactive afferent fibres (McNaughton, Douglas, & Goddard, Brain Res., 1978, 157, 277-293). In particular, synapses of the medial and lateral components of the perforant path exhibited considerably more enhancement when activated concurrently than when activated independently with the same stimulus parameters. It was of interest to determine whether such cooperativity requires simultaneity of afferent input, or whether there might still be some cooperation observed when temporal delays between the bursts of activity on the two input pathways are introduced. This question is of interest not only because of its reation to the mechanism of enhancement, but also because it relates to models of associative memory which postulate enhancement as an underlying mechanism.

Methods for extracellular recording synaptic responses of the medial and lateral perforant pathways were as described by McNaughton et al. (1978). The experimental paradigm was also essentially identical except for the stimulation parameters. In one hemisphere, 10 stimulus trains of 40 msec at 250 Hz were delivered at alternate six second intervals to each pathway. In the other hemisphere, each 40 msec train to the lateral pathway began 10 msec following the end of the equivalent train to the medial pathway. Eighteen animals were used, with the two hemispheres serving as matched samples as described previously.

There were no statistically significant differences within either pathway (paired t medial = 1.44, d.f. = 17; paired t lateral = -1.07, d.f. = 17) in the amount of enhancement produced by the two stimulus conditions. As in the previous study, however, the lateral pathway showed significantly more enhancement than the medial.

Given the magnitude and reliability of the cooperative effect observed in the previous study with simultaneous activation (e.g. in 15 animals, there were none in which the simultaneous condition gave less enhancement than the independent (six second) condition), we are inclined to accept the null hypothesis in this case and conclude that the cooperative effect requires very nearly simultaneous input from both pathways. For models of associative memory, this conclusion implies that there must be simultaneously active internal representations of two events in order for an association to be formed between them.

2150 LESION-INDUCED CHANGES OF COMPLEX CARBOHYDRATES IN THE RAT DENTATE GYRUS. E.E. Mena* and C.W. Cotman (SPON: H.C. Agrawal) Dept. of Psychobiol., Univ. of California, Irvine, CA 92707.

Glycoproteins and glycolipids have often been suggested to play a role in synapse formation. The hippocampus is a suitable system to examine the distribution of glycocomponents in a well-defined laminated system. By using lectins coupled to horseradish peroxidase, we have investigated both the normal distribution of glycocomponents in the rat hippocampal formation (HF) and also the rearrangement of these groups in response to lesions of the entorhinal cortex.

Con A (100 µg/ml) receptors in the HF are concentrated on the cell bodies of the pyramidal and granule cells. Con A binds in greater quantity to the inner third of the molecular layer of the dentate gyrus than any other dendritic zone of the HF. Very little reaction product is found in the stratum radiatum or stratum lacunosum-moleculare. At higher Con A concentrations (250 µg/ml) reaction product is homogeneously distributed throughout the molecular layer and stratum radiatum. However, Con A still fails to react with the stratum lacunosum-moleculare. Fucose binding protein (FBP) (200 µg/ml) also reacts with the granular and pyramidal cell layers. In addition, there is a band of intensified staining at the interface between the first and second thirds of the molecular layer. Otherwise the reaction product appears homogeneous throughout the remainder of the molecular layer and stratum radiatum.

Ricinus communis agglutinin (RCA) (200 µg/ml) also shows homogeneous staining throughout the stratum radiatum and outer two-thirds of the molecular layer. Like Con A and FBP, there is intensified staining in the first third of the molecular layer. Also, the stratum lacunosum-moleculare contains the lowest amount of RCA binding in the hippocampal formation. This is also the case for Con A binding.

The hippocampus was studied at 3 or 30 days after a unilateral entorhinal lesion. At 3 days post-lesion no changes were found in Con A binding. However, at 30 days post-lesion an alteration of these lectin receptors patterns was seen. The amount of Con A (100 µg/ml) binding increased greatly in the entire molecular layer and the stratum lacunosum-moleculare. FBP and RCA binding also increase throughout the entire molecular layer. The increase in binding of FBP extends past the fissure but does not appear to occupy the entire stratum lacunosum-moleculare. However, in contrast to Con A and FBP, the increase of RCA binding is largely restricted to the dentate molecular layer. These data demonstrate a plasticity of glycocomponents during active synaptic growth in the mature brain. (Supported by Grants NS08597 and MH19691)

2151 COLLATERAL-SPECIFIC LONG TERM POTENTIATION (LTP) OF HIPPOCAMPAL FIELD CA_3 OUTPUT IN THE RAT. J.J. Miller & N. McNaughton*. Dept. Physiology, Univ. British Columbia, Vancouver, Canada V6T 1W5.

Brief, high frequency stimulation of hippocampal afferents has previously been shown to result in LTP of the synaptically evoked potentials to subsequent single pulse stimulation of these inputs. While the mechanisms involved remain unclear, it has been demonstrated that the response is input specific and not a property of the target cell, implying that alterations in presynaptic or immediate postsynaptic regions may account for LTP. If a presynaptic change was involved (e.g. augmented transmitter release) then it might be expected to occur in all terminal projections of a particular neurone. To test this possibility, the characteristics of frequency potentiation and LTP of CA_3 projections to the CA_1 pyramidal and the lateral septal neurones (LS) was investigated in the urethane-anaesthetised rat.

Stimulation in the region of hippocampal field CA_3 evoked CA_1 and LS responses which exhibited similar threshold profile, strength-duration and frequency following characteristics suggesting that the same population of CA_3 neurones was being activated in both cases. CA_3 neurones were also antidromically driven from both LS/fimbria and CA_1/Schaeffer collateral stimulation sites. During low frequency (10-15 Hz) stimulation of CA_3, the CA_1 population spike exhibited potentiation while the LS response was depressed. Tetanic stimulation (100 Hz) resulted in LTP of the subsequent CA_1 population spike (400%) but no consistent change in the LS evoked field.

The collateral-specific properties of LTP demonstrated in this study, together with data indicating input-specificity suggest that changes in the immediate postsynaptic membrane are necessary to induce LTP or that presynaptic changes must be terminal specific.
(Supported by MRC of Canada and Royal Society, London.)

2152 NEURONAL PATHWAY SELECTION: DIFFERENTIAL ABILITY OF REGENERATING IDENTIFIED NEURONS TO DISCRIMINATE BETWEEN "CORRECT" AND "INCORRECT" PATHS. A.D. Murphy and S.B. Kater. Lab. Molecular Biol., Univ. of Wisconsin, Madison, WI. 53706 and Dept. Zool., Univ. of Iowa, Iowa City. IA. 52242.

The mechanisms which guide the paths of growing neurites are fundamental to the formation of specific neuronal connections and the establishment of functional neuronal circuitry. We addressed the problem of neuronal pathfinding in the context of a regenerating system by taking advantage of a well-known feature of gastropod mollusks, i.e. large identifiable neurons that can be reliably relocated within different individuals of a given species. We employed the snail, Helisoma trivolvis, for these studies and have centered our studies around two pair of large identified neurons in the buccal ganglia, neurons 4 (R&L) which innervate the salivary glands, and neurons 5 (R&L) which innervate the gut. Standard intracellular recording and staining procedures were used throughout. Neurons 4 and 5 normally have axons in the esophageal trunk (ET). The ET trifurcates to form the gastric nerve (GN) to the gut, the salivary nerve (SN) to the acinar portion of the salivary gland, and the dorsobuccal nerve (DBN) which courses along the duct of the gland and enters the buccal mass. Neuron 4 normally has an axon branch in the SN and DBN but "avoids" the GN. Neuron 5 normally has a large axon in the GN, a smaller branch in the DBN and no axon in the SN. During regeneration following crush of the ET proximal to the first branch point, neuron 4 neurites select their paths indiscriminately. Several of these neurites typically traverse each of the nerve branches including the "incorrect" GN. In only 2 of 14 cases did neuron 4 avoid the GN and in one instance neuron 4 sent all of its neurites down the incorrect GN. In contrast, neuron 5 tends to traverse its normal pathways during regeneration. In each of 13 cases neuron 5 always sent most of its neurites out the GN with a smaller number out the DBN. In only 6 of the 13 cases were any neurites seen in the incorrect SN and never more than 2 very fine neurites were seen in the SN as compared to as many as 60 which could be seen in the correct GN and DBN. While it is extremely difficult to extrapolate from regeneration paradigms to ontogeny, this study shows that processes fundamental both to ontogeny and regeneration, i.e. neurite outgrowth, can be differentially and specifically encoded in different neurons of the same nervous system and that the fidelity of regenerating neurons for selecting nerves normally containing their axons differs for different identified neurons. (Supported by PHS grants 1 RO1 NS09696 and 1 RO1 AM19858.)

2153 NON-NEURAL (HUMOURAL?) MECHANISMS ELEVATE EPSP AMPLITUDES FOLLOWING SPINAL CORD TRANSECTION. S.G. Nelson, T.C. Collatos and L.M. Mendell. Duke Med. Ctr., Durham, N.C. 27710.

Spinal cord transection at T13 or L5 elevates the amplitude of medial gastrocnemius (MG) Ia fiber-motoneuron EPSPs beginning a few hours after transection (Nelson et al, J. Neurophysiol. 41, 1979). The present experiments were undertaken to test whether this increase requires interruption of descending input which is in mono- or multisynaptic contact with the motoneurons. Cats chronically transected at T13 (9 to 13 weeks before) were subject to a second transection at T11 several hours before individual EPSPs were recorded in MG motoneurons (at L7-S1) using spike triggered averaging. Chronic transection at T13 yields EPSPs similar to those in intact preparations although a few unusually large EPSPs (400-650 µV) are observed in slow motoneurons (Nelson and Mendell, J. Neurophysiol. 41, 1979). The mean EPSP in double transected preparations was significantly larger than after chronic transection alone (201±22 µV, N=69 vs 114±8 µV, N=161, $p<0.01$). Furthermore, the mean EPSP in each of these preparations was larger than the largest mean EPSP in any single transected one. The percentage of EPSPs smaller than 100 µV was reduced whereas those larger than 400 µV became more frequent with EPSPs as large as 1150 µV being observed. However, these large EPSPs were restricted to small motoneurons and tended to have long rise times unlike EPSPs after acute transection alone which exhibited very brief rise times and which were observed in motoneurons of all conduction velocities. These features of the EPSP enlargement seen following acute transection alone seem to require interruption of fibers in synaptic contact with the test motoneurons. However, indirect, non-neural (humoural?) mechanisms affecting Ia-EPSP amplitude must also exist. The normal blood pressure in these preparations indicates this factor is not crucial. Furthermore, increases in EPSP amplitude do not require elevation of motoneuron input resistance since these were within normal limits. Control experiments revealed that these increases do not occur immediately after acute transection nor do they occur if the 2nd transection is very close (T12) to the chronic one. We speculate that this increase is triggered by neural activity which influences the recording site indirectly and that the number of functional fibers cut at T12 is too small to initiate these changes. (Supported by NIH).

2154 AGE AND HORMONES INFLUENCE GROWTH OF VOCAL CONTROL STATIONS OF THE CANARY BRAIN. F. Nottebohm. Rockefeller University, New York, N.Y. 10021.

Vocal control pathways of the canary brain include two discrete telencephalic nuclei, hyperstriatum ventrale, pars caudale (HVc) and nucleus robustus archistriatalis (RA). These two stations show gross sexual dimorphism, presumably related to the fact that males, but not females, sing complex, learned songs (Nottebohm and Arnold, Science 194 211 '76). In the first experiment described here, male canaries were sacrificed in groups of 4 at ages 15, 30, 45, 60, 75, 90 and 120 days after hatching. Their brains were weighed, fixed in formalin, embedded in gelatin albumen, sectioned at 50 u intervals and stained with cresyl violet. All age groups had adult brain volumes. The volume of HVc and RA was reconstructed as described in lit. HVc is first recognizable on day 30 after hatching. At this age RA and HVc volumes are 30% and 25%, respectively, as large as those of 1-year-old males in full reproductive condition. HVc and RA grow by a factor of 2.5 between the 30th and 60th post-hatching day. Adult volume is reached, respectively, by the end of the third and fourth month. The 30th to 60th day period of rapid growth coincides with the subsong stage of song development, a time when auditory-motor vocal experience may be integrated into HVc and RA patterns of growth and connectivity.

In a separate experiment, 10 male and 10 female canaries were gonadectomized 5-19 days after hatching. Half of the ovariectomized females received silastic cholesterol (Ch) implants, the other half received silastic testosterone (T) implants at 11 months. Both groups of gonadectomized females, 6 intact females of the same age, the castrate males and 10 intact male siblings of the latter were sacrificed at 12 months of age. Attempts were made to record song from all these birds. All intact males developed adult song. The castrate males produced subsong and plastic song, but fell silent at approximately 8 months, when their intact siblings were coming into stable adult song as well as into reproductive condition. Of the females, only the T-treated group produced male-like song. HVc and RA volumes were reconstructed for all these birds. HVc and RA were 90% and 53% larger, respectively, in the T-treated than in the Ch-treated females. HVc and RA were 45% and 54% smaller in the castrate than in the intact males. The ratio of male/female HVc and RA was comparable in intact and gonadectomized individuals, suggesting that events preceding gonadectomy had already biased development of these structures in the two sexes.

The canary HVc and RA seem to be unusual in the extent to which gross neural plasticity normally associated with early development can be induced in adulthood at the time a new behavior is acquired. (Supported by MH18343 and RF70095).

2155 **THE EFFECT OF EYE ROTATION ON VISUOMOTOR PERFORMANCE.** **Joelle Presson, Jeffrey Moran* and Barbara Gordon. Dept. of Psychology, University of Oregon, Eugene, OR 97403.**

To assess the effect of an abnormal relationship between the retina and the visual world on visuomotor behavior we have studied visually guided jumping in kittens who had one eye intorted about the time of natural eye opening. In 3 groups of animals the right eye was rotated and the left eye was either: (1) normal; (2) sutured; or (3) normal, but the animal reared with alternating occlusion. A control group had all the muscles of the right eye cut (no rotation) and a normal left eye. Starting at about 5-8 weeks of age, each animal's jumping ability was tested (under monocular control) twice a week for about 11 weeks. The kitten was required to jump from a tower of variable height onto a square platform in a pan of water without getting its feet wet. If the kitten jumped successfully 4 out of 5 times, the height was increased. Because jumping ability varied somewhat from kitten to kitten, we compared performance using the operated eye with the same kitten's performance using the normal eye, whenever possible. The muscle cut control animals performed equally well with both eyes. The rotated eye cats showed clear deficits when using the rotated eye. By 8 weeks of age they could jump from 45 cm using the normal eye, but they never attained this height using the rotated eye. The eye rotation, alternating occlusion animals showed deficits when using the rotated eye at 7 weeks, but by 13 weeks of age were jumping equally well with both eyes. The eye rotation, left eye sutured cats also did poorly (compared to other cats using their normal eye) at 7 weeks, but jumped normally by 13 weeks.

To determine whether the jumping deficits were due to an acuity deficit, the eye rotation, left eye normal, animals were trained in a two-choice acuity task. The animals were trained to choose a door with horizontal black and white stripes over a door with vertical black and white stripes. The size of the stripes was decreased until the animals performed no better than chance. When using the normal eye the cats could see, on the average, stripes as small as 11' of arc. With the rotated eye the animals could only resolve stripes as small as 21' of arc. The acuity deficit cannot account for the jumping stand deficit because the angle subtended by the platform at its highest was 12°. We conclude that rotation of the visual world following eye rotation causes a visuomotor deficit that is independent of acuity. The deficit is ameliorated if the animal is forced to use the rotated eye.

2156 RECOVERY OF ALTERNATION PERFORMANCE FOLLOWING ENTORHINAL CORTEX LESIONS. J. J. Ramirez* (SPON: D. G. Stein). Psych. Dept., Clark Univ., Worcester, MA 01610, and Dept. Neur., U. Mass. Med. Sch., Worcester, MA 01605.

Sprouting is thought to be correlated with behavioral recovery. For example, rats with unilateral entorhinal cortex (EC) damage previously were shown to be a model preparation for analysis of the relationship between collateral sprouting and recovery of alternation performance. The present study was conducted to confirm that recovery of alternation performance co-occurs with sprouting (Loesche and Steward, 1977). Thirty-six food-deprived male rats were trained to alternate for food reward in a Y-maze. Rats subsequently received unilateral or bilateral lesions and were tested for retention of the alternation task. Alternation performance was spared in rats with unilateral damage, whereas alternation performance was disrupted by perseverative responding in rats with bilateral damage. Disruption of alternation performance in rats with bilateral damage depended upon re-test paradigm: namely, massed trials (i.e. daily training) resulted in transient deficits (mean=32 days), whereas distributed trials (after Loesche and Steward, 1977) resulted in persistent deficits (58 days). Histological analysis verified that EC was damaged almost entirely in both groups of subjects.

Fink-Heimer analysis was conducted to reveal patterns of degeneration resulting from unilateral EC damage. Preliminary results indicate that there is anomalous growth into the outer molecular layer of dentate gyrus originating in intact EC. These preliminary findings corroborate those of other investigators who provided evidence for lesion-induced reorganization of entorhinal-hippocampal circuitry.

The present investigation demonstrates that impaired alternation performance is not a necessary consequent of unilateral EC damage. In addition, the preliminary histological results suggest that sprouting might be correlated with spared alternation performance following unilateral damage. Finally, the results obtained from animals with bilateral lesions indicate that variables in addition to sprouting (e.g. extensive postoperative training) may influence the recovery of alternation performance. (Supported by NIA grant 5RO1 AG 00295-03.)

2157 ELECTRON MICROSCOPIC EVIDENCE FOR ESTABLISHMENT OF SYNAPTIC CONNECTIONS BY REGROWING PYRAMIDAL TRACT AXONS IN THE INFANT HAMSTER. T. Reh and K. Kalil. Neurosciences Training Program and Dept. of Anatomy, Univ. of Wisconsin, Madison, WI 53706.

In a previous report (Kalil and Reh, '78) we presented light microscopic autoradiographic evidence demonstrating that pyramidal tract axons severed in the medulla of infant hamsters are capable of regrowing over long distances to innervate their normal targets in the medulla and spinal cord, albeit by an abnormal route. We now present electron microscopic evidence that the regrowing fibers form synaptic connections in at least two of these terminal regions, the dorsal column nuclei (DCN) and the dorsal horn of the cervical spinal cord.

Hamsters ranging in age from 4-8 days received a unilateral lesion of the pyramidal tract at the level of the inferior olive and were allowed to survive to adulthood. The sensorimotor cortex ipsilateral to the lesion was then removed and the animals sacrificed 2-8 days after the cortical lesion. The tissue was processed for electron microscopy and thin sections through the DCN and cervical spinal cord examined for degenerating axons and terminals. Sections taken from identical areas of normal animals with similar cortical lesions served as control material.

The results obtained for experimental and control animals were qualitatively similar. The fibers of both the normal and regrowing pyramidal tract showed similar rates of degeneration. After 8 days survival, myelinated axons exhibited various stages of degeneration. Some contained degenerating mitochondria and dense bodies, but their microtubules and microfilaments appeared normal. Other axons in a more advanced state of degeneration were completely filled with a dense granular matrix. Degenerating axons were similar in appearance but far less numerous at 2 days than at 8 days.

Synaptic terminals in the DCN and cervical spinal cord contained an accumulation of dense bodies and darkened cytoplasm similar to that observed in degenerating axons. At earlier stages of degeneration, synapses retained their postsynaptic contacts, while at more advanced stages glial processes frequently engulfed the terminals.

These results demonstrate that regrowing pyramidal tract fibers form synaptic terminals in their appropriate target areas. This suggests a mechanism for possible recovery of function.

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2158 FUNCTIONAL AND ANATOMICAL REORGANIZATION OF THE SOMATOSENSORY INPUT TO THE HAMSTER'S SUPERIOR COLLICULUS FOLLOWING NEONATAL ENUCLEATION. Robert W. Rhoades, Dept. of Anat., Coll. of Med. and Dent. of New Jersey, NJSOM, Piscataway, N.J. 08854.

Superior collicular neurons responsive to tactile stimuli are encountered only in the laminae ventral to the stratum opticum in normal hamsters. In the posterior part of the colliculus the vast majority of these cells can be activated both by electrical shocks delivered to the spinal cord (at the second cervical segment) and also by similar stimulation of the ipsilateral sensorimotor cortex. These data coincide well with the results of both autoradiographic and degeneration experiments which have indicated that the spinal input to the tectum in this species terminates for the most part in the stratum album intermedium and to a lesser extent in the stratum griseum intermediale in the caudal portion of the structure. Sensorimotor cortical injections of tritiated amino acids in normal animals revealed further that the projection from this cortical region to the tectum was also restricted to the laminae ventral to the stratum opticum.

In animals which have had one eye removed on the day of birth this functional and anatomical organization is clearly altered. In the posterior half of the colliculus ipsilateral to the remaining eye neurons responsive to somatosensory stimuli are encountered in all tectal layers and electrical stimulation of the type described above revealed that most of these cells received convergent input from the cord and sensorimotor cortex. The latency distributions for responses to shocks delivered at these two loci were essentially identical in the normals and the neonatal enucleates and for a given collicular cell the response latency for stimulation at the second cervical segment was invariably shorter than the latency of the discharge obtained with cortical shocks. Degeneration and autoradiographic experiments carried out in neonatally enucleated hamsters indicated that a probable anatomical substrate for the observed functional changes was a dorsal expansion of the terminal distributions of both the spinal and somatosensory cortical projections to the partially denervated colliculus. Supported in part by NJOEF grant #39-2519, NIMH grant #MS 32897-01 and grant #RR 09085 from NIH.

2159 SI CORTEX IN MOUSE: INCREASED, RANDOM DISPARITY BETWEEN THE AREAS OF BARREL FIELDS IPSILATERAL AND CONTRALATERAL TO FOLLICL ABLATIONS. F.L. Rice, D. Jeanmonod*, and H. Van der Loos*. Institut d'Anatomie, Lausanne, Switzerland CH1011.

Unilateral vibrissal follicle ablations on the muzzles of mice have been shown to produce deformations only in the contra lateral barrel field of SI cortex and only when the ablations were made between the day of birth (P-0) and P-4. The deformation is specifically located (e.g. row C follicle ablation lea to contralateral row C barrel deformations) and its form varies with the age the ablation occurs (Woolsey and Wann; JCN, 170: 53). The barrel field ipsilateral to the lesion appears normal A quantitative analysis of the tangential area occupied by the posteromedial barrel subfield (PMBSF) reveals that unilateral follicle lesions also produce a more generalized, interhemispheric abnormality. 37 experimental Swiss-Webster mice had the left row C of follicles ablated on either P-0, P-1..., or P-5 and 20 control mice had no ablations; all were sacrificed on P-12. The tangential area occupied by the PMBSF in each hemisphere was measured from reconstructions of 40 μ thick, Nissl-stained serial sections (usually a maximum of 3) cut tangential to the PMBSF. The PMBSF areas of the left hemispheres were com pared (T-test and F-test) to those of the right and no significant difference (0.05 level) was found for either the ex perimental or control population. Thus, the follicle lesions produced no consistent increase or decrease in the size of the contralateral PMBSF's relative to the ipsilateral or vice versa Next, a measure of the disparity between the left PMBSF area (L and the right (R) was derived for each animal: $(L-R) \div 0.5 (L + R) \times 100\%$. Positive percentages indicate a larger left PMBSF; negative a larger right. The disparities within both the experimental and the control populations followed a normal distribution with a mean value (-0.9% and 0.8% respectively) nearly equal to 0. However, the range of disparity was significantly greater (0.001 level) for the experimental population (range: 19% to -27%; SD = ± 10.7) than for the controls (range: 11% to -12%; SD = ± 5.8). Ablations at all ages (P-0 to P-5) produced increased disparities with no obvious age related trend. These results demonstrate that, in addition to specific contralateral effects, unilateral follicle ablations produce an increase in the range of interhemispheric disparity of PMBSF area that is unrelated to the side where or age when the ablation occurred. Perhaps a loss of peripheral symmetry results in a decrease in some kind of interdependence that may exist between the two hemispheres during development. Supported by grants 3.1350 and 3.776 from the Swiss National Science Foundation to HVdL.

2160 EFFECTS OF FIELD AND LABORATORY ENVIRONMENTS ON DEVELOPMENT OF BRAIN IN GROUND SQUIRRELS: EVOLUTION OF BRAIN PLASTICITY. Mark R. Rosenzweig, Edward L. Bennett, Paul W. Sherman*. Dept. Psych. & Lawrence Berkeley Lab., Univ. of Calif., Berkeley 94720.

We compared brain development in Belding's ground squirrels (Spermophilus beldingi) in 2 laboratory environments--enriched (EC) and impoverished (IC)--and that of feral (F) conspecifics. Laboratory young were born from pregnant females live-trapped near Tioga Pass, Calif.; they were weaned and assigned to differential environments at about 30 days of age and kept in conditions for 40 days. Just before sacrifice, feral juveniles of the same age were live-trapped near Tioga Pass; they were sacrificed together with the laboratory squirrels. There were 20 animals in each condition, half of each sex. In weights of cerebral cortex, (EC≈F)>IC; EC>IC, $p<.01$. In skeletal development (hindfoot length), F>EC>IC; F>IC, $p<.01$. In total RNA of occipital cortex, (EC≈F)>IC; EC>IC, $p<.05$. In total DNA of occipital cortex, F>(EC≈IC); F>IC, $p<.05$; this indicates a greater number of cortical cells in feral as compared to laboratory-born animals. Differences between EC and IC ground squirrels conformed in most respects to those found earlier between EC and IC laboratory rats, mice, and gerbils. Comparisons with feral animals showed EC ground squirrels to be close to the natural baseline in most cerebral and behavioral measures that we made.

Further research is under way with 2 species of ground squirrels to test the following hypotheses about evolution of brain plasticity: 1) Other things being equal, a species that has evolved in a varied and less predictable environment will show more plasticity in response to environmental demands than will a related species from a simpler and stabler environment. 2) In view of the metabolic costs of a large brain, there is survival value in reducing brain size at times in the individual's life when a low level of demand is placed on it; conversely, the brain must be large enough to meet sudden demands and plastic enough to increase its capacity when increased demand occurs. A preliminary test was made of hypothesis 2 by placing 4 juvenile ground squirrels in a cold room at 5°C where they hibernated Nov.-April while 4 others maintained normal activity at 20°C. Growth of body and brain continued at 20°C whereas the hibernators showed cessation of brain growth and even a slight loss of brain weight.

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2161 AXON SPROUTING AND SITE OF SYNAPSE FORMATION IN INNERVATED MUSCLES. Shlomo Rotshenker* and Fanny Reichert. Dept. Anat. Embryol. HU - Hadassah Med. Sch. Jerusalem, Israel.

Multiple end plate potentials (e.p.p.s.) can be recorded in single muscle fibers innervated by several motor neurons when the whole motor nerve is stimulated by successive impulses of increasing intensity. Such multiple e.p.p.s. were recorded in 16% of muscle fibers comprising cutaneous-pectoris muscles of normal frogs. The number of muscle fibers in which multiple e.p.p.s. were recorded increased in intact right muscles after injuring the motor nerve to left muscles. Is this increase in the incidence of polyneuronal innervation attained by the formation of new synapses and if so where do new axon terminals arise from and where on muscle fiber surfaces do they terminate?

Motor axon terminals stained by a mixture of zinc iodide and osmium are visible in light and electron microscopy. In normal frogs, myelinated motor axons give rise to several terminal branches that end abruptly upon the muscle fiber they innervate In contrast, in intact right muscles of experimental frogs some axons and nerve endings gave rise to sprouts that gained contact with muscle fibers apparently not innervated by their parent axons. The number of axons comprising the motor nerve to right intact muscles and left denervated muscles was similar (counts made proximal to site of injury).

In normal frogs, muscle fibers comprising cutaneous-pectoris muscles are innervated at a single end plate region that can be marked histochemically by staining for the enzyme cholinesteras which is confined to the synaptic site only. The average end plate size in right muscles is within ± 10% of that of left muscles. However, after cutting the motor nerve on the left side there was an increase in the average end plate size in right intact muscles over that in left muscles. In the same right muscles there was also an increase in the incidence of polyneuronal innervation (determined electrophysiologically). Also, electron microscopical examination of randomly chosen neuromuscular junctions revealed only a 10% incidence of neuromuscular junctions where 2 axonal profiles were identified in a single gutter.

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2162 ELECTROPHYSIOLOGICAL MAPPING OF ABERRANT AND NORMAL RETINAL PROJECTIONS TO THE SAME SUPERIOR COLLICULUS IN HAMSTERS. George M. Sachs* and Gerald E. Schneider. Department of Psychology, M.I.T., Cambridge, MA 02139

After ablation of the superficial layers of the right superior colliculus (SC) in newborn hamsters, optic tract fibers from the left retina recross the midline at the tectum and terminate in a medial portion of the intact left SC. Neuroanatomical studies have shown that this aberrant projection displaces terminals from the normal contralateral retinotectal projection. Twelve weeks to one year after early right tectum ablation, 12 hamsters underwent acute removal of visual cortex, and visually evoked multi- and single-unit responses were recorded from the left SC. Five hamsters also received injections of ^{3}H-leucine in the left eye 4 days before recording.

Electrophysiological results indicated that the tectal projections from the two eyes exhibited varying degrees of overlap. For some cases, tectal units recorded medially were driven exclusively by the left eye and there was an abrupt transition to units activated only by the right eye as more lateral regions were sampled. Autoradiography showed a dense aberrant projection with a sharp lateral border. In cases where the aberrant projection appeared less dense as seen with autoradiography, there was electrophysiological evidence for overlap of the two retinal projections. From some sites two groups of units, each driven by a different eye, could be recorded simultaneously. These sites were encountered in a region that always included the lateral part of the aberrant projection and sometimes extended to the medial edge of the left SC. The normal projection showed little compression in the mediolateral tectal axis so that, particularly in cases with segregated projections, the lowest portions of the right retina were not represented in the left SC. The aberrant projection arose primarily from the lower half of the left retina and exhibited the same polarity as the normal projection (lower retina projected medially, nasal retina projected caudally). This polarity failed for only a few pairs of recording sites, at which reversals in the mediolateral tectal axis were noted. Binocularly driven single units were encountered only rarely. Inputs to such binocular units often, but not invariably came from homologous (mirror image) regions in the visual fields of the two eyes. Although the aberrant projection to the left SC never represented the entire left retina, it did show some compression. As a result of this compression, homologous points in the two retinae were represented in two different topographic maps within the same SC.

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2163 SPARING AND RECOVERY OF FUNCTION WITHIN THE 'PREFRONTAL SYSTEM' OF THE INFANT RAT. C. L. Sahley and A. J. Nonneman. Dept. of Psychology, Univ. of Kentucky, Lexington, Kentucky 40506.

In an attempt to distinguish between sparing and recovery of function within the 'prefrontal system' of the rat, 7-8 day-old infant rat pups were given medial frontal (MF), orbital frontal (OF), caudate nucleus (CN) and sham (SH) lesions allowed to recover and then tested at 14-days of age on a variety of tasks known to be sensitive to prefrontal dysfunction in the adult rat. These tasks include resistence to extinction of a learned approach response, passive avoidance, spatial discrimination and reversal and activity in the open-field. They were also tested on an odor-aversion task. Additional rat pups 10-14 -days old without lesion were tested on the same tasks to determine if there were any age-dependent differences in performance within this age range which might interact with the lesion-dependent changes in performance. The results indicated that MF lesions produced no deficits on any of the tasks employed. In addition, only the CN operates showed an increased resistance to extinction and an increased number of trials to passive avoidance as compared to SH pups. Both CN and OF operates, however, had difficulty learning the spatial reversal and demonstrated an increase in activity as compared to shams. All lesioned and SH pups learned the odor-aversion task similarly. No age-dependent differences in performance were observed in normal pups on any of the tasks.

The results suggest that there may be a sequential encephalization of function within the 'prefrontal system' of the rat from the CN to the cortical areas. Further, both sparing and recovery of function appear to be operative in the prefrontal system. The primary determinant of the degree of sparing or recovery appears to be the degree of functional maturity of the tissue at the time of the neural damage. Finally, it appears that the CN is capable of mediating behavioral functions of the 'prefrontal system' both in the infant rat before maturation of the cortical areas is complete and following damage to the cortiareas in the infant rat.

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2164 MOVEMENT OF OPTIC TERMINALS IN GOLDFISH TECTUM AFTER LOCAL PRE- OR POSTSYNAPTIC BLOCKADE OF TRANSMISSION. John T. Schmidt. Dept.Anat.,Sch.of Med.,Vanderbilt University, Nashville, TN 37232.

Alpha-bungarotoxin(5nM) was found to be effective in blocking the synapses of all three classes of retinal fibers in goldfish tectum. It severly decrements the field potentials at all depths after either photic stimulation or electrical stimulation of the nerve. Current source-density further shows that it blocks the flow of synaptic currents into radially oriented tectal cells, although presynaptic activity remains largely unaffected. Microinjection from a pipette(10μm tip) produces a local blockade of a restricted area. One microliter blocks an area 0.6mm in diameter; two microliters increases this to 1mm. Outside this area normal size field potentials are recorded. Six to eight days later no optic terminals can be recorded within the toxin-treated area. However, construction of an extensive retinotectal map shows no corresponding scotoma in the visual field. Instead the terminals with receptive fields within the corresponding area can then be recorded from surrounding tectal areas. Usually these take the form of enlarged multiunit receptive fields from the surrounding areas overlapping into the area, so that all visual areas are still represented in the map. Apparently the optic terminals within the toxin-blocked area move outward to colonize neighboring normal areas, confirming Freeman's results in toad(Nature 269,218,1977). The time course of the exodus of terminals as well as their eventual reentry is now being determined.

Following similar local blockade by β-bungarotoxin, there is a corresponding scotoma in the visual field even after one week. Extensive mapping,however,shows complete coverage of all tectal areas including the toxin-treated area. Apparently neighboring healthy terminals move in to occupy sites vacated by toxin-damaged terminals. At the neuromuscular junction, it causes nerve terminals to degenerate. There remains to be determined whether the optic terminals also degenerate, whether they are the only terminals affected, and also whether they eventually regenerate to reclaim their usual tectal loci.

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2165 DEVELOPMENTAL ALTERATIONS IN BINOCULAR COMPETITION AND VISUAL ACUITY IN VISUALLY-DEPRIVED CATS. Douglas C. Smith, Harris D. Schwark*& Deborah M. Beaudry*. Dept. of Psychology, Southern Illinois Univ.,Carbondale, Ill. 62901, and Neural & Behav. Biol. Prog., Univ. Illinois, Champaign, Ill. 61820.

Alterations in binocular competitive interactions during postnatal development produce marked effects on the physiological properties of single cells in striate cortex of the cat. For example, if a kitten is monocularly deprived (MD) during development, almost all of the cells in striate cortex respond exclusively to visual stimulation of the nondeprived eye. In contrast, in a kitten which is binocularly deprived (BD) via lid suture, neither eye has a decided advantage over the other and the electrophysiological effects are less severe than the effects of MD. If the deprived eye of an MD cat is placed at a competitive advantage during postnatal development by removing the other eye at the same age at which MD is initiated (MDE), binocular competitive interactions are absent and only the effects of deprivation per se are present. Consequently, the response properties of cells in area 17 are less severely affected than following BD, although deprivation effects are evident in MDE cats (Kratz and Spear, 1976).

The differences which exist in striate cortex of kittens in these developmental conditions are also evident in terms of behaviorally measured visual acuity. Using the Mitchell et al., (1976) jumping stand, visual acuity for MD, BD and MDE cats was determined following 4-12 months of deprivation. The stimuli were high contrast square-wave gratings (luminance 138 cd/m^2). Threshold visual acuity (70% correct) for 3 MD cats using the deprived eye was 0.87, 0.75, or 0.3 cycles/deg ($\bar{x}$= 0.64). For 3 BD cats using a single deprived eye, threshold visual acuity was 3.7, 3.25 or 2.55 cycles/deg ($\bar{x}$= 3.17). For 2 MDE cats visual acuity was 3.94 or 3.84 cycles/deg ($\bar{x}$= 3.89). A third MDE cat is being tested. These results indicate that the nature of competitive interactions between the eyes during development determines postdeprivation visual acuity. Further, comparisons between MD and MDE cats suggest that the acuity deficits associated with developmental monocular deprivation are primarily due to abnormal competitive interactions; however, threshold acuities for MDE cats are below those found for cats using a nondeprived eye and this finding attests to the effects of deprivation per se on visual acuity. Finally, the MDE cats studied provide additional support for the correlation between visual acuity and the responsivity and specificity of single cells in striate cortex of visually-deprived cats (Smith et al., 1978).

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2166 REINNERVATION INDUCES PROLIFERATION OF A NOVEL FORM OF RADIAL GLIA IN THE OPTIC TECTUM OF GOLDFISH. James A. Stevenson, Dept. Psychology, Dalhousie Univ., Halifax, N.S., Canada.

Previously, we have described "periependymal" cell proliferation in the goldfish optic tectum. Labeling among these cells, whose nuclei are located between the ependyma and the neuronal stratum periventriculare (SPV), is seen when ^{3}H-thymidine injections are made 25-35 days after optic nerve section, the time at which functional reinnervation of the tectum occurs. Tecta denervated by eye enucleation do not show such proliferation (Stevenson and Yoon, '78, Brain Res., 153).

The present report describes the periependymal (PE) cells. In the light microscope these cells are easily recognized by their location and thymidine labeling properties. Unlabeled PE cells are distinguished from their neighbors by their deeply invaginated nuclei with eccentric nucleoli, moderately basophilic cytoplasm and large perikarya. Golgi preparations display a single peripherally directed process extending from such perikaryá. The process reaches the stratum opticum where it divides into several oblique branches which terminate at the tectal surface as subpial end feet. Along its course the process is covered in mossy branches, giving it the appearance of a spruce tree. Neither the Golgi rapid nor the Kopsch technique demonstrates an apical process connecting the PE cell perikaryon with the ventricle. Ultrastructurally, the PE cell perikaryon contains its invaginated nucleus, abundant Golgi apparatus, mitochondria which often contain single dark granules, rough and smooth endoplasmic reticulum, free ribosomes, and a great number of microtubules which funnel into the process where they run longitudinally.

The notable absence of fine filaments and an apical process connecting the perikaryon with the ventricle distinguish the PE cells from other radial glia in the goldfish tectum.

2167 CHANGES IN THE UPTAKE OF 3H-2-DEOXYGLUCOSE ACCOMPANYING DENERVATION AND REINNERVATION OF THE RAT'S DENTATE GYRUS. Oswald Steward and Lodi K. Smith*. Depts. of Neurological Surgery and Physiology, Univ. of Va. Sch. Med., Charlottesville, VA 22908.

Following the removal of the major excitatory projection to the rat dentate gyrus, which originates in the ipsilateral entorhinal cortex, the dentate granule cells are reinnervated as a result of the sprouting of several surviving afferent systems. The present study measured 2-deoxyglucose (2DG) uptake in the dentate gyrus to determine, a) if the removal of a major excitatory pathway would decrease 2DG uptake in the denervated dentate gyrus, and b) whether any decreases would be reversed as a consequence of the reinnervation. 2DG uptake was measured autoradiographically at 1,2,4,8,10, and 14 days post-lesion by injecting the animals intravenously with 3H-2DG 45 min. prior to sacrifice, and processing the tissue on emulsion-coated slides, permitting a quantitative evaluation of 2DG uptake through grain counting. For comparison, a few animals were also injected with 14C 2DG, and processed on x-ray film. 2DG uptake in the denervated dentate gyrus ipsilateral to the lesion was compared with that in a comparable region on the contralateral side. 2DG uptake was reduced in the denervated dentate gyrus during the early post-lesion intervals (1,2, and 4 days), although the extent of the decrease is surprisingly slight (approximately 10-20%) given the density of the entorhinal projection system. At 8-10 days post-lesion, 2DG uptake in the denervated zone was not only not decreased, but was actually somewhat increased relative to the contralateral control. This increased uptake was restricted to the outer portion of the dentate stratum moleculare. The enhanced uptake was not evident at the 14 days post-lesion. These results suggest that denervation results in decreases in 2DG uptake, while reinnervation (which occurs predominantly around 8-10 days post-lesion) is accompanied by a reversal of these decreases, and even a slight increase in uptake relative to a contralateral control during the period of active synaptogenesis. The question of whether this increase at 8-10 days post-lesion reflects a metabolic accompaniment of the active phase of sprouting, or simply a return of excitatory innervation will require a further analysis at longer post-lesion intervals, after the lesion-induced synaptogenesis has reached completion. Because the extent of the changes was so slight given the density of the entorhinal projection system, it is probable that most of the 2DG uptake in areas of neuropil in the hippocampus is associated with elements other than afferents and terminals. The results demonstrate the usefulness of the 3H-2DG method for quantitative studies.

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2168 SENSITIVITY OF MYELINATED PATHWAYS TO ENVIRONMENTAL MANIPULATION. Frank Szeligo, Gerald F. McCarthy*, Jeannine Ewen*. Dept. Psyc., UNB, Fredericton, Canada E3B 5A3.

Comparison of the brains of rats raised for a month or more in a stimulating environment with those of siblings raised in a non-stimulating environment reveal a number of anatomical differences. In this way a variety of components of the neural microenvironment have been found to be susceptible to the differences between an enriched environment which contains a number of rats plus toys and an impoverished environment which houses rats in isolation. Axons were added to the list by an abstract in the Anat. Rec., 187: 726-727 (1977) which reported differences in the thickness of the myelinated pathway at the base of the occipital cortex, above the corpus callosum. This size difference was apparently the result of greater numbers of axons in enriched cortex. The generality of this finding is being investigated by studying the sensitivity to environmental manipulation of the size of other myelinated pathways. Pathways for which there presently are adequate samples include the cingulum, the fornix and the stria terminalis. The tissue was prepared by perfusion with mixed aldehydes and fixation in osmium and was then embedded in Epon. Semithin sections of the tissue were stained with toluidine blue. There is data available for the cingulum in 9 sibling pairs. The cingulum was sampled in the coronal plane at the level of the posterior edge of the midline corpus callosum. The dorsal-ventral extent was measured at .2 mm intervals over an .8 mm extent. Enriched rats had a thicker cingulum by 11% ($p<0.01$). The fornix was sampled in the cross sectional horizontal plane, at the level of the anterior commissure in 12 pairs of rats. The area of the entire fornix was determined. There were no significant differences. The stria terminalis was sampled in the same plane as the fornix. Again, area measurements were used. For 11 pairs of animals, there were no significant differences.

2169 INTERNEURONS AS POSSIBLE SOURCE FOR THE RETURN OF SUBSTANCE P (SP) AFTER LUMBOSACRAL DEAFFERENTATION. Alan Tessler,* Roman Artymyshyn,* Marion Murray and Michael Goldberger. The Medical College of Pennsylvania, Philadelphia, PA 19129.

Dorsal root afferents are the major known source of SP in the spinal cord. Using the unlabeled antibody (PAP) technique for demonstrating SP, we have shown that following lumbosacral deafferentation of the cat hindlimb by dorsal root section, the amount of SP reaction product in the dorsal horn of lumbar segments progressively decreases for the first 10-11 days after surgery. However, the SP never disappears altogether from the dorsal parts of the dorsal horn. The quantity of SP then increases and by 1 month has returned in substantial amounts. We have interpreted these changes to indicate sprouting by SP containing processes which remain in the deafferented dorsal horn. The reaction product which returns is different in appearance from that of control animals, being finer, more regular, and less intense. The source or sources of these sprouting terminals is unknown. Deafferentation combined with transection at the L1 level did not prevent SP reaction product from returning in amounts comparable to those seen after 1 month survival with deafferentation alone. This suggests that long descending tracts are not the sources of the SP which returns after deafferentation. Deafferentation combined with midlumbar (L4) transection did not prevent the return of SP reaction product to the dorsal horn of segments rostral to the transection. This suggests that ascending fibers are not a major source of sprouting SP axons. However, L4 transection abolished most or all SP staining from the dorsal horn on the chronically deafferented side. Thus, one origin of the sprouting fibers may be interneurons with descending collaterals. In fact, SP containing cell bodies are seen in the lumbar grey matter in normal control and operated animals. They are located in several laminae, including VII, VIII, and X, of lumbar segments both on the side of deafferentation and on the side with intact dorsal roots. The SP containing cell bodies do not constitute a specific nucleus since they lie scattered within several laminae. Moreover, the fact that they are also dispersed rostrocaudally while the return of SP is found in all deafferented lumbar segments, suggests that the SP cells maintain widespread projections which extend rostrocaudally for several segments. Sprouting by interneurons may provide one explanation for the hyperreflexia which occurs in chronic, deafferented animals.

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2170 EVIDENCE FOR THE INVOLVEMENT OF THE RETROGRADE REACTION IN SUSTAINED COLLATERALS DURING RECOVERY OF FUNCTION FOLLOWING BRAIN DAMAGE. Roy G. Thompson* and Fred H. Gage. Chemistry of Behavior Program, Texas Christian University, Fort Worth, Tx 76129.

Present understanding of neural mechanisms subserving recovery of function following brain damage stress various means of compensating for the loss of neural input, e.g., collateral sprouting and receptor supersensitivity. Reversible consequences of nerve damage are poorly understood. Several lines of evidence have recently demonstrated that nerve terminals proximal to the site of damage exhibit a transient decrease in both their neurotransmitter content and biosynthetic enzymes. These data have been interpreted to reflect the occurrence of a functional denervation at the terminal sites of axon collaterals which remain structurally intact following axotomy. The purpose of the present experiment was to further investigate the consequences of axotomy on the functional integrity of sustained collaterals. The ability of hypothalamic nerve terminals to accumulate and retain their neurotransmitter was determined at various times following septal lesions and compared with changes in uptake occurring in nerve terminals exhibiting anterograde degeneration. Crude synaptosomes were prepared from hippocampal and hypothalamic tissue at 1, 5, 10 and 15 days following septal lesions and incubated in the presence of tritiated norepinephrine, serotonin or choline. The results show that uptake of all three transmitter substances is decreased by 20-30% on the first day after septal lesions in both hippocampus and hypothalamus. Maximal loss of uptake was observed by day 5. While loss of hippocampal uptake remains depressed at 10 and 15 day survival times, hypothalamic uptake returns to normal by day 15. The initial decrease in uptake observed in the hypothalamus lends support to the hypothesis of a disruption in the functional integrity of proximal nerve terminals following axotomy. Recovery of hypothalamic uptake can be interpreted to reflect one of two events; 1) collateral sprouting of intact axons in response to degenerating septo-hypothalamic projections, or 2) recovery of uptake within nerve terminals exhibiting the initial loss. A kinetic analysis of neurotransmitter uptake is currently being performed in order to further elucidate the neural events contributing to this recovery.

2171 AGE-RELATED COMMISSURAL SPROUTING IN THE DENTATE GYRUS DEMONSTRATED BY ANTEROGRADE TRANSPORT OF HORESRADISH PEROXIDASE. James R. West, Asa C. Black, Jr., and Terence H. Williams. Department of Anatomy, University of Iowa, College of Medicine, Iowa City, Iowa 52242.

Using a modification of the horseradish peroxidase technique (West and Black, Neuroscience Letters, in press), we studied post-lesion growth in the dentate gyrus.

In accord with Lynch and co-workers, we found moderate growth of the commissural terminal band into the outer molecular layer in rats receiving unilateral entorhinal lesions as adults (group A). In rats lesioned at 11 days of age and examined as adults (group B), we observed prolific growth throughout all but the most distal edge of the outer molecular layer.

The density and distribution of label in group A suggests that the sprouting probably results from direct expansion of fibers and terminals into the proximal portion of the lesion-induced deafferented zone. In group B, particularly in the dorsal leaf of the dentate gyrus, the label appeared as two dense bands (the proximal band corresponded to the normal commissural terminal field), separated by an area of lighter labeling. This disparity in patterns of re-innervation between groups A and B suggests that the mechanisms underlying the reorganization might not be the same.

We suggest that one of the following mechanisms is responsible for these results: (1) The commissural fibers grow into the normal zone and then sprout into the outer molecular layer. Their terminals are distributed to two different portions of the dendritic tree, creating the appearance of two separate bands. (2) There is a division of the commissural fibers as they grow into the altered dentate gyrus during development, creating two adjacent bands. (3) A combination of the above mechanisms is also possible, such that the two bands result from the division of the commissural fibers as they enter the dentate gyrus. The inner fiber plexus could then sprout; the sprouts occupying the space between the two bands. (Supported by NS 11650 to THW).

2172 INCREASE IN ^{3}H-GLUTAMATE ACCUMULATION FOLLOWING INDUCTION OF LONG-TERM SYNAPTIC POTENTIATION IN HIPPOCAMPAL SLICES. A. Wieraszko*[1], M. Baudry*, R. Creager*, R. Finn* and G. Lynch. Dept. of Psychobiology, Univ. of California, Irvine, CA 92717 and [1]Dept. of Biochemistry of Nervous System and Muscle, Nencki Inst. of Exp. Biology, Warsaw, Poland.

Long-term potentiation (LTP) in the hippocampus has been the subject of considerable interest in recent years as it represents a potential model of learning and memory. LTP is routinely produced in the hippocampal slice preparation by a brief repetitive electrical stimulation of the Schaffer collaterals and commissural fibers. However, the biochemical processes underlying this long-term change in synaptic efficacy are not yet understood.

The present study examined the possibility that LTP in the hippocampal slices is accompanied by changes in the accumulation of the excitatory amino acids glutamate and aspartate, which are possible neurotransmitters in the potentiated pathways.

Potentiated slices incubated with a low (50 nm) concentration of ^{3}H-glutamate accumulated more radioactivity (+20%) than did control non-stimulated slices. Further, this increase was still present 30 minutes after the stimulation. A similar increase in accumulation of radioactivity was exhibited in potentiated slices incubated with ^{3}H- or ^{14}C-aspartate. It is not likely that the increased accumulation found with the excitatory amino acids represents a non-specific effect since potentiated slices incubated with ^{14}C-tyrosine or ^{14}C-glucose showed no increase in the accumulation of radioactivity. In addition, the increased accumulation of ^{3}H-glutamate was more pronounced in the regio superior, the locus of the potentiated synapses, than in the dentate gyrus. Under the present conditions, the accumulation of ^{3}H-glutamate by slices is not likely to simply reflect uptake by presynaptic elements since, after commissural lesions, the accumulation by slices is not changed whereas the uptake in homogenate is decreased by 30%. Thus, the present results are consistent with the hypothesis that LTP induces an increased number of binding sites for glutamate.
(Supported by NIH grant 19793 and NSF grant 72077137).

2173 AUDITORY DEPRIVATION AFFECTS DENDRITIC SPINE DENSITY IN THE MONGOLIAN GERBIL CORTEX: A QUANTITATIVE GOLGI STUDY. Robert W. Williams*, Michael D. McGinn*, Richard G. Coss*, Albert Globus* (SPON: A. J. Sillman). Department of Psych., University of California, Davis, CA 95616.

We have assessed the effects of auditory deprivation in the cortex of the mongolian gerbil. Compared to littermate controls 32 day old experimental animals showed significantly lower spine densities along pyramidal cell apical dendrites. Ligation of the external meatuses at day 12 caused acoustic deprivation of more than 25 decibels. Groups of gerbils were sacrificed 12, 22 and 32 days after birth and cerebral blocks were processed for rapid Golgi. The auditory cortex was mapped in a parallel electrophysiological study using single unit and evoked response criteria. Electrolytic lesions in Nissl stained material provided a reference for Golgi quantification. This quantification was restricted to pyramidal cells of layer V in primary auditory cortex. Drawings of all well impregnated neurons were made at a magnification of 1000 X using a drawing tube and spine densities were determined from these drawings. A 50 µm long segment of the apical shaft beginning 150 µm above the soma was quantified. In 32 day old material spine densities were lower an average of 38% in experimentals ($p < .005$; n of cells: 14 control, 15 experimental). A comparison of the entire 200 µm segment also yielded significantly lower densities in experimentals ($p < .025$). An insufficient amount of 22 day old control material precluded a similar analysis at this age. However, a comparison between the 22 and 32 day old deprived groups showed significantly higher densities in the younger material. Additionally there is no significant difference between 22 day old deprived and 32 day old control material (mean per 10 µm is 12.1 and 10.7 spines respectively). These data suggest that the lower spine density seen in the month old gerbils is due to a reduction from a state of higher spine density rather than being the result of delayed maturation.

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2174 CHANGES IN THE CORTICAL BARREL FIELDS SUBSEQUENT TO VIBRISSAL CAUTERIZATION IN THE NEONATAL AND ADULT MICE DEMONSTRABLE WITH CYTOCHROME OXIDASE HISTOCHEMISTRY. Margaret Wong-Riley and Carol Welt. Dept. Anat., Univ. Calif., San Francisco, CA 94143, and Central Wisconsin Center for the Developmentally Disabled, Madison, WI 53704.

Cytochrome oxidase histochemistry has been applied previously in the study of functional changes within the auditory and visual systems of cats (Wong-Riley, Merzenich & Leake, Brain Res. 141: 185-192, 1978; and Wong-Riley, Brain Res., in press). The present study examines the applicability of this technique in the somatosensory system of rodents. The barrels in layer IV of normal mouse SmI face cortex were found to have a high level of cytochrome oxidase activity. Surrounding each barrel was a clear zone of very low enzyme reaction. Preliminary electron microscopic examination indicated that most of the reactive mitochondria within the barrels reside in the neuropil, rather than in the cell bodies. Thus, the barrels normally have a higher level of oxidative metabolism, most likely reflecting a higher level of functional activity, than surrounding regions. In order to determine whether vibrissal damage induces any change in the oxidative activity of the neonate and adult animals, and whether the C.O. technique is sensitive enough to detect such functional changes in the somatosensory cortex, two groups of mice were tested. In the first group, selective row or rows of mystacial vibrissae were cauterized on postnatal day 1 (within hours after birth), and the animals allowed to survive for 2-3 months. In the second group, vibrissal cauterization was performed in adult animals, and they survived for 2-3 months post-operatively. The results indicated that neonatal removal of vibrissae caused severe shrinkage and often fusion of affected cortical barrels, with concomitant expansion of neighboring barrels (confirming previous reports by others), and the level of cytochrome oxidase activity of the shrunken barrels was lower than that of normal. Removal of vibrissae in the adult did not cause size changes of cortical barrels. However, there is a significant decrease in the level of oxidative enzymatic activity within these barrels. Thus, the removal of sensory input through the destruction of peripheral sensory organ causes distinct functional changes at the level of the cortex. Such changes are more severe in the neonate, where cortical fields actually shrink in size. Changes, however, can still be induced in the adult animal, where the levels of oxidative activity of cortical fields are demonstrably reduced. These functional changes are detectable morphologically with the cytochrome oxidase technique.

PSYCHO-PHARMACOLOGY

2175 EFFECTS OF APOMORPHINE ON ELICITED AND OPERANT PECKING IN PIGEONS. Joanne S. Abelson* (SPON: B.P.H. Poschel). Psychology Department, University of Michigan, Ann Arbor, MI. 48104.

Apomorphine (APO) elicits stereotyped behavior in a number of species, but its effects on operant behavior are less consistent. In general, APO causes decreases in rates of operant responding, but some subjects show paradoxical rate-increases. To account for such findings,it has been postulated that an occasional subject might redirect its stereotyped responding toward the operant manipulandum and thereby generate high operant rates. The relationship between the effects of APO on elicited (stereotyped) and operant behavior was studied by examining the effects of the drug on both behaviors in the same subjects.

Fifteen test- and drug-sophisticated pigeons were used as subjects in both experiments. In the pigeon, behavior induced by APO takes the form of persistent and continuous pecking. Examination of elicited pecking was conducted in the home cage during a 1 hr observation test. APO, in half-log unit doses from 0.032 to 32.0 m/k administered I.M. immediately prior to testing, caused dose-related increases in elicited behavior in all subjects, as has been observed previously. Pecking was first observed at 0.1 m/k and reached a maximum at 3.2 m/k. The stereotypy induced by APO was remarkably similar across subjects.

In contrast to the effects on stereotyped behavior, operant responding on a multiple FI-5, FR-30 schedule revealed individual differences in response to the drug. These differences were used to form descriptive groups based on the lowest dose which would eliminate operant responding. Doses of 0.32 m/k eliminated all operant responding in four subjects (Group 1); 3.2 m/k was needed in nine other subjects (Group 2); and two subjects required 32.0 m/k to eliminate responding (Group 3). Groups 1 and 2 showed similarities in overall drug effects and differed only in the sensitivity of operant behavior to disruption by APO. These groups showed dose-related decreases in operant responding with concomitant increases in elicited behavior. In contrast, data from group 3 showed increases in operant responding which were highly correlated with increasing stereotypy.

It is concluded that the effects of APO on operant behavior were dependent on induced stereotypy. Rate-decreasing effects appeared to be related to the disruption of ongoing operant behavior by elicited pecking aimed elsewhere in the chamber, while rate-increases seemed to be produced by the redirection of elicited pecking to the operant key. Further, since operant behavior showed individual differences in sensitivity to APO while elicited behavior did not, operant and elicited pecking may be differentially mediated by dopaminergic mechanisms. This separation of the key-peck response into two components may provide a model for the screening of neuroleptic drugs.

2176 EFFECT OF STRAIN DIFFERENCES IN TYROSINE HYDROXYLASE ON PHENCYCLIDINE-INDUCED LOCOMOTOR ACTIVITY

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Phencyclidine-induced locomotor activity was studied in three strains of albino rat that differ in the level of tyrosine hydroxylase (TOH) in the brain (Segal, et al, 1972). The strains included: F344 (high in TOH), Sprague-Dawley (moderate level of TOH), and BuF (low in TOH level). Activity level following 0.5-4.0 mg/kg phencyclidine (PCP) was recorded for a 30 minute period in a circular horizontal runway with each animal receiving a baseline session 24 hours prior to drug treatment. A control group for each strain received saline on the day of PCP treatment. There was a significant dose-response curve for each strain. Activity increased in a linear way with increasing dosage level. Further, there were significant differences between the strains in the amount of activity induced by PCP. Because of baseline differences in activity level across strains a transformation of the data was made and the treatment effects analyzed through analysis of variance methods. The F344 strain was significantly more responsive to PCP at 2.0 and 4.0 mg/kg. The BuF strain was the least responsive to the effects of PCP.

These results when interpreted in view of the TOH differences across these strains and the response of these strains to norepinephrine infusion as previously reported suggest that PCP does act like a sympathomimetic. It is consistent with this suggestion that strain differences in catecholamine receptor activity associated with the TOH differences would account for the observed distinction in responsiveness to PCP as measured in the present study.

2177 LOW LEVEL HYPERBARIC ETHANOL ANTAGONISM IN MICE. Ronald L. Alkana and Richard D. Malcolm. School of Pharmacy, University of Southern California, Los Angeles, CA 90033.

Recent studies in our laboratory demonstrated that exposure to hyperbaric oxygen-helium environments at 6 and 8 but not 10 atmospheres absolute (ATA) antagonized the narcosis in mice induced by 3.2 g/kg ethanol. The antagonism did not result from the direct effects of oxygen nor enhanced ethanol elimination. The present study further explored the mechanism and strength of the antagonism using the hypothesis that the increased heat loss induced by helium and/or hypoxia during compression may have obscurred demonstration of a pressure related antagonism. Drug naive, male C57 Bl/6j mice were injected i.p. with 3.2, 3.6 or 4.0 g/kg ethanol (20% w/v). Upon loss of their righting reflex, they were placed in a hyperbaric chamber. The atmospheric pressure was taken to 1 ATA air or 1, 4, 6, 8, 10 or 12 ATA oxygen-helium at the rate of 1 ATA/30 sec using procedures designed to keep the compression oxygen partial pressure between 0.2 and 0.7 ATA to avoid hypoxia. Oxygen partial pressure at the final testing pressure was 0.2 ATA. The chambers were immersed in a water bath and maintained at 33 ± 3°C using a pyrometer probe to reduce helium-induced hypothermia. A 20 µl tail vein blood sample taken at wake-up was used for gas chromatographic ethanol determination. Hyperbaric treatment significantly reduced sleep-times and increased wake-up blood ethanol concentrations at all doses tested ($p<0.05$, t-test). The degree of antagonism was pressure and dose related. The mean sleep-times and wake-up blood ethanol concentrations (BECs)±S.E. following 3.2 g/kg ethanol are shown below. The sleep-time reduction was greatest

Pressure (ATA)	Sleep-time (min)	BEC (mg/dl)	N
Air			
1	27.1 ± 3.8	368 ± 8	9
Oxygen-Helium			
1	22.2 ± 2.4	383 ± 5	9[A]
4	16.0 ± 3.6**	384 ± 5	9
6	15.1 ± 3.3**	391 ± 8*	9
8	18.5 ± 4.1	384 ± 5	10
10	13.6 ± 2.6**	398 ± 6****	9[A]
12	12.3 ± 2.6****	391 ± 6**	10

A - BEC N=8 *p<0.05; **p<0.025; ***p<0.01; ****p<0.005

at 12 ATA oxygen-helium. There was also a non-significant trend ($p<0.1$) toward reduced sleep-time in the 8 ATA treated mice. These findings indicate that the antagonism is not limited to a small range of hyperbaric pressures nor to low hypnotic ethanol doses. The implications of these results regarding the mechanisms of low and high pressure ethanol anesthesia antagonism are discussed.

2178 ONTOGENY OF STRAIN DIFFERENCES OF NIGROSTRIATAL TYROSINE HYDROXYLASE ACTIVITY AND SPONTANEOUS AND DRUG-INDUCED BEHAVIORS IN MICE. H. Baker, J.S. Fink, T.H. Joh, A. Swerdloff*, and D.J. Reis, Dept. Neurol., Cornell Univ. Med. College, New York, N.Y. 10021.

Adult mice of the BALB/cJ strain have more midbrain dopamine (DA) neurons and greater tyrosine hydroxylase (TH) activity in the substantia nigra-A10 (SN) region, caudate nucleus (CN), and mesolimbic brain areas than mice of the CBA/J strain (Nature 264:964, 1976). These differences correlate with greater spontaneous and amphetamine-induced locomotion and lesser sensitivity to stereotypy induced by apomorphine in BALB/cJ than CBA/J mice (Neurosci. Abst. 4:492, 1978). We sought to determine: (a) if the strain-dependent differences in TH activity in SN and a major terminal field, the CN, develop postnatally; and, (b) whether there is a temporal correlation between the ontogeny of the differences in TH and the behavioral differences in these two strains. In both strains, TH activity in SN increased rapidly, peaked at 125% of adult levels 15 days postnatally (P15) and decreased gradually to adult levels (P56) at P28. TH in the CN increased gradually, with TH activity at 65% of adult levels at P15 and 90% of adult levels at P21. Strain differences in TH activity (BALB>CBA) were not present before P7 but first appeared in the SN at P9 and in the CN at P11. Choline acetyltransferase (CAT) activity, an index of the maturity of intrinsic neurons of the CN, was 40% of adult levels at P15 and 70% of adult levels at P21. In early development (P8-P15), spontaneous and amphetamine-induced locomotion were less than, and apomorphine stereotypy greater than or equal to, the adult values. At this time, in contrast to adults, BALB/cJ mice were less active, less responsive to amphetamine, and more responsive to apomorphine than CBA/J mice. Between P15 and P21 spontaneous and amphetamine locomotion increased markedly in both strains. During this same period apomorphine stereotypy decreased in BALB/cJ mice and increased in CBA/J mice. By P21, then, evident strain differences in spontaneous and drug-induced behaviors which resembled the adult patterns were present. We conclude: (1) strain differences in TH activity in the nigrostriatal system and the adult pattern of DA-mediated behaviors first appear postnatally; and, (2) the development of strain-differences in TH activity in the nigrostriatal system precede the adult patterns of strain differences in DA-mediated behaviors. These data suggest that the phenotypic expression of DA-mediated behaviors is dependent not only on the level of TH activity in the nigrostriatal system, but may also depend on the maturation of its major afferent nucleus, the CN.

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2179 BLOOD PLASMA OSMOLALITY AND MANIC-DEPRESSIVE ILLNESS
Claude F. Baxter, Ken Tachiki* and Lawrence F. Gosenfeld*. Neurochemistry Labs (151B2), VA Medical Center, Sepulveda, CA 91343, and Affective Disorders Clinic, Brentwood VA Medical Center, Los Angeles, CA 90073.

A variety of physiological, nutritional and pathological conditions are known to alter the osmolality of blood plasma. In turn, these changes in plasma osmolality can affect the biochemistry and biochemical composition of the Central Nervous System. In particular, changes can be produced in the cerebral levels of urea, carbohydrates, keto acids, amino acids and amines. The latter two categories include several putative neurotransmitters and neurotransmitter precursors.

A preliminary clinical study has been conducted with manic and depressed patients, most of whom were kept under well controlled conditions in a hospital ward. All patients were on lithium therapy with blood levels in the range of the "therapeutic window" 0.8 to 1.2 meq/liter. All blood samples were drawn before a meal, usually before breakfast on the day that the mood and condition of the patient was tested using the Research Diagnostic Criteria of Spitzer and Endicott. Blood plasma osmolality of normal volunteers and staff ranged from 285 to 295 mOs, whereas the range in patients extended from 270 to 310 mOs. Although the highest plasma mOs were found in some depressed patients and the lowest mOs in some manic patients, no absolute correlation between plasma osmolality and clinical condition could be demonstrated. However, clinical improvement of manic patients, as measured by research diagnostic criteria, was correlated in a highly significant manner with an increase in plasma osmolality. Similarly, clinical improvement of depressed patients was correlated in a highly significant manner with a decrease in plasma osmolality. The clinical improvement was not correlated with fluctuations within the "therapeutic window" of plasma lithium concentrations.

It is conceivable that the small changes in plasma osmolality observed in these patients can be translated into small but significant metabolic and compositional changes in brain tissue and, thus, influence mood and behavior. (Supported by the Medical Research Service of the Veterans Administration.)

2180 USE OF CONDITIONED SUPPRESSION AND EXTINCTION TO EVALUATE THE NATURE OF NEUROLEPTIC-INDUCED AVOIDANCE DEFECITS. Richard J. Beninger* (Spon: Anthony G. Phillips) Dept. Psychology, Univ. British Columbia, Vancouver, B.C., Canada V6T 1W5.

Numerous studies have shown that animals with brain catecholamines (CA) depleted are impaired in their ability to acquire a conditioned avoidance response (CAR) and the results of additional experiments indicate a critical role for dopamine (DA). To evaluate the nature of this CAR defecit, in Experiment 1 rats were injected with the neuroleptic pimozide (1.0 mg/kg) and received five sessions of CAR training in which a tone signalled shock on each trial (and were observed to fail to avoid). These same rats when in an undrugged state received food-reinforced lever-press training until responding stabilized. The tone that had signalled shock in CAR training was then presented. Significantly greater conditioned suppression to the tone was observed in the rats that had received CAR training while drugged, than in unshocked controls, indicating that the neuroleptic-treated rats had learned the tone-shock association in spite of their failure to avoid. Thus, neuroleptic-induced avoidance defecits were not related to an impairment in associative learning. To determine if the CAR deficit was related to an impairment in response learning, in Experiment 2 pimozide-injected rats received five sessions of CAR training and were then tested while undrugged. One group was tested in extinction (i.e., shocks were no longer presented) and another continued to receive shocks. Both groups showed gradual acquisition of the CAR indicating that they had failed to learn the appropriate response during training. The fact that the extinction group acquired the CAR indicated that they must have learned the significance of the tone while drugged; this observation was consistent with the results of Experiment 1. These data indicate that DA is not required for learning the association between stimuli. They further suggest that normal DA functioning is required for conditioned (i.e., shock-associated) stimuli to acquire the ability to elicit specific responses. (Supported by the Medical Research Council of Canada).

2181 EFFECTS OF ANTICHOLINERGICS ON VISUAL ACUITY OF MACACA MULATTA. C. T. Bennett*, G. D. Callin*, D. N. Farrer*, C. Link* and P. Garcia* (SPON: James King). USAF School of Aerospace Medicine/RZW, Brooks AFB, TX 78235.

Rhesus monkeys were trained to discriminate between left- and right-opening Landolt rings. The test apparatus presented stimuli at 1 meter in seven discrete sizes corresponding to Snellen acuities 20/200, 20/100, 20/50, 20/40, 20/30, 20/20 and 20/15; a titration paradigm was designed to elicit performance at maximum acuity. Upon establishment of stable baseline performance, the subjects were injected IM with the following drugs prior to testing: (1) benactyzine, 0.054 to 0.54 mg/kg; (2) atropine, 0.014 to 0.14 mg/kg; (3) TAB (a compound consisting of atropine, benactyzine, and the oxime TMB-4 in a ratio of 1:4:40), 0.64 to 6.4 mg/kg; and (4) a diluent control. Injection of active substances caused dose-related decreases of near visual acuity. Onset times and effect durations differed among drugs.

2182 INCREASED AMPHETAMINE POTENCY FOLLOWING CHRONIC NALTREXONE ADMINISTRATION IN RATS. Richard S. Blair*, Shimon Amir* and Zalman Amit. Department of Psychology, Concordia University, Montreal, Quebec, Canada.

There is evidence to suggest that enkephalins may modulate the normal functioning of the nigro-striatal dopamine system by interacting with opiate binding sites in the brain. Given this and the recent finding that chronic naloxone administration produces an increase in opiate receptor binding sites, we undertook to investigate the effect of chronic naltrexone pretreatment on the effects produced by the dopamine agonists, d-amphetamine and apomorphine.

Thirty male Wistar rats received a daily injection (subcutaneous) of saline (1ml/kg) or naltrexone (1mg/kg) for eight consecutive days. Forty-eight hours after the last naltrexone injection, animals then received an intraperitoneal injection (I.P.) of either d-amphetamine (1mg kg), apomorphine (1mg/kg) or saline (1ml/kg). Fifteen minutes after the I.P. injection, the animal's locomotor activity in an open field was measured by the number of photo-beams broken during the 30 minute testing session. The animals were retested seven and 14 days after the termination of the naltrexone pretreatment using the same experimental procedure.

An analysis of variance revealed a significant overall increase in the activity by naltrexone. Furthermore, a significant drug effect, as well as a significant overall decrease in activity over the three testing sessions were noted. Multiple t-tests revealed that amphetamine produced a greater activity in animals given naltrexone pretreatment than in animals that received saline pretreatment.

The increase in locomotor activity observed in rats chronically treated with naltrexone, as well as the marked potentiation of d-amphetamine's excitatory effect, suggests that prolonged blockade of the opiate receptor may lead to the development of a supersensitivity in the dopamine systems that mediate motor functions. If, as previously suggested, endogenous enkephalins excite striatal dopamine neurons, then an increase in the number of opiate binding sites produced by chronically administered naltrexone, may have potentiated the excitibility of the nigro-striatal dopamine system.

2183 INTRASTRIATAL AND INTRALIMBIC INFLUENCES OF DOPAMINE, PHENYLETHYLAMINE AND AMPHETAMINE. R.L. Borison, J.E. Comaty and B.I. Diamond. Mt Sinai Hosp & Ill St Psych Inst, Chicago, Il 60608 & 60612.

Stereotyped behavior in animals is believed to result from stimulation of striatal dopaminergic mechanisms, however, it is now widely held that this behavior can be modulated by limbic dopaminergic and noradrenergic influences. Stereotyped behavior in rodents elicited by d-amphetamine (dA) has been used as a pharmacological paradigm for schizophrenia because this behavior is blocked by neuroleptics, and amphetamine administration in man can result in a paranoid schizophreniform psychosis. We have shown that the administration of phenylethylamine (PEA), an endogenous biogenic amine, is also capable of producing stereotypy. Dopamine receptor blocking antipsychotics antagonize both dA and PEA elicited stereotypy, however thioridazine and clozapine, which are proposed to be more limbic than extrapyramidal system site specific, selectively antagonize PEA behavior. Moreover, upsetting the striatal dopamine-acetylcholine balance by administering an anticholinergic potentiates dA but not PEA stereotypy. Based on these data, we have suggested that PEA stereotypy may more specifically involve limbic mechanisms. To test this hypothesis we have studied the effects of intrastriatal and intralimbic injections of dopamine, dA and PEA. Subjects were white male Sprague-Dawley rats with bilateral stereotactically placed 0.8mm stainless steel cannula in the caudate-putamen nucleus and the nucleus accumbens. Animals were rated for stereotypy using a 5 point scale of ascending intensity of stereotypy. The administration of dopamine (100µg) into the striatum produced stereotyped sniffing, head-weaving and occassional gnawing resulting in a stereotypy score of 3.37 ± 0.12. In comparison, intrastriatal dA (100µg) elicited a stereotypy score of 4.25 ± 0.16 and increased locomotor activity. In contrast, PEA (100µg) administration resulted in a score of 2.00 ± 0.01 with animals exhibiting stereotyped grooming and sniffing lasting 20 min. We found that intralimbic dopamine produced increased locomotion, occassional gnawing, stereotyped grooming and sniffing. At a dose of dopamine of 100µg, the stereotypy score was 2.8 ± 0.12, whereas at 200µg the score reached 3.5 ± 0.14. The administration of dA (100µg) produced stereotyped sniffing, achieving a score of 2.62 ± 0.13. In contrast, intralimbic injection of PEA (100µg) resulted in a stereotypy score of 3.50 ± 0.11 with increased locomotion, and stereotyped grooming, sniffing and head-swaying. These results demonstrate that PEA, as compared with dA, exerts more potent effects on stereotyped behavior via the activation of mesolimbic mechanisms.

2184 EFFECT OF DIETARY LIPID COMPOSITION ON LOCOMOTOR ACTIVITY AND PSYCHOMOTOR STIMULANT RESPONSE. Douglas E. Brenneman* and Charles O. Rutledge. Dept. Pharmacol. and Toxicol., Sch. Pharm., Univ. Kansas, Lawrence, KS 66045.

Spontaneous locomotor activity was compared in rats fed semi-synthetic diets enriched in either polyunsaturated fat (sunflower oil) or saturated fat (coconut oil). Control animals were given Purina rodent chow. Neonatal rats received the dietary lipid through the maternal milk. After weaning, the pups were maintained on the same diet given to the dam. There were no significant differences in body weights among the dietary groups. Cumulative counts as measured in an Animex activity chamber were monitored every 6 min. for one hour. All experiments were performed on naive animals. Developmental studies revealed that animals fed sunflower oil had a 40-60% increase in locomotor activity as compared to animals given coconut oil. Significant differences were observed on days 20, 30, and 65. No changes in activity were found on day 10. The activities of rats given sunflower oil were also increased when compared to animals receiving the standard rodent chow.

Locomotor activity responses to i.p. administration of d-amphetamine, methylphenidate, and atropine were compared in day 30 animals fed the various regimens. Animals receiving sunflower oil had a decreased response to d-amphetamine in comparison to rats fed coconut oil. Maximal activity was observed at 3 mg/kg for the coconut oil fed group and at 10 mg/kg for those receiving sunflower oil. A dose-response study for methylphenidate revealed no differences in activity among the dietary groups. Atropine (2 mg/kg) decreased activity 40% below basal levels in rats fed sunflower oil. High doses of atropine (15 mg/kg) were necessary to achieve an increase in locomotor activity in this group. In contrast, coconut oil fed animals exhibited an increase in activity with 1 mg/kg atropine. Maximal response to atropine occurred at 15 mg/kg in both dietary groups but the activity of the sunflower oil fed group was 30% less than in rats fed coconut oil. Thus, the animals given sunflower oil had higher basal levels and responded to a lesser extent to d-amphetamine and atropine. These results indicate that sunflower oil can induce a hyperactive state in developing rats and that the response to psychomotor stimulant drugs are differentially affected by dietary fat composition. Supported by USPHS Grants NS 12760 and RR 5606.

2185 AN AMPHETAMINE-INDUCED DYSKINESIA IN MONKEYS MEDIATED BY PERIPHERAL SYMPATHETIC STIMULATION. P. J. Bushnell* and C. M. Baysinger* (SPON: W. T. McKinney). University of Wisconsin Primate Laboratory, Madison, Wisconsin 53706.

A highly characteristic behavioral response to d-amphetamine injections (0.3 or 0.6 mg/kg, SC) in infant rhesus monkeys was identified. This response consisted of bizarre, catatonic-like postures and dyskinesias in which the animals' hands and/or feet appeared to float for prolonged periods of time. This "floating limb" developed over the first 1 - 10 biweekly injections in monkeys tested beginning at 4 weeks of age, and over the first 2 - 4 semiweekly injections in monkeys one year of age. When fully developed, the response to drug occurred with an average frequency of 7/min., and occupied on the average 30% of the animals' total ongoing behavior. While floating limb was most dramatically induced by d-amphetamine, peripherally-acting sympathomimetics including hydroxyamphetamine (0.6 mg/kg) and epinephrine (25 ug/kg) also produced a qualitatively similar response. The increased intensity of the dyskinesia following d-amphetamine may have resulted from its central, attention-focusing action, added to its stimulation of the peripheral sympathetic nervous system. The peripheral mechanism(s) mediating floating limb are presently being explored with sympathomimetic drugs having specific alpha- and beta-receptor activities.

Since hydroxyamphetamine and other sympathomimetics lacking CNS activity have rarely been used as control substances in psychopharmacological research with amphetamine, these results call into question the assumption that the behavioral effects of amphetamine are mediated solely by its activity in the CNS.

2186 AN IMMOBILIZING STIMULUS SIGNIFICANTLY ATTENUATES BOTH AMPHETAMINE AND APOMORPHINE-INDUCED STEREOTYPIES. A.R. Caggiula and L.A. Chiodo. Psychobiology Program, Dept. of Psychology, Univ. of Pittsburgh, Pittsburgh, PA 15260.

We have recently suggested that the behavioral effects of activating and immobilizing stimuli depend, at least in part, on their reciprocal influence on the functioning of brain dopamine (DA)-containing systems. For example, an activating stimulus, such as tail pressure (TP), can induce a variety of appetitive behaviors and this effect is attenuated by pharmacological blockade of brain DA activity. Moreover, the same TP procedure dramatically alters the firing rate of DA neurons in the substantia nigra of anesthetized rats. Conversely, an immobilizing stimulus, such as cervical probing (CP), counteracts both the behavioral and electrophysiological effects of TP. Consistent with our view that CP can block the behavioral effects of DA activation, we now report that CP drastically attenuates stereotyped responses produced by two DA agonists, amphetamine and apomorphine.

Ovariectomized female rats received 50 µg/kg (s.c.) of estradiol benzoate 48 and 24 hours prior to testing. The test consisted of three 30 sec. bouts of CP which were preceeded and separated by one minute baseline observation periods. The tests were conducted 30 and 120 minutes after amphetamine and 30 minutes after apomorphine. CP, which was administered with a saline lubricated glass rod (3 mm diameter) inserted into the vagina and pressed firmly against the cervix, attenuated all aspects of stereotypy for 6, 12 and 18 mg/kg of amphetamine and 3 and 6 mg/kg of apomorphine. For example, 60 minutes after 12 mg/kg of amphetamine, 85% of the animals were sniffing and 70% licking prior to CP, whereas percentages were 15 and 18%, respectively, during CP, and 72 and 62% after. Similarly, the values for 6 mg/kg of apomorphine were 100% sniffing prior to CP, 5% during CP and 95% after (N=7 per group).

It therefore appears that an immobilizing stimulus, such as CP, is able to dramatically attenuate both drug and sensory-induced activated behaviors which are thought to be the result of increases in mesencephalic DA function.

2187 SELECTIVE ATTENTION DISRUPTED BY APOMORPHINE IN THE MONGOLIAN GERBIL. MaryLou Cheal. Neuropsychol. Lab., McLean Hospital, Dept. Psychiatry, Harvard Medical School, Belmont, MA 02178.

In previous research, selective attention was intact in gerbils following injections of amphetamine (Phys. Behav., 1978, 21, 299-305). Amphetamine-treated gerbils (2.0-3.0 mg/kg) selectively responded to objects that were novel even though the amphetamine-induced competing stereotypies resulted in an attenuation of the amount of active investigation of the object. Additionally, when a large dose (6.0 mg/kg) of d-amphetamine was administered, intense stereotypy occurred and very little active investigation was seen. However, "passive" attention could be inferred from data collected the next day when the acute effects of the drug had passed (Soc. Neurosci. Abst., 1978, 4, 487). Because habituation can be demonstrated in normal gerbils up to two weeks following a single 60 sec nonreinforced trial (J. Biol. Psychol., 1978, 20, 26-32), it was possible to test amphetamine-treated gerbils for habituation on the second day. The responses of these gerbils reflected habituation similar to habituation in controls that had actively investigated the object following injection. Thus, the stimulus-elicited investigation paradigm allows the separation of active investigation or alertness from selective attention.

Several lines of research implicate the action of amphetamine in increasing dopamine (DA) activity as the mechanism whereby amphetamine affects exploratory behaviors and motor activity. However, in the stimulus-elicited investigation paradigm, the DA receptor stimulant, apomorphine, produced results that differed from those of amphetamine. Selective attention was disrupted following doses as low as 1.0 mg/kg apomorphine. At this dose, the gerbils moved to the object as frequently as controls on the first trial, but subsequent trials did not reflect rapid habituation as seen in controls. Further, although "passive" attention was found following 0.3 mg/kg apomorphine, at higher doses (1.0, 3.0 or 10.0 mg/kg) the gerbils showed no evidence of earlier exposure to the object when retested the second day. It was also demonstrated that failure of habituation following apomorphine injections was not due to amnesia or retrieval problems. Additional gerbils were tested for habituation to the object prior to injections. Following subsequent apomorphine treatment, habituation levels of responding occurred indicating that once the memory was stored, it was not disrupted by apomorphine. The data indicate that apomorphine can disrupt selective attention at doses where active investigation, or alertness, can be exhibited. (Supported by the Scottish Rite Schizophrenia Research Program, N. M. J., U. S. A. and by the Biomedical Research Support Program, D. R. R., N. I. H.)

2188 ESTROGEN MODULATION OF DA DEPENDENT BEHAVIORS L.A. Chiodo, A.R. Caggiula and C. F. Saller.* Psychobiology Program, Depts. of Psychology and Biological Sciences, Univ. of Pittsburgh, Pittsburgh, PA 15260.

We have previously shown that estrogen potentiates two behavioral consequences of drug-induced changes in DA activity. That is, ovariectomized (OVX) female rats treated with estradiol benzoate (EB; 100 µg/kg, s.c.) 48 h before testing showed significantly longer durations of spiperone-induced catalepsy (0.5 mg/kg) and more intense amphetamine-induced stereotypy (3 and 9 mg/kg) than OVX/oil controls (Chiodo and Caggiula, 1978). The present study demonstrates that OVX female rats receiving the same hormone treatment also show significantly more intense apomorphine-induced stereotypy (3 and 6 mg/kg) than OVX/oil controls. In all cases stereotypy was rated on a 7 point scale.

We also examined the effects of EB on the uptake of radioactively labelled spiperone, amphetamine and apomorphine in order to determine whether estrogen may have increased the behavioral effects of these drugs by altering their peripheral metabolism or uptake into the brain. We now report that an EB treatment which increased spiperone-induced catalepsy (0.5 mg/kg) also elevated levels of ^{3}H-spiperone in both blood and whole brain. EB increased whole brain tritium levels, relative to oil controls, at both three (129%) and six hours (129%). Tritium levels in blood were also elevated at these same times (125% and 146% respectively). Similar results were obtained after chromatography of brain and blood, indicating that most of the radioactivity was accounted for by ^{3}H-spiperone. These findings suggest that at least some of the increased catalepsy may have resulted from estrogen's ability to increase the levels of spiperone reaching the brain.

In the same manner, we examined the effects of EB on the uptake into the brain of tritiated amphetamine (3 mg/kg) and apomorphine (3 mg/kg) administered intraperitoneally. EB did not increase whole brain or blood levels of labelled apomorphine, amphetamine or its active metabolite, p-hydroxynorphedrine. Similar results were obtained with chromatography. In fact, there was a non-significant trend for OVX/EB animals to show lower tritium levels than controls. In contrast to the changes in peripheral metabolism of spiperone, increased uptake into the brain cannot account for EB's effects on amphetamine or apomorphine stereotypy.

In summary, both central and peripheral effects of estrogen must be considered when assessing its role in the behavioral and neurochemical consequences of drug-induced alterations in DA function.

2189 COCAINE-INDUCED STEREOTYPED BEHAVIOR; ONGOING RESPONSES DETERMINE DRUG EFFECTS. Jeremiah P. Collins and Henry Lesse. Department of Psychiatry and Brain Research Institute, School of Medicine, University of California, Los Angeles, CA 90024.

Stereotyped behaviors result following the administration of a number of different drugs, primarily the psychomotor stimulants. The stereotypies consist of responses occurring repetitively with little variation, have no apparent source of reinforcement, and are relatively insensitive to changes in ambient stimuli. Examples of these behaviors are repetitive orienting or arousal reactions such as head bobbing, rearing, sniffing, grooming, or in man, complex response chains including speech and cognition. Interactions of ongoing behaviors with the actions of psychomotor stimulants have also been reported. We investigate in the present study whether ongoing operant responses occurring at the time of cocaine administration become stereotyped. Cats were trained in a discrimination task in which each bar-press emitted when a tone (S+) was present resulted in milk reinforcement. When the tone was absent (S-) milk was not delivered. The duration of S+ and S- periods varied randomly from 1 to 5 mins and a 1-min DRL contingency was in effect during S-. Training continued until a high level of discrimination was achieved. Drug testing was then begun with injections of cocaine hydrochloride (1 mg/kg). The ongoing behavior was manipulated by scheduling either a 5-min S+ or S- period immediately following cocaine administration. When the S+ *period followed cocaine administration, stereotyped bar-*pressing developed, i.e. bar-pressing continued during subsequent S- periods despite the absence of reinforcement. When the S- period followed cocaine, marked suppression of bar-pressing resulted. This effect was reversible in the same subjects when stimulus conditions were later reversed. A similar study conducted with rats yielded similar results. Whatever the mechanism rezponsible for these drug-induced behaviors, it is clear that the stimuli and the behaviors occurring at the time of cocaine administration are powerful determinants of subsequent stereotyped behaviors.

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2190 CENTRAL AMINERGIC NEURONAL SYSTEMS AND THE BEHAVIORAL EFFECTS OF HALLUCINOGENS. R. Commissaris*, W.H. Lyness, R.H. Rech and K.E. Moore. Dept. of Pharmacol. & Toxicol., Michigan State Univ., East Lansing, MI 48824.

The mechanisms by which a variety of drugs produce hallucinations are not well understood, but it has been suggested that these drugs interact with 5-hydroxytryptamine (5HT) and dopamine (DA) neuronal systems in the brain. The present study was designed to examine the role of 5HT and DA neurons in modulating the behavioral effects of two hallucinogens having different chemical structures: d-lysergic acid diethylamide (LSD), an indolealkylamine analog, and 2,5-dimethoxy-4-methylamphetamine (DOM), a phenylethylamine analog.

Male rats, maintained at 70-80% of their free-feeding weight, were trained to press a bar for food pellets on a fixed ratio-40 (FR-40) operant schedule. When trained, these rats respond at a constant, rapid rate (approximately 100 responses/min) during daily test sessions of 40 min duration. The rats continue to respond when central stimulant or depressant drugs are administered, but the rates of responding change. On the other hand, the administration of hallucinogens causes an abrupt cessation of responding followed sometime later by an equally abrupt reinstatement of responding. The duration of the "hallucinatory pause" was found to be dose-dependent for both LSD (25, 50, 100 and 200 µg/kg, i.p.) and DOM (0.25, 0.5, 1 and 2 mg/kg, i.p.). The same tests were repeated in rats that had previously received an intraventricular injection of 5,7-dihydroxytryptamine (5,7-DHT; 200 µg/10 µl). This neurotoxin destroyed 5HT neurons, as evidenced by the marked reduction in the uptake of ^{3}H-5HT by brain synaptosomes, but did not alter regional brain concentrations of DA or norepinephrine. In the 5,7-DHT-treated rats the dose-response curves for the "hallucinatory pause" induced by both LSD and DOM were shifted to the left. On the other hand, *the behavioral effects of LSD and DOM were not altered in rats* in which DA nerve terminals in nucleus accumbens were selectively destroyed by microinjections of 6-hydroxydopamine (8 µg/2 µl) into this nucleus; the locomotor stimulant effects of d-amphetamine are reduced in these animals.

In summary, the hallucinatory pause induced by both LSD and DOM is enhanced when 5HT neurons in the brain are destroyed, but not altered by destruction of DA nerve terminals in nucleus accumbens. (Supported by USPHS grant DA1836.)

2191 THE PRECLINICAL IDENTIFICATION OF PSYCHOTOMIMETIC BENZOMORPHAN ANALGESICS. A. Cowan, F.C. Tortella and M.W. Adler. Dept. Pharm., Temple University School of Medicine, Philadelphia, PA 19140.

A large number of benzomorphan narcotic antagonists possess clinically useful analgesic properties; unfortunately, many have been discarded because of their psychotomimetic side-effects. Reliable preclinical tests for identifying such activity are currently lacking. In the present study, we defined the profile of SKF 10,047 (N-allylnorphenazocine), the prototype (benzomorphan) σ receptor agonist, in terms of rat Y-maze behavior and EEG activity. The corresponding profiles of cyclazocine, ethylketocyclazocine (EK), morphine, 8-methoxycyclazocine (8-MC), and Win 42,156 (2'-hydroxy-2,5,9α-trimethyl-9β-(3-oxooctyl)-6,7-benzomorphan) were then compared.

Groups of 6-8 male, S.D. rats (100-120 g) were injected s.c. with vehicle or test compound and 30 min later were individually placed in a Y-maze for 3 min. Only the known hallucinogenic compounds, SKF 10,047 (2.5-40 mg/kg), cyclazocine (0.63-5 mg/kg), and 8-MC (2.5-10 mg/kg), caused the following dose-related and naloxone-insensitive "psychotomimetic" profile: increased locomotion, perseveration of exploratory behavior during the 3rd min, circling, absence of grooming, and side-to-side head swaying at the end of each runway.

Additional rats (150-200 g) were prepared with cerebrocortical electrodes for continuous recording of the EEG. Three to 5 days later, the rats were placed in individual glass chambers and allowed to acclimate and move freely for 3-4 hr during which time control EEG recordings were taken. Drugs were injected i.p., and EEG and behavior were monitored until the onset of slow-wave sleep. Morphine (2.5-10 mg/kg) induced biphasic EEG and behavioral effects characterized by an initial phase of high-voltage EEG slow-wave activity (slow bursts) and associated stupor followed by EEG activation and behavioral arousal. EK (0.63-10 mg/kg) caused similar effects. The profile with SKF 10,047 (10-40 mg/kg) was different. There was an initial "psychotomimetic" phase of EEG activation with increased locomotion and side-to-side head swaying lasting 20-50 min followed by intermittent periods of stupor (associated with EEG slow bursts) and behavioral arousal (and EEG activation). Cyclazocine (2.5-10 mg/kg) induced similar effects. 8-MC gave a morphine-like profile at 2.5 mg/kg and a SKF 10,047-like profile at 10 mg/kg. In all cases, pretreatment with naloxone (10 mg/kg, s.c. at -10 min) antagonized the morphine-like EEG slow bursts and stupor but it had no effect on the "psychotomimetic" component. Preliminary results indicate that the profile of Win 42,156 (2.5-40 mg/kg) is not like that of SKF 10,047 and allows us to predict that this interesting narcotic antagonist analgesic will not possess SKF 10,047-like subjective effects in man. (Supported by Grant DA00376 from NIDA.)

2192 EFFECTS OF PHENOBARBITAL ON THE ACTIVITY OF HYPERACTIVE AND NON-HYPERACTIVE ANIMALS. J.Diaz, K.Watanabe*, and J.Zagun*. Dept. of Psychology, Univ. of Washington, Seattle, WA 98115, Dept. of Psychiatry, NPI/MR, Los Angeles, CA 90024.

Studies show that stimulants may not only improve the attention span of children including hyperactive children, but may also reduce their activity. In addition, most central nervous system depressants will not sedate but rather agitate hyperactive children. The purpose of the present study is to examine the effects of a CNS depressant - phenobarbital - on the activity of a normally very active rodent - the Mongolian gerbil (Meriones Unguiculatus) and a considerably less active rodent - the rat.

Thirty-day old gerbils were assigned to either a phenobarbital group (n=20) or to a vehicle group (n=19) and were injected daily with phenobarbital (60 mg/kg,s.c.) or the vehicle for 4 months. After approximately 14 weeks of drug administration, the activity of the animals in a 5 minute open field test was recorded once a week at varying times after injection (1,2,4,6,8,16,24 hours). During this testing period blood was drawn from the drug animals to determine phenobarbital levels at the time of testing. Vehicle treated animals were subjected to the same proceedure to control for the stress of the bleeding. Thirty-day old male Wistar rats were also assigned to either a phenobarbital group (n=22) or to a vehicle group (n=17) and were given drugs and tested in the same way as the gerbils (after 1,2,4,6,8,24 hours).

The results indicate that the control gerbils and rats show a stable level of locomotor activity for all the time intervals after injections. Moreover, the activity scores for the control gerbils were more than double the activity scores for the control rats. Even though phenobarbital did not appreciably change the activity of rats, it did dramatically alter the activity of gerbils. Animals tested within a few hours after injections were sedated but the handling involved in moving them into the testing device seemed to rouse them. Once in the open field the activity of the phenobarbital treated gerbils was 3 times higher than that of control gerbils. This heightened level of activity remained for at least 8 hours, and by 16 hours after injection the drug group returned to activity levels which were similar to control gerbils. The phenobarbital plasma levels of the drug animals indicate that even though gerbils clear phenobarbital at a slightly faster rate, both rats and gerbils show peak plasma levels at one or two hours after injections.

There are striking similarities between the effects of CNS depressants on the behavior of hyperactive children and the behavior of gerbils. The data from this preliminary study suggest that the gerbil may be a useful model for hyperactivity.

2193 SENSITIZATION OF DRINKING DURING CHRONIC AMPHETAMINE ADMINISTRATION. A.J. Eichler*, S.M. Antelman* and P. Longstreet.* (SPON: G. Werner). Psychobiology Program, Dept. of Psychology, Univ. of Pittsburgh, Pittsburgh, PA 15260.

It is believed that the progressive enhancement (sensitization) of behaviors following repeated administration of stimulants such as amphetamine (AM) may mimic the development of AM psychosis in humans, a condition markedly similar to paranoid schizophrenia. The behaviors typically studied with regard to sensitization in animals have been locomotion and various motor stereotypies. We now report that chronic AM can induce a polydipsic response in rats which shows significant sensitization over time.

Rats were injected daily I.P. with saline, 2, 4, or 8 mg/kg of d-AM sulfate. Water intakes were measured for the 5 hrs immediately following drug administration on different days into the chronic regimen. No significant drinking occurred acutely. However, by day 6 animals were drinking significant amounts to all doses of AM (2 mg/kg: 5.4 ml; 4 mg/kg: 5.9 ml; 8 mg/kg: 4.7 ml; V = 1.0 ml) and by day 35, the response had risen to 15.7 (2 mg/kg), 21.8 (4) and 18.8 ml (8). AM also increased urine output during the same 5 hr period. However, this diuresis was dissociable from the polydipsia, since it showed no sensitization over time. The drinking response is unlikely to be due to conditioning or an accumulation of AM, since the polydipsia could also be seen following only 2 injections of AM spaced at a 20-day interval.

While we have yet to address directly the mechanisms underlying AM-induced drinking, we have observed a significant drinking response which sensitizes during chronic electrical stimulation of the medial frontal cortex, a terminal area of the mesocortical dopamine system. Since electrical stimulation of this region also produced a sensitization to AM stereotypy, it is possible that the medial frontal cortex may be involved in sensitization phenomena in general, as well as in the progressive polydipsia reported here.

We suggest that AM-induced drinking may serve as a suitable measure for studying AM sensitization. The simplicity of the procedure coupled with its objectivity offer distinct advantages over previously employed sensitization paradigms. Finally, it should be noted that the induction of drinking following repeated AM may be clinically relevant, since Bell (1973) has reported increased thirst in individuals manifesting AM psychosis.

2194 SEROTONIN INVOLVEMENT IN MURICIDE BLOCKADE BY IMIPRAMINE. Norman Eisenstein*, Donald Clody* and Louis C. Iorio*. (Spon: W. Gray). Department of Pharmacology, Schering-Plough Corporation, Bloomfield, New Jersey 07003.

The role of serotonin (5HT) in muricidal behavior has been well documented. Decreases in central 5-HT levels produced by p-chlorophenylalanine, fenfluramine or raphe lesions induces killing in non-killer rats; increasing 5-HT by reuptake inhibition (fluoxetine) and administration of precursors (l-tryptophan, 5-HTP) and 5-HT agonists (quipazine) blocks muricide in established spontaneous killers. It has been reported that imipramine and other tricyclic antidepressants block muricidal behavior. These drugs also increase 5-HT, norepinephrine (NE) and dopamine (DA) by inhibiting reuptake mechanisms. This study was done to test the hypothesis that imipramine and other antidepressants may produce their antimuricidal effects through alterations in 5-HT.

Groups of established killers with and without a tryptophan-free diet were tested with various doses of imipramine. The tryptophan-free diet, which decreased whole brain levels of 5-HT, produced a shift to the left of the dose-response function of the antimuricide activity of imipramine. This shift was also produced by treatment with p-chloroamphetamine, a specific 5-HT neurotoxin. L-5-hydroxytryptophan, at doses which did not reduce mouse-killing, potentiated the antimuricide activity of imipramine.

Therefore, it appears that the antimuricidal effect of imipramine is dependent, in part, on its ability to block reuptake of 5-HT. However, it is important to note that no antiserotonergic treatment completely blocked the imipramine effect, suggesting that other neurotransmitter systems may also be involved.

2195 FOLLOWING SEVERAL DAYS OF CONTINUOUS ADMINISTRATION D-AMPHETAMINE ACQUIRES HALLUCINOGEN-LIKE PROPERTIES. Gaylord Ellison, Erik B. Nielsen*, and Arlene Stark*. Dept. Psychology, UCLA, Los Angeles, CA 90024

Rats injected with LSD or mescaline show the behavioral syndrome which has been previously reported to be hallucinogen-specific in higher mammals: limb flicks and whole body shakes. Although these behaviors are decreased in a dose-dependent manner by acute injections of d-amphetamine, they are present 4-5 days after rats are implanted with slow-release silicone pellets producing continuous d-amphetamine administration. These behaviors can be elicited at a high frequency in rats which have been pretreated for 4 1/2 days with an amphetamine pellet, given a 12 hr. rest period, and then injected with a low dose of d-amphetamine. Such pellet-pretreated animals also groom their body surface excessively. We propose that this novel behavioral syndrome which follows continuous amphetamine administration can serve as a new animal model of amphetamine psychosis, since it is based on the same drug regimen which most reliably produces a model psychosis in humans. This behavioral syndrome is altered by concurrent injections of dopamine or serotonin antagonists.

2196 STABILIZATION OF BLOOD ETHANOL LEVELS BY SUBCUTANEOUS RELEASE OF ETHANOL IN RATS DRINKING PREFERRED LIQUID DIETS. Carlton K. Erickson, Kathe I. Koch* and Joseph E. Knipper*. Dept. Pharmacol. Coll. Pharmacy, Univ. Texas, Austin, TX 78712

Liquid diets containing ethanol are a popular method of producing long-term exposure, tolerance and physical dependence in rats and mice. The diets consist of formulations developed a) for weight reduction or nutritional supplementation in humans, or b) expressly for rodent nutrition. We have tested four human nutritional supplements (Nutrament chocolate and vanilla, Sustacal chocolate and vanilla) and two rodent formulations (Lieber-DeCarli, Bioserv; Shorey AIN, custom formula) during 5-day preference tests in female Sprague Dawley rats. Ethanol or (in controls) dextrin supplied 37% of calories in each diet. Animals were housed individually and liquid diets in sterile bottles equipped with Richter drinking tubes were the sole source of food and water. Fresh diet was provided daily. Rats in general preferred chocolate-flavored diets over vanilla-flavored diets, and dextrin-containing diets over ethanol-containing diets. The Lieber-DeCarli diet with dextrin was preferred over the Shorey AIN diet with dextrin, but when ethanol isocalorically replaced dextrin the preference was reversed. Severe withdrawal symptoms could not be evoked after the 5-day exposure. Blood ethanol levels (BEL) cycled during the diet drinking periods, as determined by gas chromatographic analysis of arteriovenous (tail) blood samples.

Stabilized BEL (less cycling compared to diet-induced BEL in the above study) were obtained by using diet in combination with subcutaneous silastic tubes (Sustained Ethanol Release Tubes, SERT; Pharmacologist 20: 159, 1978) which slowly released 95% v/v ethanol. These were refilled once a day. An initial intragastric dose of ethanol in liquid diet was used to "set" the desired BEL. The SERT sustained the BEL, while an available diet added supplemental ethanol and calories for maintenance of stable BEL and body weight. After 4 days of high BEL (above 300 mg/dl), severe withdrawal symptoms were seen upon removal of ethanol, indicating the development of physical dependence. This combination of two models is therefore useful for studying physical dependence in rats or for studying the effects of stable (vs. cycling) BEL in rats over short or long-term exposure to ethanol.

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2197 EFFECTS OF d-AMPHETAMINE AND NALOXONE ON BRAIN-STIMULATION REWARD. Ralph U. Esposito, William Perry*, and Conan Kornetsky. Laboratory of Behavioral Pharmacology, Boston University School of Medicine, 80 E. Concord St., Boston, MA. 02118 and Hampshire College, Amherst, MA. 01002 - WP

Rats were stereotaxically implanted with bipolar electrodes aimed at either the medial forebrain bundle or the ventral tegmental area. Self-stimulation thresholds were determined by means of a modification of the psychophysical method of limits (Esposito, R., & Kornetsky, C., Science, 195:189, 1977). Reinforcement values were determined after the administration of d-amphetamine alone, naloxone alone and naloxone administered 2 minutes prior to the injection of d-amphetamine. d-Amphetamine yielded dose related decreases in the threshold (0.25 - 1.00 mg/kg, i.p.), while naloxone alone (2.0 - 16 mg/kg, i.p.) caused no consistent changes. For each animal, the dose of d-amphetamine that yielded the maximum threshold lowering effect was then selected to be administered with varying doses of naloxone. The results indicate that naloxone blocks the threshold lowering effect of d-amphetamine at doses as low as 2.0 or 4.0 mg/kg of naloxone. This finding suggests the possible involvement of an opiate receptor in the mediation of the reward enhancing action of d-amphetamine on brain-stimulation reward.

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2198 BRAIN WEIGHT, COCAINE AND SEIZURE SUSCEPTIBILITY. R. J. Fanelli, P. J. Donovick, R. G. Burright*, B. A. Symchowicz*, and A. N. Ritz. Dept. Psych., SUNY Binghamton, Binghamton, N.Y. 13901.

From the Binghamton heterogeneous (HET) stock of mice, Fuller selected three lines on the basis of their brain weight (high, medium and low). These lines, stabilized for some time now, have been shown to differ in both the rate of development of brain weight and behavior as assessed by a variety of tasks. One overriding theme is that the brain weight lines may differ in sensitivity to their environment. We therefore examined the susceptibility of these lines to seizures and how it might be affected by a single administration of cocaine. While seizure susceptibility and its interaction with drug sensitivity is a diffuse measure, it may nonetheless indicate differential cortical excitability.

Male and female mice of the three brain weight lines and HET strain were weaned (ca. 21 days of age) and injected with either saline or 5 or 10 mg/kg cocaine hydrochloride. Litters were distributed across the three dosage groups. Fifteen minutes after the mouse was injected a 3 ma transcorneal electroconvulsive shock was applied for 0.5 sec. We recorded both duration and severity of seizures. All testing was conducted between 10 a.m. and noon.

In saline injected groups HETs and low brain weight mice were most susceptible to seizures and high brain weight mice least. Medium brain weight line mice lay approximately half way between these two groups. Surprisingly, cocaine appeared to provide some protection against seizure in the more susceptible lines of mice (HETs and low brain weight). That is the higher the dose the less frequent the seizure. In contrast neither of the less susceptible lines (medium brain weight and high brain weight) showed clear changes in seizure susceptibility following cocaine administration.

While repeated administration of cocaine has been shown to increase seizure susceptibility, our results indicate that this is not true following a single administration at 21 days of age. Further, seizure sensitivity was clearly affected by genotype and may be critically related to stage of development.

2199 ALTERATIONS IN STRIATAL NEUROTRANSMITTER RECEPTOR BINDING FOLLOWING CHRONIC ADMINISTRATION OF PSYCHO ACTIVE AGENTS. J.W. Ferkany* and S.J. Enna. Dept. Pharmacol and of Neurobiol. & Anat., Univ. Texas Med. Sch., Houston, Tx. 77025

Numerous studies have indicated that chronic activation or blockade of neurotransmitter receptors may lead to alterations in receptor sensitivity. In a previous study we have reported that the chronic administration of inhibitors of 2-oxyglutarate aminotransferase (GABA-T), the enzyme responsible for the metabolism of GABA in brain, causes an increase in ^{3}H-spiroperidol binding, a decrease in ^{3}H-muscimol binding but no change in cholinergic muscarinic receptor binding in homogenates of rat corpus striatum (Fed. Proc. 38:747, 1979). Kinetic analysis of ligand binding indicated that these changes were due to alterations in receptor number and not receptor affinity. The increase in dopamine receptors correlated with an increase in stereotypic behavior following an apomorphine challenge in mice treated chronically with a GABA-T inhibitor. In the present investigation, studies were undertaken to further study neurotransmitter receptor interactions in this brain region following chronic administration of drugs. Rats were administered either aminooxyacetic acid (AOAA) (10 mg/kg, i.p.), atropine (At)(1 mg/kg i.p.) or AOAA (10 mg/kg i.p.) and At (1 mg/kg, i.p.) for 15 days, and 24 hr following the last injection the brains were removed, dissected into regions and receptor binding assayed using ^{3}H-ligand binding procedures. In agreement with previous results, AOAA caused an increase in ^{3}H-spiroperidol binding in the corpus striatum of treated animals. However, in the At and At and AOAA treated animals, ^{3}H-spiroperidol binding was significantly decreased (38%) with respect to control animals. These findings suggest a cholinergic influence on dopaminergic activity in this brain area. In additional studies, animals were treated chronically with ethanolamine-O-sulfate (EOS) (500 mg/kg i.p.), a peripheral inhibitor of GABA-T, muscimol (Mus) (0.6 mg/kg, i.p.) a direct acting GABA receptor agonist, or EOS (500 mg/kg, i.p.) plus Mus (0.6 mg/kg, i.p.). At the doses studied, neither GABA nor dopamine receptor binding were significantly altered in the corpus striatum following any of these treatments. This finding suggests that, at this dose, systemically administered muscimol may not act as a GABA receptor agonist in brain. These results indicate that neurotransmitter receptor binding studies may be useful for investigating neurotransmitter interactions in brain and may provide information with regard to the therapeutic potential and side effect liability of pharmacological agents. (Supported in part by USPHS grants NS-13803, MH-07688 and an RCDA NS-00335 (S.J.E.))

2200 GENETIC DIFFERENCES IN ^{3}H-SPIROPERIDOL BINDING IN CAUDATE NUCLEUS AND CATALEPTIC RESPONSE TO NEUROLEPTIC DRUGS IN INBRED MOUSE STRAINS WITH DIFFERENT NUMBERS OF MIDBRAIN DOPAMINE NEURONS J. Stephen Fink, Alissa Swerdloff, Tong H. Joh and Donald J. Reis. Laboratory of Neurobiology, Dept. Neurology, Cornell Univ. Medical College, New York, NY 10021.

Mice of the BALB/cJ strain have 25% more midbrain dopamine (DA) neurons than mice of the CBA/J strain. This difference is reflected in a correspondingly higher activity of tyrosine hydroxylase (TH), the rate-limiting enzyme for DA biosynthesis, in the caudate nucleus (CN) (Nature 264:654, 1976) and mesolimbic nuclei, and correlates with greater exploration and behavioral response to amphetamine (Neurosci. Abst. 4:492, 1978). We sought to determine whether these strains also differ in: (a) binding of a radiolabelled neuroleptic, ^{3}H-Spiroperidol (^{3}H-SPIRO), in the CN; and (b) the cataleptic response produced by neuroleptic drugs. ^{3}H-SPIRO binding was measured by the method of Creese and Snyder (Eur. J. Pharm. 49:201,1978) using 0.05-2.0 nM ^{3}H-SPIRO. In membrane preparations from CN the maximal number of ^{3}H-SPIRO binding sites determined by Scatchard analysis was 24% greater in BALB/cJ than in CBA/J mice (B_{max}: BALB/cJ=279 ± 4, CBA/J=224 ± 11 fmole/mg protein; n=15, $p<0.01$). There was no difference in K_d between the two strains (K_d: BALB/cJ=0.22 ± 0.05, CBA/J=0.22 ± 0.03 nM; n=15, $p>0.05$). Neuroleptic drugs of three chemical classes [butyrophenones (spiroperidol and haloperidol), phenothiazines (trifluoperazine) and diphenylbutylpiperidines (pimozide)] produced 3-20 times greater catalepsy in BALB/cJ than in CBA/J mice:

	Catalepsy Score (secs/300 min test)		
Drug	BALB/cJ (n=10)	CBA/J (n=10)	P
Spiroperidol (1 mg/kg)	849 ± 239	131 ± 85	<0.05
Haloperidol (4 mg/kg)	755 ± 292	39 ± 26	<0.05
Trifluoperazine (12 mg kg)	1139 ± 174	193 ± 65	<0.001
Pimozide (4 mg/kg)	881 ± 167	275 ± 166	<0.05

Time action curves for a given drug were similar between strains. A greater number of midbrain DA neurons and greater TH activity in CN correlates with a correspondingly greater number of ^{3}H-SPIRO binding sites and greater cataleptic response induced by several classes of neuroleptic drugs. Because ^{3}H-SPIRO binding probably labels a population of DA receptors, BALB/cJ mice may also have more DA receptors in CN. Genetically-determined differences in the number of midbrain DA neurons are associated with a greater number of neuroleptic receptors in the CN and may contribute to a greater cataleptic response to neuroleptic drugs. (Supported by NIH grants HL18974, MH24285, NS03346).

2201 COMPARISON OF CENTRAL BIOCHEMICAL AND BEHAVIORAL ACTION OF ETHANOL AND 1,3-BUTANEDIOL. G.D. Frye, R.A. Vogel, R.B. Mailman, M.G. Ondrusek, R.A. Mueller and G.R. Breese. University of North Carolina School of Medicine, Chapel Hill, North Carolina 27514.

The aliphatic alcohol, 1,3-butanediol (1,3-BD), like ethanol, has been reported to effectively suppress signs of ethanol withdrawal in the rat. Unlike ethanol, this action of 1,3-BD is reported to occur at doses which do not impair motor function in alcohol-naive animals (Majchrowicz et al., Science 194: 1181, 1976). The present studies were conducted to further evaluate the relative potencies of ethanol and 1,3-BD in several behavioral and biochemical tests sensitive to ethanol. During ethanol withdrawal, a dose of 1,3-BD as low as 1.0 g/kg reduced the severity of motor tremor and the frequency of audiogenically-induced seizures, while higher doses completely suppressed them. Impairment of the aerial righting reflex and decreases in cerebellar cyclic GMP were similar after doses of 1,3-BD and ethanol that were equivalent on a molar basis. In addition, 1,3-BD and ethanol both increased punished drinking in a conflict paradigm in which licking was suppressed in water deprived rats that received shock after every 20 licks. Whereas ethanol (1.0 - 2.0 g/kg) stimulated the locomotor activity of HA/ICR female mice, no stimulatory effect of 1,3-BD was seen over doses ranging from 1.0 - 10.0 g/kg. Withdrawal of 1,3-BD administered to rats for 12 days via a liquid diet vehicle (used to produce physical dependence on ethanol) resulted in severe motor tremor in all animals and susceptibility to audiogenically-induced seizures in 8 of 10 rats. Thus these studies indicate that 1,3-BD shares many pharmacological properties with ethanol, including the ability to induce an ethanol-like withdrawal syndrome. (Supported by USPHS Grants AA-02334 and AA-05047 and funds from the Alcohol Research Authority.)

2202 ALTERATION OF THE DISCRIMINATIVE STIMULUS PROPERTIES OF ETHANOL DURING CHRONIC ETHANOL TREATMENT. S. Gibbs* and H.L. Altshuler. TRIMS and Baylor College of Medicine, Texas Medical Center, Houston, TX 77030

This study evaluated the effects of chronic ethanol (ETOH) administration on the discriminative stimulus properties of ETOH in rats trained to perform a double lever operant drug discrimination task. Twenty-five male Sprague-Dawley rats ranging in weight from 240 to 260 gm were used in these studies. These were reduced to 80 per cent of free feeding weight and gradually shaped to perform operant responses for food reinforcements under a variety of schedules culminating in the performance of the double lever choice paradigm during a DRL-10 schedule of reinforcement. The animals were then shaped to preferentially respond on one of the levers when they had been pretreated with ETOH, 1.0 gm, Kg, IP, 15 min. prior to testing. Criteria for correct responding in this portion of the shaping schedule was that the group mean ETOH correct lever responses was at least 85% of total responses. Following the acquisition of this behavior at the ETOH training dose was gradually reduced from 1.0 gm/Kg, IP to 0.75 gm/Kg, IP during a 30 day period. Following acquisition of the 0.75 gm/Kg discrimination, a dose response curve was generated to establish the generalization of correct discriminations across a range of ETOH doses from 0.5 gm/Kg-1.5 gm/Kg. The animals were divided into three groups, Group A received saline chronically. Group B, ETOH, 2.5 gm/Kg. three times daily and Group C ETOH 5 gm/Kg, 3 times daily. Drug was administered by intragastric intubation, 7 days per week. The animals were tested every ten days to assess levels of correct responses after saline or ETOH (0.75 gm/Kg, IP) pretreatment. Chronic ETOH administration continued for sixty days and dose-response, generalization curves were established after 30 days and 60 days. These experiments demonstrated that chronic ETOH administration produced significant reductions in the discriminative stimulus properties of ETOH at all points in the dose response curve, producing a highly significant shift to the right of the curve. The differences between the two groups were significant. No significant changes in correct responding were noted in the saline control group. These data demonstrate that the chronic administration of ethanol to rats trained to perform a double lever operant task produced dose related alterations in the discriminative stimulus properties of ethanol, suggesting that the development of tolerance to ethanol is associated with reduction in the animal's perception of the drug's effects.

2203 THE SITE SPECIFIC EFFECTS OF NALOXONE ON INTRACRANIAL SELF-STIMULATION RATES IN THE RAT. F. Gimino* R. Farrell*, A. Tempel*, S.S. Steiner, and S.J. Ellman. Dept. Psych., City College, City University, N.Y. 10031.

Male sprague-Dawley rats were stereotaxically implanted with bipolar electrodes aimed at various self-stimulation sites. After recovery from surgery, all rats were shaped to respond for .1 msec. monophasic rectangular pulse-pairs of intracranial stimulation (ICS) at a variety of current levels (150-500 microamps). The first pulse in a pair (C-conditioning pulse) was followed by a second (T-test pulse) which was parametrically varied in time (.5-5.0 msec.). A condition in which the T-pulse was omitted was also randomly presented with these values in a latin square design.

All subjects were then tested until ICS response rates were stable. These animals were then entered into a drug paradigm of 3 days .9% saline followed by a fourth injection day of naloxone hydrochloride. The dosage of naloxone to be administered (1.0,10.0, or 40 mg./kg.) was determined by a counterbalanced design. This sequence of naloxone and saline was repeated until all animals completed three tests at each dose level. All subjects were then given three more days of saline followed by a fourth injection day of morphine at 1.25 mg./kg.

Naloxone was found to differentially affect ICS response rates. Most subjects demonstrated response suppression at the higher doses (10 mg.,40 mg) and at the middle range of C-T intervals (.5-2.5 msec) In other subjects,response rates were either enhanced or remained the same under naloxone. Thus far, subjects showing a response suppression have enhanced their response rates under morphine, while subjects showing no naloxone induced rate suppressions have evidenced no effect to morphine. The data also suggest some evidence for a reverse tolerance to naloxone (progressive sensitivity) from some sites.

2204 ORAL DYSKINESIA IN BRAIN-DAMAGED RATS WITHDRAWN FROM ANTIPSYCHOTIC DRUGS. Robert B. Glassman and Harriet N. Glassman*. Dept. Psychol., Lake Forest College, Lake Forest, IL 60045

Many schizophrenic patients who are maintained on antipsychotic medication (neuroleptics) eventually develop a syndrome called tardive dyskinesia, which consists primarily of active movements of lips, jaw, and tongue (Tarsy & Baldessarini, Biol. Psychiat., 1977). While the use of neuroleptics is widely considered indispensable, this iatrogenic disorder is recognized as a major problem. Animal models may help to understand it.

To date, schizophrenia has not been shown to be associated uniformly with a specific, gross brain pathology but some symptoms of schizophrenia resemble those of frontal cortical damage (Glassman, Behav. Sci., 1976). For this and other reasons based on known loci of action of neuroleptics, it was hypothesized that frontal damage would predispose an organism to drug-induced dyskinesia. Three groups of rats were maintained chronically on chlorpromazine (0.5 mg/ml in drinking water) for six weeks; (a) frontally damaged, (b) occipitally damaged controls, and (c) unoperated controls. Numbers of rats surviving in each group were 7, 4, and 5, respectively. Eight additional operated and unoperated rats were kept undrugged for an equal amount of time. Before and after withdrawal each rat was observed for 5-minute periods during which tabulations were made of occurrences of chewing and of other behaviors.

The frontally damaged, drugged rats showed more vacuous chewing on withdrawal than did any other group. The mean (and range) numbers of occurrences of chewing were: frontal 8.6 (1-23); occipital, 1.0 (0-2); unoperated 1.6 (0-3); undrugged, 0.3 (0-1). A t-test comparing frontals with the combined occipitals and unoperated drugged groups yielded $p < .05$. The same differences were obtained on retesting, by an observer who did not know to which group each animal belonged. Additional observations showed the dyskinesias to last for at least a month after withdrawal. The effect was not changed significantly by a subsequent reintroduction of chlorpromazine for six weeks, at the same dose, nor by a later six week course of treatment with haloperidol (0.02 mg/ml in the drinking water). Other behavioral measures (e.g., grooming, walking, rearing, shaking) did not show an effect of drug treatment.

The results suggest that oral dyskinesia is due to release of parts of the nervous system, that control certain fragments of behavior, from higher level organization. Frontal cortical damage and chronic neuroleptic treatment, which is thought to disrupt striatal function, combine to yield this effect.

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2205 EVALUATION OF FLUNITRAZEPAM, AMITRIPTYLINE, MAPROTILINE, CLOZAPINE AND PROPRANOLOL IN A SQUIRREL MONKEY CONFLICT PROCEDURE. Fred S. Grodsky* and Jerry Sepinwall. Dept. Pharmacol., Hoffmann-La Roche Inc., Nutley, New Jersey 07110

We have previously used a sensitive behavioral procedure to define the antipunishment (anticonflict) effects of benzodiazepine antianxiety agents (JPET 204: 88, 1978) and of barbiturates (Fed. Proc. 37: 617, 1978). In the present experiments, the benzodiazepine compound, flunitrazepam (FLNTZ), the antidepressant agents, amitriptyline (AMIT) and maprotiline (MAPR), and the antipsychotic agent, clozapine (CLOZ), were studied. In addition, the ß-adrenergic antagonist propranolol (PROP), which has been claimed to have some antianxiety activity clinically, was studied alone and in combination with diazepam (DIAZ) or chlordiazepoxide (CDAP).

Compounds were administered by intragastric tube (three to eight monkeys per dose level) 30 min. before a 90 min. test session to twenty-one squirrel monkeys working on a food-maintained two-lever concurrent VI 1.5' VI 6' schedule. Responses in the VI 1.5' component were also punished intermittently (VR 24) with footshock.

Like other benzodiazepines, FLNTZ increased responding in the punished component (Min. Effect. Dose = 0.04 mg/kg), but was 8 and 16 times more potent than DIAZ and CDAP, respectively; however, FLNTZ was effective over a narrower range of doses. In agreement with its clinical efficacy as an hypnotic agent, FLNTZ at 0.62 mg/kg appeared to induce sleep rapidly in 5 out of 6 monkeys. When retested 24 hrs. later, these animals had recovered to baseline levels of performance. CLOZ exhibited significant anticonflict activity of a low magnitude within a limited dose range (1.25 to 2.5 mg/kg). At 5 and 10 mg/kg, dose-related depression of both punished and unpunished responding was seen; in some monkeys these effects persisted for at least 24 hours.

Neither AMIT (0.62 to 10 mg/kg) nor MAPR (2.5 to 20 mg/kg) showed any anticonflict activity. At 10 mg/kg AMIT decreased both punished and unpunished responding although no overt symptoms of sedation were seen. At 20 mg/kg, MAPR decreased responding in some monkeys and produced copious salivation. Thus, neither antidepressant agent exhibited antianxiety-like activity in this test up to doses that produced side-effects.

PROP (1.25 to 40 mg/kg) showed no anticonflict activity; depressed responding and salivation were observed at 40 mg/kg. Nor did PROP (10 mg/kg) show any additive or potentiative effects when it was given in combination with either 2.5 mg/kg of DIAZ or CDAP. These results are consistent with previous animal experiments suggesting that the antianxiety effects of propranolol, if real, are of a different kind than those of benzodiazepines.

2206 GENERALIZATION OF MORPHINE AND PENTAZOCINE TO THE APOMORPHINE DISCRIMINATIVE STIMULUS COMPLEX: DOSE-RESPONSE CURVES. Linda L. Hernández, Alice M. Holohean* and James B. Appel. Behavioral Pharmacology Lab., Dept. of Psychology, U. of South Carolina, Columbia, SC, 29208, USA.

In rats trained to discriminate intraperitoneal doses of apomorphine (0.25 mg/kg) from saline in a two-lever bar-pressing task, both morphine (1.25-5.0 mg/kg, i.p.) and pentazocine (2.50-15.0 mg/kg, i.p.) produce dose-dependent responding on the lever associated with apomorphine. However, morphine is two to three times more potent than pentazocine (ED_{50}'s = 2.76 and 7.55 mg/kg, respectively), and the (log_{10}) dose-response curves for the two opiates differ in form. The dose-response curve for morphine is steep (slope = 132.1 percent per log dose), linear, and reaches 97% apomorphine-lever responding following a dose of 5.0 mg/kg of morphine. The dose-response curve for pentazocine is less steep than that for morphine (slope = 90.5), linear up to a dose of 10.0 mg/kg, but reaches a maximum of only 63% apomorphine-lever responding. Increasing the dose of pentazocine beyond 10.0 mg/kg produces no further increase in bar-pressing on the apomorphine-appropriate lever, but produces a decrease in the number of animals responding. The results replicate and extend our previous report of generalization of the morphine stimulus complex to the apomorphine cue (PB&B, 1978, 9: 459) and suggest that morphine and pentazocine differ with respect to the dopaminergic components of their stimulus complexes.

2207 EFFECTS OF PRENATAL AND/OR EARLY POSTNATAL EXPOSURE TO ETHANOL ON OFFSPRING OF RATS. Joan A. Holloway and Walter N. Tapp*. Dept. Psychiatry & Behav. Sci., Univ. Okla. Health Sci. Ctr., P.O. Box 26190, Oklahoma City, OK 73190.

Nutritionally matched pregnant female rats received ethanol or sucrose in liquid diets from the third or 15th day of gestation. Another group received ad lib lab chow and water. Litters were reduced to eight and cross-fostered on the third day after birth. Thus, there were groups whose mothers had ethanol diets during gestation and lactation, during gestation only, or during lactation only. Animals born to mothers receiving the ethanol diet or the sucrose diet were smaller and lighter than the offspring of the ad lib lab chow mothers. Developmental patterns of activity indicated all groups except the ad lib lab chow group showed abnormal development, and no increased activity in the experimental offspring was found. The weight and activity effects are explainable as malnutrition effects or as being due to the confounding of ethanol exposure and malnutrition. Offspring of animals receiving the ethanol diet showed an increased ethanol preference at 28 days of age, an effect not explainable by malnutrition effects. At 70 days of age all groups showed reduced ethanol preference, especially those whose mothers received ethanol diets. Additionally, males showed a greater reduction in ethanol preference than did females.

2208 REVERSIBILITY OF TARDIVE DYSKINESIA SYMPTOM. Chuong C. Huang. Department of Psychiatry and Mental Health Sciences, Medical College of Wisconsin, Milwaukee, WI. 53226

In a double blind controlled study of 30 Tardive Dyskinesia (T.D.) patients, alpha-methyldopa at doses of 750 to 1500 mg daily and Reserpine at doses of 0.75 to 1.5 mg daily provided statistical significant improvement of the symptomatology of T.D. in comparison to that of placebo. The results are compatible with the "postsynaptic receptor hypersensitivity" theory. It is therefore believed that the T.D. symptom is a functional disorder, not a structure change and should be eliminated completely by desensitization processes. To test this hypothesis, it is necessary to have a long term follow up study. The author had previously proposed a gradual desensitization method as the treatment of T.D. which includes reduction or discontinuation of neuroleptics, discontinuation of antiparkinson drugs and desensitization with reserpine. Six chronic T.D. patients (2 female and 4 male) who had had persistent, severe T.D. symptoms for 3 to 10 years were treated with this method. The treatment period lasted for 4 months to one year and 5 months. They had shown remarkable improvement of their symtoms--2 cases had complete recovery, 4 cases had minimal involuntary tongue movement remained. The tongue movement is always the intractable and last symptom to be eliminated. Four other subacute T.D. patients who had had T.D. symptoms for one month to 3 months had improved completely, simply by discontinuation of causative agents, neuroleptics and antiparkinson drugs. The treatment period lasted for one month to 12 months. In conclusion, the T.D. symtom is a functional disorder and is reversible. The results of this study are contrary to the general belief that T.D. symptoms become irreversible if they have lasted for more than six months. This study reaffirms the postsynaptic receptor hypersensitivity theory. However the remaining intractable minimal involuntary tongue movement could be due to a structure change. Further study is necessary to answer this question.

2209 COMPARISON OF BEHAVIORAL EFFECTS OF 6-HYDROXYDOPAMINE, RESERPINE, AND TETRABENAZINE. C. W. Hughes, S. H. Preskorn, B. K. Hartman, and P. Veit*. Depts. of Psychiatry, Washington Univ. School of Medicine, St. Louis, MO, and Dept. Psychology, Univ. Missouri - Rolla, MO 65401.

Motor deactivation has been postulated to occur as a result of norepinephrine depletion (e.g., Weiss, J. M., et al., Animal Models in Human Psychobiology, Plenum Press, 1974). To test this hypothesis, three different ways of depleting norepinephrine centrally were used. This approach also permits assessment for non-adrenergic effects. Adult rats were compared on a battery of behavioral tests following stereotaxic administration of 125 µg of six hydroxydopamine (6-OHDA) injected intraventricularly in 10 µl of artificial cerebrospinal fluid (csf) over a five minute period. Sham-treated animals received an equal volume of the artificial csf only. Additional behavioral comparisons included groups of rats that received intraperitoneal injections of tetrabenazine, reserpine (2.5 & 5.0 mg/kg), or saline. The behavioral effects of these latter two drugs are often related to their depletion of catecholamines; however, they also have other CNS effects.

If a behavioral effect is mediated by depletion of norepinephrine specifically, then the effect should be observed with all three treatments. Whereas behavioral measures of shuttle-avoidance, open field, and swimming performance were all severely impaired by tetrabenazine and reserpine, the only effect found for the 6-OHDA treated animals was a small decrease in the total amount of open field activity. Although immunohistochemistry indicated that our 6-OHDA treatment resulted in a fairly specific depletion of noradrenergic fibers with little damage, if any, to surrounding tissues or other fiber systems, our behavioral data and finding of no body weight differences in 6-OHDA treated and sham animals (measured over a 45 day period) bring into question the role of norepinephrine depletion in behavior. Previous studies of 6-OHDA-induced deficits in behavior may be due to the nonspecific cytotoxic effects of 6-OHDA on brain tissue. Similiarly, pharmacologic studies reporting motor deactivation due to norepinephrine depletion with agents such as tetrabenazine and reserpine may be a result of drug effects on other central systems.

2210 DISRUPTIONS IN THE SLEEP-WAKE CYCLE OF MICE CAUSED BY N,N-DIMETHYLTRYPTAMINE. P.R. Hyde*, L.J. Bearden, D.L. Weiler* and G.V. Pegram. Neurosciences Program, Univ. Ala. in Birmingham, Birmingham, Ala. 35294.

N,N-Dimethyltryptamine (DMT) is an endogenous compound which produces behavioral disruptions in animals, and hallucinations in humans. Most of the attention regarding the neural role of DMT has focused on the possibility that DMT might precipitate or accentuate various psychiatric disorders. Recent work indicates that there are specific recognition sites for DMT on synaptic plasma membranes (Rosengarten and Friedhoff, Neurosci. Abst. 4:519, 1978; Bearden, et al., Neurosci. Abst. 4:419, 1978), and that the conversion of DMT to inactive metabolites in vitro is accelerated by nicotinamide (Barker et al., in preparation), a compound which exerts benzodiazepine-like effects in vivo (Möhler, et al., Nature 278:563, 1979). These findings could be interpreted as additional evidence suggesting the involvement of DMT in behavioral disorders. To examine further the behavioral effects of DMT, we have studied the effects of this compound on the sleep-wake cycle of mice.

Mice were implanted with chronic cortical electrodes, and following recovery, were given either no treatment, saline (i.p.) DMT (10 or 20 mg/kg; i.p.), or 1,5-diazabicyclo (4.3.0)non-5-ene (DBN; 300 mg/kg, orally; an indoleamine-N-methyltransferase inhibitor). Fronto-parietal electrocorticograms were recorded for seven hour periods (900-1600 hrs.), and scored for awake, slow wave sleep (SWS) and rapid eye movement (REM) sleep. DMT given at either 10 or 20 mg/kg produced a transient increase in total awake time, and a decrease in both SWS time, and in REM sleep time as a percentage of total sleep time. These effects were dose dependent, and disappeared within three hours, with the sleep pattern returning simultaneously to that seen in controls. Inhibition of the synthesis of DMT by DBN produced no observable changes in the sleep-wake pattern of these animals. These results show that DMT administered i.p. produces a short-term disruption in the normal sleep-wake activity of mice. Other workers have observed that LSD, tryptamine and other tryptamine analogs exert similar effects on sleep-wake activity.

2211 MULTIPLE NALOXONE EFFECTS IN VIVO IN MICE. Yasuko F. Jacquet and Michael Jimenez*. Center for Neurochemistry, Rockland Research Institute, Ward's Island, New York City, NY 10035.

Swiss Webster male naive mice (n = 48) were randomly assigned to one of 4 groups and given intraperitoneal (i.p.) injections of either saline or naloxone hydrochloride (1-,10- or 100-mg/kg in a volume of 0.005 cc/gm body weight) twice daily for 4 days. Following each injection, the mice were placed, 4 cagemates together, in an unfamiliar glass jar (30.5 cm in diameter, 61 cm high) and their behavior observed for 30 min. The saline group showed an unexpectedly high incidence of hyperactivity and jumping behavior (mean jumps = 17.49 ± 2.91). Additional control groups of mice (n = 4 ea) given either i.p. needle puncture of no needle puncture prior to being placed in the glass jar also showed a high incidence of this hyperactivity and jumping behavior. This suggested that this behavioral syndrome was due to an "emotional" reaction to the unfamiliar environment.

Naloxone-treated mice showed a dose-dependent reduction in this behavior. At the highest dose (100-mg/kg), naloxone induced sleep in mice, the sleep occurring within 2-3 min, and lasting for the 30-min observation period. Otherwise, there were no detectable adverse effects on mice receiving this high dose of naloxone twice daily for this period. The inactive enantiomer, (+)-naloxone, at 100-mg/kg, resulted in behavior no different from the saline group.

Mice injected with morphine sulphate (25- or 50-mg/kg) or etorphine hydrochloride (0.5-mg/kg) showed a typical opiate syndrome characterized by Straub tail and compulsive, robot-like ambulation around the perimeter of the glass jar. These opiate-injected mice never showed jumping behavior. The opiate behavioral syndrome was fully reversed by naloxone at 1-mg/kg.

These results emphasize the strikingly different responses to opiates by different species (as noted previously by other workers).

The present results suggest that naloxone in vivo in mice have two distinct effects: (1) It antagonized the behavioral effects of opiates at low doses; (2) It inhibited behavior arising from the "emotional" effects of being placed in an unfamiliar environment. This latter suppression required higher doses of naloxone, being only partially effective at the low dose. At the highest dose (100-mg/kg), this "sedative" action of naloxone resulted in inducing sleep, an effect not previously reported for naloxone. Both actions of naloxone were stereospecific, occurring only with the (-)-enantiomer, and not with the (+)-enantiomer.

2212 REDUCED PHYSIOLOGICAL AND SUBJECTIVE EFFECTS AFTER REPEATED ADMINISTRATION OF COCAINE IN HUMANS. J. I. Javaid, M. W. Fischman*, J. M. Davis* and C. R. Schuster*. Illinois State Psychiatric Institute, IL 60612 and Department of Psychiatry, University of Chicago, IL 60637.

Cocaine is a potent central nervous system stimulant and local anesthetic. Although, it is believed that cocaine use produces psychological dependence, most studies suggest that there is no tolerence developed to any of cocaine effects. In fact, it has been suggested that repeated use of cocaine devlops supersensitivity to its behavioral and physiological effects.

In this study, cocaine HCl was administered intranasally followed by intravenous injection. Eight volunteers, who had given their informed consent, were included in the study. Each received, either 4 mg or 96 mg of cocaine HCl, intranasally. Sixty minutes later, either 16 mg, 32 mg or 48 mg of cocaine were given intravenously. Blood was withdrawn for cocaine determinations prior to the drug administration and at different times after drug administration. Physiological measures were monitered continuously and subjective effects of the drug were measured by the stimulant sections of the Addictive Research Center Inventory (ARCI) questionnaire and the Profile of Mood Scales (POMS).

The plasma concentrations of cocaine were always related to dose administered. This was not true for changes in heart rate and subjective effects. When intavenous cocaine was given after 96 mg intranasal administration the increase in heart rate was not as great as when an intravenous injection followed 4 mg inhalation. Comparable changes were obtained in subjective effects. The heart rate changes were plotted against plasma concentrations of cocaine in a scattered diagram. When an intravenous injection was given after high dose of intranasal cocaine the slope of the line fitted to the data was smaller than the slope obtained when an intravenous injection followed low dose inhalation. These results suggest that there is a decrease in physiological and subjective effects of cocaine when administered repeatedly in humans. (Supported in part by PHS grant DA01491)

2213 DECREASED LOCOMOTION IN THE OPEN FIELD AND AFTER AMPHETAMINE IN RATS WITH UNILATERAL DENERVATION OF DOPAMINERGIC MESOLIMBICO-CORTICAL TERMINAL FIELDS. D. Jeste* and G.P. Smith. Dept. Psychiatry, Cornell Univ. Med. College, New York and NIMH Lab. of Clin. Psychopharmacol., Saint Elizabeth's Hospital, Washington, D.C.

Severe bilateral denervation occurs in all dopaminergic (DA) mesolimbicocortical terminal fields after bilateral injections of 6-hydroxydopamine (6-OHDA) into the anterolateral hypothalamus (Fink, Greenfield and Smith, Neuroscience Abstract 660, 1976). Such denervation has been correlated with abolition of the locomotor response to amphetamine (Ervin et al, Brain Res. 132:507, 1977) and with decreased activity in the open field (Fink et al, 1976). To determine if bilateral denervation is necessary to produce these behavioral deficits, we injected 6-OHDA into right (n=4) or left (n=4) anterolateral hypothalamus (A7.0, L2.0, 8.0 mm down according to de Groot). Vehicle injections were also made into the right (n=4) or left (n=3) anterolateral hypothalamic site. 6-OHDA damage of ascending NE fibers was prevented by pretreatment with DMI. Behavioral testing began 15 days after surgery. Rats were tested in the open field (OF) for 5 min on 3 consecutive days. 6-OHDA rats traversed less than half the number of squares vehicle rats traversed on all 3 days ($p < .05$ on all 3 days). Right and left 6-OHDA injections produced the same results. After OF testing was completed, 6-OHDA and vehicle rats were injected with d-amphetamine (1.5 mg/kg) and the locomotor response was recorded by interruptions of photocell beams for 2 hours. The locomotor response of 6-OHDA rats was about 50% of the locomotor response of vehicle rats ($p \ll .05$). No circling was observed and right and left 6-OHDA injections produced the same results.

The significant decrease in activity in the OF and in response to amphetamine observed in unilateral 6-OHDA rats demonstrates that bilateral denervation of any one mesolimbicocortical DA terminal field is not required for significant decreases in response to novel stimuli of an OF and to a low dose of amphetamine. The fact that unilateral 6-OHDA rats responded significantly more to amphetamine than bilateral 6-OHDA rats suggests that the extent of mesolimbicocortical DA denervation correlates with the magnitude of the decrease of the amphetamine response. All the results are consistent with an arousal function of the mesolimbicocortical DA system for locomotor responses to a novel OF and to a low dose of amphetamine.

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2214 EFFECTS OF NEONATAL LEAD EXPOSURE ON SPONTANEOUS ALTERNATION PERFORMANCE IN RATS.

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One frequent consequence of subacute lead toxicity is hyperactivity, usually viewed as a result of diminished ability to inhibit responding and/or lack of habituation to environmental stimuli. Spontaneous alternation performance is a behavioral measure which could be used to assess this effect. Alternation performance is thought to be a result of habituation to the cues of the maze arm most recently visited, leading to exploration of the relatively novel stimuli of the arm least recently visited. Alternation rates of rats normally average 70-75%. The apparatus used for these measurements is a symmetrical Y maze. The sequence of arm entries as well as the total number of entries is noted, and a percentage alternation score is calculated. An alternation response is defined as the animal's leaving one arm of the maze and entering the least recently visited of the other two.

Accordingly, the effect of neonatal lead exposure on spontaneous alternation performance in mature albino rats (N=16) was studied. The lead was delivered orally in a concentration of 225 mg/kg body wt, from day 1 following birth through day 20. The lead solutions were made up each day so that the appropriate dosage of lead was delivered in a .0025 ml/kg volume of distilled water. Beginning on day 70, the animals were tested daily in 15 minute sessions. After completion of the testing program, the animals were sacrificed and the brains assayed for lead by atomic absorption spectrophotometry. Although the brain lead levels of the experimental animals did not differ from those of controls, both behavioral measures revealed statistically significant differences between lead and non-lead animals, the lead animals exhibiting lower rates of alternation ($p<0.05$) and higher total arm entries ($p<0.05$).

In addition, although littermates were assigned randomly to treatment conditions, hierarchal analysis of the data revealed a significant litter effect ($p<0.05$) on the number of arms entered. This litter effect, which is presumably a result of environmental influences, should be of consideration in any behavioral investigation. It appears that differences in mothering by the dam and other neonatal variables produce relatively long-lasting behavioral effects.

2215 ENHANCED ACUTE TOLERANCE IN RATS TREATED CHRONICALLY WITH OPIATES. H. Kalant* and R.F. Mucha* (SPON: K.E. Livingston). Dept. Pharmacol., University of Toronto, and Addiction Research Foundation, Toronto, Ontario, Canada M5S 2S1.

Tolerance occurs in naive subjects during the action of a single opiate injection. The present study suggests that such acute tolerance is important for the understanding of chronic tolerance.

In Expt. 1, male Wistar rats treated with morphine-SO4 (MS) for 49 days (0, 20 or 200 mg/kg maintenance doses) were tested for analgesia (tail immersion test, 48°C water) after i.p. etorphine-HCl (5-160 µg/kg for 20 and 200 MS groups). Tests at 0.5 hr intervals showed faster within-session loss of analgesia in chronically tolerant than non-tolerant rats. In Expt. 2 these effects were replicated in similarly-treated rats after injection of etorphine into the lateral ventricle (0.1-5 µg, 0.24-4 µg, and 0.6-10 µg/rat for 0, 20 and 200 mg/kg MS groups respectively).

The rapid acute tolerance was not due to repeated testing. Rats treated chronically with MS, 20 and 200 mg/kg, received either saline or MS (10 mg/kg i.v.) pretreatment, followed 1 hr later by i.v. injection of 25 or 50 mg/kg MS and a single tail-flick test 2 min later. Latencies were 50% lower in the MS pre-treated than in saline pre-treated rats. In contrast, MS pre-treatment in chronic saline controls increased the latencies slightly. Similarly, pretreatment with MS (30 µg into the III ventricle), in rats treated chronically with etorphine (200 µg/kg twice daily), reduced the analgesia at 30 min after a test dose of etorphine (200 µg/kg i.p.).

Thus, a very rapid acute tolerance was found in chronically opiate-treated rats. Earlier, we reported (Psychopharmacology, 1978, 60, 59-65) similar findings in ethanol experiments. We suggest that chronic tolerance is an enhancement of acute tolerance. This effect may be critical for estimating treatment-produced changes in opiate sensitivity; log dose/response (LDR) curves for data from Expt. 1 and 2 indicated a tolerance-related shift to the right at short test intervals and shift plus flattening at intermediate intervals. In addition, the nature of the acute tolerance suggested that it could be used to localize sites in the brain responsible for high levels of tolerance to systemically administered opiates. Plugging the cerebral aqueduct with Nivea cream attenuated acute tolerance produced by 30 µg MS injected into the III ventricle in chronic etorphine-treated rats. This attenuation was not seen when MS was injected into the IV ventricle.

2216 ROLE OF THE PERIAQUADUCTAL GRAY IN THE DISCRIMINATIVE STIMULUS PROPERTIES OF METHODONE. M. Kallman, G. Krynock* and J. Rosecrans. Dept. of Pharmacol., Med. Col. of Va., Richmond, VA 23298.

Rats, trained to discriminate methodone injections (1.5 mg/kg, s.c.) from saline injections on a standard two-bar operant task, were assessed for generalization to central administration of methodone and morphine at the site of the periaquaductal gray (PAG). Initially, animals were shaped to respond on one lever following methodone injections (1.5 mg/kg) and on the opposite lever following saline (1 ml/kg) injections during the 15 min. daily sessions. Reinforcement was delivered on a variable interval (VI)-15 sec. schedule for correct-lever responding (0.1 ml sweetened milk). Training continued until discrimination of each drug state was reliable when assessed during 2½ min. extinction sessions. Extinction sessions for generalization testing occurred once a week with continued training under both drug states between generalization tests. Subsequent to discrimination training, animals were anesthetized (40 mg/kg Nembutal) and implanted with stainless steel (24 ga.) cannulae in the PAG for direct drug injection. Surgical recovery and restabilization on the VI-15 sec. schedule were followed by testing for generalization to the methodone cue. PAG injections were accomplished by inserting a 31 ga. needle which extended 1 mm beyond the guide cannulae. All drug doses tested were suspended in a total volume of 1 µl saline for injection. Both dose response and time course data were collected following the direct application of morphine and methodone at the site of the PAG.

Peripheral injections of methodone produced 89.1 ± 6.7% drug-lever responding and saline injections produced 5.1 ± 3.2% drug-lever responding prior to generalization testing at the PAG site. The peripherally administered methodone training cue could not be mimicked by PAG injections of methodone (0, 10, 20 or 40 µg/1 µl) at any of the time parameters examined (0, 15 or 30 min. postinjection). Although the PAG was insensitive to methodone, direct morphine application at the site of the PAG did generalize to the methodone training cue (1.5 mg/kg) at a mean dose of 3.8 ± 1.9 µg. These findings suggest that morphine and methodone produce similar discriminable cues but the role of the PAG in the methodone cue is not clear. The PAG has been implicated in the action of morphine and in the discriminable cue of morphine but the discriminable cue of methodone may be dependent on other central sites. (Supported by U.S.P.H.S. grant #DA-00296-05).

2217 DIETARY TRYPTOPHAN MODULATION AND AGGRESSIVE BEHAVIOR IN MICE. Kathleen M. Kantak, Linda R. Hegstrand and Burr Eichelman. Univ. of Wisconsin and William S. Middleton Veterans Medical Center, Madison, WI 53706.

The effects of a tryptophan-free diet on isolation-induced fighting and predatory cricket killing in mice were examined. The results demonstrated that consumption of a tryptophan-free diet for 18 days 1) decreased both the number of fighters and duration of isolation-induced fighting; 2) increased the number of cricket-killing mice and decreased the latencies to attack and to kill crickets; 3) reduced brain serotonin 27%; 4) increased water intake 38%; and 5) decreased body weight 27%, without affecting food intake. To determine if these effects were due specifically to the lack of dietary tryptophan, other groups of mice were fed a 5% tryptophan load in the standard chow; a 0.15% tryptophan supplement in the tryptophan-free diet; or a 3 grams/day calorically-restricted chow diet. The lack of tyrptophan in the diet specifically produced the almost total inhibition in isolation-induced fighting, the reduction in brain serotonin, and the large decrease in body weight. The other non-specific effects appeared to be related to general factors such as dietary need for the cricket killing or diet composition for the water intake.

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2218 LITHIUM DECREASES SELF-STIMULATION AND ESCAPE IN RATS INDEPENDENT OF LOCOMOTOR ACTIVITY. Nilda M. Keene, James J. Keene and Brenda Doble*. Dept. Psychiat. & Dept. Physiol., Sch. Med., Univ. Puerto Rico, San Juan, PR 00936.

The effects of lithium chloride (2 meq./kg.) on self-stimulation and escape behavior elicited by intracranial electrical stimulation of the medial forebrain bundle and the midbrain reticular formation respectively, were tested in chronically implanted rats. In the same test periods, locomotor activity was also measured. A "tilt-box" method allowed the animals to control the amount of brain stimulation (0.25 sec, 100 Hz trains of cathodal pulses delivered at 1 Hz) by its position in the box. Experimental tests began with two hours of pre-drug performance to establish baseline measures for self-stimulation (or escape) and locomotor activity level during the test. Stimulation current was set to elicit moderate performance (40 - 300 uA). Post-drug performance was followed over up to four hours. Throughout, data was tabulated at 8 min. intervals. In 7 experiments (4 rats), pre-drug self-stimulation increased activity compared to periods without brain stimulation; and lithium caused both self-stimulation and activity to decrease. In 7 escape experiments (4 rats), lithium decreased escape although locomotor activity was not changed. At higher stimulus currents eliciting better performance, both self-stimulation and escape were less affected by this dose of lithium. Thus, lithium may be acting to a significant extent directly on affective mechanisms of the brain, and may not be merely sedative in its action, since escape decreased while locomotor activity did not. Supported by NIH Grant RR-08102.

2219 BEHAVIORAL EFFECTS OF LESIONS TO THE MESOLIMBIC DOPAMINE NEURONS. G.F. Koob, L. Stinus, M. Le Moal, D.C.S. Roberts, and F.E. Bloom.. A.V. Davis Ctr. for Behav. Neurobiol., Salk Inst., La Jolla, CA 92037 and Laboratoire de Neurobiologie des Comportements, Universite de Bordeaux, Talence, France.

Previous work has established that 6-hydroxydopamine (6-OHDA) lesions to the nucleus accumbens septi (NAS) produce transient hypoactivity and block the increase in locomotor activity induced by d-amphetamine (AMPH), whereas radio-frequency (RF) or 6-OHDA lesions to the ventral tegmental area (VTA) produce a significant hyperactivity and fail to block AMPH induced locomotor activity. An attempt was made in the present study to determine the substrate for these differential effects. Rats pretreated with pargyline (50 mg/kg) were injected intracerebrally with 6-OHDA dissolved in saline containing 0.2 mg/ml of ascorbic acid. Animals were divided into 8 lesion groups: Sham-operated, 6-OHDA (8ug/2ul of vehicle)-medial NAS, 6-OHDA (8ug/2ul)-posterior NAS, 6-OHDA (2ug/1ul)-VTA, 6-OHDA (3ug/1ul)-VTA, 6-OHDA (4ug/1ul)-VTA, RF-VTA, and RF-VTA with 6-OHDA (8ug/2ul)-medial NAS. All rats were tested for nocturnal activity on day 8 post-lesion, their locomotor response to 1.5 mg/kg AMPH i.p. on day 13 post-lesion, and their locomotor response to 0.1 mg/kg of apomorphine (APO) s.c. on day 15 post-lesion. All testing took place in circular corridor photocell cages. 6-OHDA lesions of the VTA produced significant increases in spontaneous nocturnal activity at the 2ug/1ul dose but no change in nocturnal activity at the 4ug/1ul dose. RF lesions to the VTA produced even greater increases in nocturnal activity which were blocked by the addition of 6-OHDA to the medial NAS. NAS 6-OHDA lesions alone failed to significantly alter nocturnal activity. 6-OHDA lesions to the VTA produced a block of AMPH induced activity that was directly related to the dose of 6-OHDA used with the 4ug/1ul dose producing a 90% decrease. The posterior and medial NAS lesions produced an identical block of AMPH induced activity. The rats with the RF lesions to the VTA were spontaneously hyperactive and remained hyperactive after injection of AMPH. In contrast, the rats with the combined RF lesion and medial NAS 6-OHDA lesion showed a block of AMPH activity identical to that observed with medial NAS lesion alone. All lesion groups that showed a block of AMPH locomotor activity exhibited supersensitivity to APO as measured by a significant increase in locomotor activity. The RF-VTA lesion rats showed a significant decrease in their spontaneous hyperactivity after APO. These results suggest that limited destruction of the mesolimbic dopamine system can produce hyperactivity but that more extensive destruction of this system produces no hyperactivity and an attenuation of the locomotor stimulating effects of AMPH.

2220 EFFECT OF ETHYL ALCHOHOL AND d-AMPHETAMINE ON SOCIAL BEHAVIOR AND THE RESPONSE TO PEER SEPARATION IN RHESUS MONKEYS. G.M. Kraemer, D. Lin‡ N. Kalin‡ M.H. Ebert, and W.T. McKinney. University of Wisconsin, Primate Laboratory, Madison WI 53706 and NIMH, Bethesda MD, 20014 (MHE).

Previous studies from our laboratory have shown that drug treatments which interfere with catecholamine metabolism can significantly alter the response of peer grouped rhesus monkeys to separation from their peer group, in the absence of demonstrable drug effects on group social behavior(J. Affective Disorders, 1,33-54,1979). Specifically, pretreatment of monkeys with alpha-methyl-para-tyrosine(AMPT) in dosages as low as 25 mg/Kg/day appears to increase the levels of despair behavior following separation, while pretreatment with fusaric acid(DBH inhibitor), or tricyclic antidepressants, decreased the level of despair behavior. These results suggested an important parallel between the effects of drugs on the production of separation despair in monkeys and their possible depressant or antidepressant effects in humans.

In subsequent experiments we have examined the effects of a wider spectrum of psychoactive drugs in the peer separation paradigm, and the effects of these drugs on cerebro-spinal fluid(CSF) metabolites of norepinephrine and dopamine. The results of studies with ethyl alchohol and d-amphetamine suggest that these agents alter both social and separation behavior of rhesus monkeys. However, the sedative or activating effects of these agents appeared equally in both social and separation housing conditions. Therefore, the biochemical effects of these agents do not significantly augment or ameliorate the behavioral effects of peer separation in the absence of prior effects on group social behavior. These results provide additional evidence that the interaction of drugs with despair following social separation may depend on specific characteristics of the biochemical alterations produced rather than the overt sedative or activating effects of the drug. Specific alterations in brain catecholamine metabolism may be an important factor. However, our data indicate that important changes in social and separation behavior can occur with very little alteration in the turnover of CSF catecholamine metabolites.

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2221 EFFECTS OF L-DIHYDROXYPHENYLALANINE AND D,L-5-HYDROXYTRYPTOPHAN ON TYROSINE FACILITATED AGGRESSION. N. R. Kramarcy[1] and J. B. Thurmond. Dept. of Psych., Univ. of Louisville, Louisville, KY 40208

The administration of tyrosine via the diet has been reported to increase aggression and brain dopamine concentrations in mice (Lasley et al. Neurosci. Abstr. 4, 1978). Employing a resident-intruder aggression test, we have investigated the effects of acute administration of L-dihydroxyphenylalanine (L-DOPA) and D,L-5-hydroxytryptophan (D,L-5-HTP) to resident mice receiving a 4% tyrosine supplemented diet (TYR) or the 12% casein basal diet (CAS) for one week. Residents in both diet conditions were matched for aggression and locomotor activity (test 1) prior to drug test (test 2).

The TYR residents receiving saline in test 2 displayed increased aggression and elevated dopamine concentrations relative to the CAS residents. L-DOPA administration (12.5, 25, 50, 100 mg/kg) increased the aggression of the CAS residents. The TYR residents exhibited increased aggression after 12.5 and 25 mg/kg, but decreased aggression after 50 and 100 mg/kg L-DOPA. The concentration of dopamine (DA) was elevated in all groups. Treatment with D,L-5-HTP (50, 100, 200 mg/kg) produced a marked decrease in aggression in the CAS residents at all doses with little change in locomotion. In contrast, the TYR residents' aggression decreased markedly only at 200 mg/kg D,L-5-HTP. The concentrations of serotonin (5-HTP) and 5-hydroxyindoleacetic acid (5-HIAA) were elevated at all doses in both diet groups, but the increase in the TYR group was reduced relative to the CAS group.

These studies demonstrate that administration of the neurotransmitter precursor tyrosine via the diet can produce changes in aggressive behavior and neurochemistry, alter behavioral responses to L-DOPA and D,L-5-HTP, and reduce the synthesis of serotonin after D,L-5-HTP administration. The increase of dopamine concentrations relative to norepinephrine appears responsible for increases in aggression and the increase of serotonin concentrations relative to dopamine appears important for inhibition of aggression. (Supported in part by NIMH grant no. MH26677.)

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2222 EFFECTS OF INTRASTRIATALLY INJECTED THIORIDAZINE ON APOMORPHINE-ELICITED BEHAVIORS. H. Lai* and A. Horita* (SPON: R. M. Quock) Dept. Pharmacol. and Psychiat. & Behav. Sci., Univ. Washington, Seattle, WA. 98195.

Bilateral injection of thioridazine (10 μg) into the neostriata of rats enhanced stereotypic behavior elicited by subcutaneously injected apomorphine (1 mg/Kg), while motor activity of the animals was not affected. This enhancing effect of thioridazine on apomorphine-elicited stereotypic behavior was attenuated by pretreating the rats with α-methyl-p-tyrosine. These results suggest that thioridazine, when injected directly into the neostriatum, can enhance dopaminergic activity probably by increasing release of dopamine from the nigrostriatal dopaminergic pathway.

2223 CORRELATION OF ANTI-PSYCHOTIC DRUG POTENCY AND NEUROLEPTIC RECEPTOR INHIBITION IN POST-MORTEM HUMAN BRAINS. Tyrone Lee and Philip Seeman. Department of Pharmacology, University of Toronto, Toronto, Canada M5S 1A8.

In order to investigate whether anti-psychotic drugs inhibit the binding of ^{3}H-spiperone to neuroleptic/dopamine receptors in human brain in accordance with their clinical potencies, we determined the effect of 22 different neuroleptics in inhibiting the specific binding of ^{3}H-spiperone to caudate and putamen of post-mortem human brain.

All clinically active neuroleptics effectively inhibited the binding of ^{3}H-spiperone. β-flupenthixol, trans-thiothixene and (-)-butaclamol were ineffective even at micromolar concentrations. The potencies of these drugs for inhibiting ^{3}H-spiperone binding correlated very well with their clinical potencies for treating schizophrenia. The 50% inhibitory concentrations (IC_{50}) of these drugs were as follows:

DRUGS	nM	DRUGS	nM
Spiperone	0.3	Metiapine	70
Benperidol	1	Clozapine	150
Trifluperidol	1	Molindone	150
Pimozide	5	Sulpiride	400
Haloperidol	5	cis-thiothixene	3
Moperone	10	trans-thiothixene	>1000
Lenperone	22	(+)-butaclamol	5
Perphenazine	3	(-)-butaclamol	>1000
Fluphenazine	4	α-flupenthixol	9
Triflupromazine	17	β-flupenthixol	600
Prochlorperazine	20		
Trifluoperazine	22	Dopamine	>1000
Chlorpromazine	30	Apomorphine	>1000
Thioridazine	40	Noradrenaline	>1000
Promazine	200	5-HT	>1000
		Carbachol	>1000
		GABA	>1000

It remains to be determined whether such a profile also holds in schizophrenic brain tissues where higher numbers of neuroleptic receptors have been found (Lee and Seeman, Proc. Soc. Neurosci. 1977, 1978). Scatchard analysis using ^{3}H-spiperone indicated that there was no change in receptor affinity in schizophrenic tissues compared to those from normal controls.

(Supported by the Ontario Mental Health Foundation and the Medical Research Council of Canada.)

2224 IN VIVO EFFECT OF PHENCYCLIDINE ON SYNAPTOSOMAL UPTAKE OF DOPAMINE AND 5-HYDROXYTRYPTAMINE. D. E. Leelavathi, and Robert C. Smith. Texas Res. Inst. Mental Sci., Houston, Texas, 77030.

Phencyclidine (PCP) is a potent psychetomimetic agent and some of this action has been presumed to be mediated by effects on catecholamines. Earlier results (Smith, et.al., Biochem. Pharm., 26:1435, 1977) indicated that PCP significantly inhibited the in vitro uptake of dopamine (DA) and 5-hydroxytryptamine (5-HT) in crude synaptosomal preparations of rat brain. The present investigation was undertaken to study the effects of a single dose of intraperitoneal administration of PCP to rats, at which behavioral effects are observed on the in vivo uptake of DA in striatum and 5-HT in brain stem. Fifteen minutes after administration of PCP (10 mg/kg body weight), there was no effect on the DA uptake in striatum whereas 5-HT uptake was inhibited in brain stem to an extent of 10%. Forty-five minutes after administration of PCP, the behavioral effects are more prominent but there was no effect on 5-HT uptake. This probably indicates that inhibition of uptake of 5-HT at presynaptic level may not be related to the observed behavioral effects by PCP.

2225 BEHAVIORAL AND BIOCHEMICAL STUDIES OF AMPHETAMINE TOLERANCE AND REVERSE TOLERANCE. Nancy J. Leith & Ronald Kuczenski, Dept. Pharmacol., Vanderbilt School of Medicine, Nashville, TN 37232

Leith & Barrett (Psychopharm, 46, 1976) have demonstrated that following chronic administration of d-amphetamine, (increasing doses, 3 times daily for 4 days) tolerance is seen to the facilitating effects of the drug on self-stimulation responding. On the other hand, Segal & Mandell (Pharmacol., Biochem. Behav. 2, 1974) showed that repeated administration (constant dose once daily for 7-14 days) of the drug results in an enhanced response (reverse tolerance) to the locomotor stimulatory and stereotypy - producing effects. These data suggest that the drug effects on self-stimulation responding and motor behavior reflect different actions of amphetamine. However, the chronic regimens used in the two studies were quite different and could have attributed to the dissimilar results obtained. The present study compared both drug regimens on both behaviors to determine which interpretation is accurate.

For the motor activity studies, male Sprague-Dawley rats were injected with .75, 2.0, 3.0 or 5.0 mg/kg d-amphetamine and activity monitored for 3 hours. Half of the animals in each dosage group then received 3.0 mg/kg daily for 6 days and were retested on day 7. The other half received 3 injections daily for 4 days beginning with 1 mg/kg and incrementing by 1 mg/kg at each injection and were retested on Day 7. Regardless of the chronic injection regimen, all animals showed an enhanced response to d-amphetamine when retested. Self-stimulation animals were implanted with electrodes in the MFB and after training, were tested with an acute dose of 0.3 mg/kg. The animals were then treated chronically as stated above. Animals receiving 3 mg/kg daily showed no change in the amphetamine response when retested whereas those treated 3 times daily with increasing doses demonstrated tolerance as previously reported. These data, then, suggest that the two behaviors do in fact reflect different aspects of amphetamine's action.

As a first step in assessing possible biochemical changes that might underlie the behavioral phenomena occurring after chronic administration of amphetamine, we have measured DOPAC and HVA levels in the caudate and mesolimbic area. The chronically treated animals showed a significant decrease compared to controls in both DOPAC and HVA in the caudate but not the mesolimbic region when killed 48 hours after the last drug injection, but the two groups were not significantly different from each other after a challenge dose of 3 mg/kg d-amphetamine. In addition, in an attempt to further characterize the nature of the changes in dopamine function, the responses to Haldol and apomorphine are being evaluated in animals treated chronically with amphetamine.

(Supported by NIH Grants MH29217 and MH29106 and MH00091).

2226 A PROFILE OF THE CAPSAICIN DESENSITIZED RAT. Marciá G. Levin*, Rodney J. Pelchat and Nancy Wzontek* (SPON: D. Moulton). Dept. Psych., Univ. of Penna., Philadelphia, PA 19104.

Jancsó-Gábor et al. (J. Physiol. 206:495, 1970) reported that rats given large injections of capsaicin (8-methyl-6-nonenoyl-vanillamide) show deficits in thermoregulatory and pain responses which last for a year or more. These animals show abnormally elevated body temperature in warm environments but in neutral and cool environments their body temperature is normal (i.e. matches controls). These animals are also insensitive to peripherally applied chemical irritants. Jancsó-Gábor called this set of symptoms capsaicin desensitization. When we placed desensitized and control rats at an ambient temperature of 32°C, the groups showed equal amounts of saliva spreading. Controls showed large amounts of prone extension whereas desensitates showed none. At an ambient temperature of 38°C desensitates and controls showed equal amounts of prone extension but desensitates had a much longer latency to prone extend. These studies suggest that desensitized rats have elevated thresholds for prone extension and, therefore, that their thermoregulatory deficits are, in part, of central origin. This is in contrast to the contention of Cabanac et al. (Pflugers Archiv. 366: 217, 1976) that the hyperthermia in desensitates at high ambient temperatures is due only to effector deficits. In another study we investigated the specificity of the analgesia observed in desensitates. Latencies to tail flick in desensitized rats were found to be identical to their own pre-desensitization baselines and were also identical to latencies in matched controls. Thus, this measure reveals no effects of capsaicin desensitization on reactions to pain due to heat.

2227 PROPERTIES OF HIGH AFFINITY ^{3}H-DOPAMINE BINDING SITES IN RAT STRIATUM. S. List, M. Titeler and P. Seeman, Department of Pharmacology, University of Toronto, Toronto, Ontario M5S 1A8.

Although a high affinity binding site for ^{3}H-dopamine in rat striatal tissue had been reported by Seeman et al. (Fed. Proc. 33, 1974) to have a K_D of 0.6 nM, the work of Burt et al. (Proc. Nat. Acad. Sci. 72, 4655, 1975) only revealed a low affinity site for ^{3}H-dopamine (i.e. 50% displaced by 380 nM dopamine).

In order to examine this further, we studied the inhibitory effects of various drugs on the binding of ^{3}H-dopamine to rat striatal homogenates. The total binding of ^{3}H-dopamine was reduced by 60% at 500 to 1000 nM dopamine with little further reduction at 10,000 nM dopamine. Thus, specific binding of ^{3}H-dopamine was defined as that displaceable by 500 nM dopamine.

A Scatchard analysis of the specific ^{3}H-dopamine binding sites revealed a K_D of 3 nM and a maximum number of binding sites of 100 fmoles/mg protein. The site appeared to be similar to the ^{3}H-dopamine site labeled in the calf caudate, this site having high affinity for dopaminergic catecholamines and low affinity for neuroleptics (see Table). (Supported by the Medical Research Council of Canada and the Ontario Mental Health Foundation).

	^{3}H-Dopamine IC_{50}	
	Rat Striatum	Calf Caudate
Apomorphine	1.5 nM	1 nM
ADTN	3 nM	10 nM
Dopamine	12 nM	8 nM
LSD	15 nM	40 nM
Haloperidol	200 nM	900 nM
Chlorpromazine	600 nM	1500 nM

2228 ASSESSMENT OF THE ROLES OF RESPONSE RATE AND REINFORCEMENT RATE IN PRODUCING AMPHETAMINE'S SCHEDULE-DEPENDENT BEHAVIORAL EFFECTS. I. Lucki and R. E. DeLong*.
Dept. Psychol., Univ. Iowa, Iowa City, IA 52242.

The rate dependency hypothesis states that baseline rates of responding determine the effects of amphetamine on schedule-controlled operant behavior. However, the correlation between response rate and reinforcement rate under most reinforcement schedules confounds attributing the schedule-dependent effects of amphetamine solely to differences in rates of responding. Two comparisons were performed to independently evaluate the roles of response rate and reinforcement rate in the schedule-dependent effects of amphetamine. The comparisons were complementary, in that multiple schedules were arranged where one variable, either response rate or rate of reinforcement, was held constant and the other variable was allowed to vary.

In the first comparison, a procedure yoked reinforcement availability under a variable-interval (VI) schedule to the temporal pattern of reinforcement under a differential reinforcement of low rate (DRL) schedule (mult DRL 7 sec yoked VI). VI response rates were 3.2 times greater than DRL response rates, at rates of reinforcement that were held relatively constant. Amphetamine increased DRL responding at the same doses that decreased VI responding. This agrees with previous demonstrations of rate-dependent amphetamine effects when these schedules were studied individually, but further indicates that different rates of reinforcement between schedules are not necessary to produce schedule-dependent amphetamine effects.

The second comparison examined the hypothesis that different reinforcement rates could produce independent effects on amphetamine action. A multiple random ratio (RR) schedule (mult RR20 RR50) held rates of responding constant while rates of reinforcement varied by 2.7-fold. Amphetamine affected responding under both random ratio schedules equally, indicating the different reinforcement rates exerted no effects on responding under amphetamine.

The results of the complementary procedures agreed in assessment of the rate dependency hypothesis. The primary determinant of amphetamine's schedule-dependent behavioral effects is the control rate of responding, rather than the control rate of reinforcement.

2229 INFLUENCE OF DOPAMINERGIC NERVE TERMINALS IN NUCLEUS ACCUMBENS ON THE ACQUISITION AND MAINTENANCE OF d-AMPHETAMINE SELF-ADMINISTRATION. W.H. Lyness and K.E. Moore. Dept. Pharmacol. & Toxicol., Michigan State Univ., East Lansing, MI 48824.

The objective of this study was to determine the role of mesolimbic dopaminergic neurons, which terminate in nucleus accumbens, in mediating the self-administration of d-amphetamine. Rats were trained to lever press for i.v. self-injection of 0.125 mg/kg d-amphetamine (FR-1 schedule) during daily 16 hr test sessions. They acquired a stable injection rate in 7-14 days and were tested thereafter in 8 hr test sessions (50-90 injections per 8 hr). In trained rats, noncontingent injections of 1.0 mg/kg d-amphetamine or 0.5 mg/kg apomorphine reduced the number of self-injections for periods consistent with the duration of action of the drugs (90 min for d-amphetamine, 30 min for apomorphine). Replacement of d-amphetamine with saline resulted in a 4-6 fold increase in response rate for several hours followed by cessation of lever pressing. Rats previously trained to self-administer d-amphetamine were given bilateral injections of 6-hydroxydopamine (8 µg/2 µl) or vehicle (0.1 mg ascorbic acid/ml saline) into the nucleus accumbens under Equithesin anesthesia and 25 mg/kg desipramine pretreatment. 6-Hydroxydopamine reduced the dopamine content of the nucleus accumbens to 10% of control. A 14 day recovery period was allowed before self-administration studies were resumed. After this recovery period, vehicle-injected rats immediately resumed rates of self-administration which were comparable to those before the 14 day rest (range 50-95 self-injections per 8 hr). 6-Hydroxydopamine-lesioned rats, failed to reinitiate self-administration behavior (range of only 0-12 self-injections per 8 hr), even when examined for up to 21 days. Replacement of d-amphetamine with saline increased self-injection rates only in rats administered vehicle in nucleus accumbens; rats given 6-hydroxydopamine failed to alter their self-injection rates.

Naive rats with 6-hydroxydopamine lesions of the nucleus accumbens failed to acquire self-administration behavior when examined over a 21 day period following the lesion. The data indicate that dopaminergic nerve terminals in the nucleus accumbens are essential for both the acquisition and maintenance of self-administration of d-amphetamine. (Supported by USPHS grant MH 13174.)

2230 β-ADRENERGIC AGONISTS AND BENZODIAZEPINES STIMULATE MEMBRANE PHOSPHOLIPID METHYLATION IN RAT ASTROCYTOMA CELLS. Pierre Mallorga*, Warren J. Strittmatter*, Fusao Hirata*, John F. Tallman and Julius Axelrod. Biological Psychiatry Branch and Laboratory of Clinical Science, NIMH, Bethesda, MD 20205

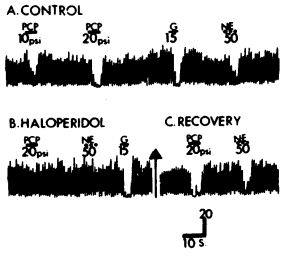
C_6 rat astrocytoma cells contain β-adrenergic and benzodiazepine receptors. The β-adrenergic receptor has been characterized by specific binding of l-[^{3}H]dihydroalprenolol, displacement of this radioactive ligand by various β-adrenergic agonists and increased 3':5'cyclic AMP production in intact cells after incubation with l-isoproterenol. The benzodiazepine receptor has been characterized by specific binding of [^{3}H]diazepam and displacement of this ligand by various benzodiazepines: Ro5-4864 is more potent than clonazepam and chlordiazepoxide.

l-Isoproterenol and Ro5-4864 are able to stimulate the methylation of membrane phosphatidylethanolamine to form phosphatidylcholine in intact cells. Half-maximal stimulation is obtained with approximately 10^{-8} M l-isoproterenol or 10^{-8} M Ro5-4864. If both compounds are added together to the incubation, methylation of phosphatidylethanolamine is additive. This suggests that two different sets of methylating enzymes, and probably two domains of the membrane are involved in the mechanism of action of β-adrenergic agonists and benzodiazepines.

A two-hour exposure of the cells to l-isoproterenol (10^{-5} M) results in a 80-90% loss of responsiveness to a subsequent challenge by l-isoproterenol, although the loss of binding sites is 20%. We were able to block this agonist-induced refractoriness by adding mepacrine, an inhibitor of the phospholipase A_2, to the medium of incubation. Mellitin, an activator of the phospholipase A_2 caused the cells to become refractory to l-isoproterenol.

2231 PHENCYCLIDINE - NEUROLEPTIC INTERACTIONS IN A CENTRAL NORADRENERGIC PATHWAY. J. Marwaha*, M. Palmer, B.J. Hoffer* and R. Freedman* (SPON: T. Dunwiddie). Dept. of Pharmacology, University of Colorado Medical Center, Denver, Colorado, 80262.

The interactions between the "dissociative anesthetics", phencyclidine (PCP), ketamine (K) and the antipsychotic drugs, fluphenazine (F), haloperidol (H), α-flupenthixol (αF) were studied in the cerebellum of urethane-anesthetized rats. The inactive isomer β-flupenthixol was used as a control. Activity of single Purkinje (P) neurons was recorded extracellularly. Since aqueous solubility is a problem with many of these agents, they were ejected from multibarrel micropipettes using air pressure. Locally applied PCP and K decreased the spontaneous discharge of P neurons in a dose dependent manner. In general, the latencies for the depressions were 2 to 3 seconds and recovery was seen 2 to 3 seconds following termination of drug ejection. Ketamine was about one fifth as potent as PCP. Parenteral (0.5 mg/Kg) or local application of F, H and αF reversibly antagonized the depressant effects of PCP and K on P cell discharge whereas βF was ineffective. F, H and chlorpromazine also antagonized the depressant effects of iontophoretically applied norepinephrine (NE) and locus coeruleus stimulation on these same neurons, but not the effects of iontophoretic application of GABA (G).



These results taken together with previous reports that antipsychotic drugs block noradrenergic transmission in the cerebellum suggest that PCP and K depress P cell discharge by altering noradrenergic neurotransmission in the cerebellum and that the cerebellar noradrenergic pathway is a convenient model system to analyze these drugs. (Supported by DA 07043, AA 03527, and Pharmaceutical Manufacturer's Association.)

2232 DIFFERENCES IN BINDING AND ACCUMULATION OF AMPHETAMINE ISOMERS IN HYPOTHALAMIC VERSUS STRIATAL SYNAPTOSOMES. R.T. Matthews and P.A. Shore. Dept. of Pharmacol., University of Texas Health Science Center, Dallas, TX 75235.

A recent study in this laboratory (Fed. Proc. 38:524, 1979) demonstrated stereoselectivity in the accumulation and binding of amphetamine (AMPH) isomers by synaptosomes of the rat corpus striatum. At 37°, accumulation of ^{3}H-d-AMPH was inhibited much more than ^{3}H-l-AMPH by ouabain, unlabeled d-AMPH (but much less so by unlabeled l-AMPH) or by dopamine (DA) neuronal uptake inhibitors. The norepinephrine (NE) uptake inhibitor, desipramine, showed little effect. In the cold (4°), considerable binding of ^{3}H-d-AMPH remained; this was also inhibited by DA uptake inhibitors or by unlabeled d-AMPH. It was concluded that the DA uptake carrier is the site of stereoselective binding and accumulation of d-AMPH in the striatal DA nerve terminal. The stereoselectivity for d-AMPH correlates with the greater electrophysiological and biochemical potency of d-AMPH on nigrostriatal DA neurons.

In the present study, similar experiments were performed using a crude synaptosomal preparation of rat hypothalamus, an area rich in NE nerve terminals. Accumulation of ^{3}H-AMPH isomers (10^{-7}) was observed at 37°, but while this was inhibited by cold, the pattern of inhibition by drugs differed markedly from that seen in the striatal preparation. Thus at 37°, little or no effect of ouabain (10^{-5}M) was observed on d-AMPH. Desipramine (10^{-7}-10^{-6}M) or unlabeled AMPH isomers inhibited d-AMPH accumulation more than l-AMPH, but more meaningful was the finding that ^{3}H-d-AMPH accumulation was inhibited equally by unlabeled d- or l-AMPH (10^{-6}M) as was ^{3}H-l-AMPH accumulation.

These preliminary findings are suggestive that, unlike striatal DA neurons, little active accumulation or stereoselectivity of binding of AMPH isomers exists in the case of hypothalamic NE neurons. The findings are consistent with the known electrophysiological and biochemical equipotency of AMPH isomers on NE neurons as contrasted with nigrostriatal DA neurons where the d-isomer is much more potent than the l-isomer. (Supported by USPHS Grant MH-05831).

2233 EFFECTS OF ATROPINE OR BENACTYZINE ON DRL PERFORMANCE OF MONKEYS. John H. McDonough, Jr.* Neuropsych. Br., U.S. Army Biomedical Laboratory, Aberdeen Proving Ground, MD 21010.

In humans, one of the consistently reported psychopharmacological effects of cholinergic blocking agents is a loss of the "sense of passage of time." The ability of two anticholinergic drugs, atropine and benactyzine, to produce this effect was assessed in Rhesus monkeys using a differential reinforcement of low rates (DRL) operant conditioning schedule. Four adult Rhesus monkeys (2 males, 2 females) were trained to respond for food pellet rewards on a DRL 28 sec schedule for 60 min each day while housed in a sound-attenuated booth. The monkeys had been trained for more than two years to a stable performance baseline prior to these drug tests. They had had some previous experience with these compounds, but had not received any drug for at least 3 months prior to this study. Atropine SO_4 (0.014, 0.044, 0.14, 0.44 mg/kg) or benactyzine HCl (0.054, 0.17, 0.54, 1.7 mg/kg) was administered i.m. on alternate tests with doses administered according to a randomized design. Atropine was given 45 min prior to the sessions, benactyzine 15 min prior to the session. The two sessions prior to each drug test were regarded as controls. The eight control sessions for each drug were averaged to obtain a mean control performance with which to compare the effects of drug treatment. At least one week, and four test sessions, intervened between drug tests. Both anticholinergic drugs had a similar pattern of effect: total responding was not effected by any dose of atropine ($F(4,12) = 0.35$) or benactyzine ($F(4,12) = 0.14$) while both drugs produced reliable reductions in the number of rewards earned (atropine $F(4,12) = 48.3$, $p < .001$; benactyzine $F(4,12) = 10.51$, $p < .01$). The two highest doses of both drugs were responsible for the loss of rewards and reduction in efficiency. While group mean total responding did not reliably change at any of the doses tested, there was a dose-related increase in the variability of responding. When compared to control performance the variance for total responses was increased 16-fold by the high dose of atropine, and 11-fold by the high dose of benactyzine. In all cases the two highest drug dosages produced a flattening and a shift to shorter interresponse times of the unimodal response distribution curves that are typical of well trained DRL performance. There was some evidence of a within-session behavioral tolerance, in that errors under drug tended to occur most frequently in the first half of the session. Atropine was by far the more potent of the two compounds, producing a 71% reduction in rewards earned at the 0.44 mg/kg dose as contrasted with a 39% reduction in rewards produced by 0.54 mg/kg benactyzine. The results indicate the primary effect of these anticholinergic drugs was to produce an underestimation of the DRL interval which resulted in a decrement of efficient performance.

2234 AREA POSTREMA LESION ELIMINATES THE ANTIAGGRESSIVE AND AVERSIVE CONDITIONING PROPERTIES OF LITHIUM. John J. McGlone, Sue Ritter and Keith W. Kelley*. Dept. of Animal Science and College of Veterinary Medicine, Wash. State Univ., Pullman, WA 99164.

Previous experiments have demonstrated the ability of lithium (Li) to reduce aggression in animals. Doses of Li used in animal aggression studies, however, are also effective in producing conditioned taste aversions (CTA), suggesting that malaise and toxicity may contribute to Li's antiaggressive effect. We attempted to test this hypothesis in two experiments. Since many chemicals that produce emesis or malaise do so by their action on the chemoreceptive zone of the area postrema (AP), it seemed possible that some of the side effects of Li might also be mediated at this site. Therefore, in the first experiment, we investigated the possibility that thermal lesions of the AP would reduce Li-induced malaise, as indicated by the CTA test. In the second experiment, the antiaggressive effect of Li was evaluated in AP-lesioned rats. Fifteen female and 12 male rats were used in these experiments. Lesions were confirmed by histology.

In the first experiment, AP-lesioned and sham-operated rats were tested for CTA after pairing of a novel palatable food with lithium chloride (3mEq/kg), scopolamine methyl nitrate (1 mg/kg), d-amphetamine sulfate (2 mg/kg) or saline (.15M). Although strong aversions were demonstrated by sham-operated rats after Li and scopolamine, aversions were not induced by these substances in AP-lesioned rats ($p<.05$). AP-lesioned rats, however, formed strong taste aversions to a novel solution paired with amphetamine ($p<.01$), indicating that their taste aversion deficits after Li and scopolamine cannot be attributed to generalized behavioral or sensory disruption resulting from the lesion. These results suggest that AP lesions may significantly attenuate Li-induced malaise.

In the second experiment, sham-operated and AP-lesioned rats, previously screened in the taste aversion test, received daily intraperitoneal injections of saline or Li (5 mEq/kg) for 5 days prior to aggression tests. Rats were paired by surgical procedure, sex and weight. Pairs were tested for latency and frequency of shock-induced aggression in a chamber where constant current shocks were delivered through the floor grid. Attacks were scored during 2 one-min trials. Our results reveal that Li increased the latency to attack in sham-operated rats, compared to saline-treated shams (18.3 + 3.3 sec vs 7.3 + 3.7 sec, $p<.05$). In contrast, AP-lesioned rats treated with Li attacked with a latency similar to AP-lesioned rats treated with saline (6.2 + 4.3 vs 5.5 + 4.3, $p>.1$). These data suggest that at the doses of Li used in these studies, drug-induced malaise may mediate Li's antiaggressive effects.

2235 INTERACTION OF ℓ-DOPA OR 5-HTP AND IMIPRAMINE ON SCHEDULE PERFORMANCE IN THE RAT. P.S. McGuire* and L.S. Seiden. University of Chicago, Chicago, IL. 60637.

Administration of 10.0 mg/kg imipramine (IMI, 1 hour prior to the experimental session) to rats performing on a differential-reinforcement-of-low-rate schedule (DRL > 18-sec.) of water reinforcement increases reinforcements and decreases responses. To investigate the possible involvement of neurotransmitters in this effect, rats performing on a DRL > 18-sec. schedule were treated with dihydroxyphenylalanine (ℓ-DOPA, 2.5 - 200.0 mg/kg, i.p. and 50 mg/kg RO4-4602, i.p.) or 5-hydroxytryptophan (5-HTP, 2.5 - 200.0 mg/kg, i.p. and 50 mg/kg RO4-4602, i.p.). ℓ-DOPA decreased both response rate and reinforcement rate at doses of 50.0 - 200.0 mg/kg; lower doses had no significant effect. Interresponse time (IRT) distributions for individual rats showed variable effects. In some rats, doses of 2.5 and 25.0 mg/kg ℓ-DOPA decreased the number of short IRTs and increased the number of IRTs longer than 18-sec. Other rats were unaffected. Higher doses of ℓ-DOPA flattened the IRT distributions. 5-HTP increased response rate and decreased reinforcement rate at doses of 50.0 - 200.0 mg/kg. These doses of 5-HTP increased the number of IRTs shorter than 18-sec. and decreased the number of reinforced IRTs. Following determination of dose-response curves for each drug alone, all doses of ℓ-DOPA and 5-HTP were given in combination with 10.0 mg/kg IMI. IMI potentiated both the response rate and reinforcement rate decreases seen with ℓ-DOPA. The response rate increases produced by 5-HTP (50.0 - 200.0 mg/kg) were blocked when 10.0 mg/kg IMI was administered. These data suggest a differential disruption in DRL performance as a function of interference with specific neurotransmitter systems. (Supported by USPHS GRants MH-11191; MH-14274; and 5-K05-MH-10562).

2236 CHARACTERISTICS OF APOMORPHINE-INDUCED HYPOTHERMIA IN MICE. M. K. Menon, Psychopharmacol. Res. Lab., VA Medical Center, Sepulveda, CA 91343.

In male Swiss mice, apomorphine HCl (APO) caused a dose-dependent (0.5--20.0 mg/kg) decrease in the core temperature, a 10 mg/kg dose caused a 2° 8° C drop at 30 min. This dose and time schedule was used in all the following studies. A second dose of APO administered 3 hr after the first dose caused only a 46 percent response. Chlorpromazine (3 mg/kg, 10 min), haloperidol (1 mg/kg, 10 min), pimozide (1 mg/kg, 2 hr) and (+) butaclamol (1 mg/kg, 10 min) blocked both the hypothermia as well as the behavioral responses of APO, but αMT (280 mg/kg, 4 hr), clozapine (3 mg/kg, 10 min) and (-) butaclamol (5 mg/kg, 10 min) failed to do so. Piribedil (20 mg/kg, 10 min) and bromocryptine (3 mg/kg, 10 min) neither produced hypothermia nor did they block APO hypothermia. Phenoxybenzamine (15 mg/kg, 2 hr), propranolol (10 mg/kg, 10 min), methysergide (3 mg/kg, 10 min), cinanserin and xylamidine (both 10 mg/kg, 10 min) and PCPA (3 X 100 mg/kg) potentiated APO effect. The 5-HT receptor agonists quipazine and MK-212 (both 3 mg/kg, 10 min) specifically blocked the hypothermic response. Desipramine (20 mg/kg, 30 min) blocked the APO effect even in animals pretreated with αMT.

A 0.2 mg/kg haloperidol (10 min prior) blocked the hypothermic response of a 5 mg/kg dose of APO, but not that of 20 mg/kg APO which demonstrated that the blockade is surmountable. Chronic haloperidol treatment did not modify the intensity of APO-induced hypothermia but potentiated its behavioral responses. The doses (mg/kg) of neuroleptics required to block 90-95 percent response of APO were as follows: Chlorpromazine, 3.3, (+) butaclamol, 1.0 and haloperidol, 1.0. Trifluperazine, even in doses of 5 mg/kg caused only a 63 percent blockade of APO response. In this regard, the potencies of the two phenothiazines did not correlate with their antipsychotic potency.

This work was supported by the Veterans Administration.

2237 DIFFERENTIAL ATTENUATION OF AMPHETAMINE- AND COCAINE-INDUCED STEREOTYPIES AND SUPPRESSION OF PRIMATE SOCIAL BEHAVIOR BY ANTIPSYCHOTIC DRUGS. Klaus A. Miczek and Hiroyuki Yoshimura*. Dept. Psychol., Carnegie-Mellon Univ., Pittsburgh, PA 15213.

Withdrawal from social interactions is one of the striking symptoms of psychosis that has received little experimental attention. The preclinical study of antipsychotic drugs has focussed mainly on their ability to block stereotyped motor acts induced by dopaminergic agonists. It was our objective to induce "psychotic-like" changes in social behavior in members of established troops of squirrel monkeys by repeated administration of d-amphetamine or cocaine, to study the effects of haloperidol, chlorpromazine and physostigmine on psychostimulant-induced suppression of social communication, and to compare these effects to those on motor stereotypies. In the first series of experiments, either d-amphetamine (1 mg/kg) or cocaine (10 mg/kg) were injected p.o. three times within 24 hrs to one of the adult male members of each of four colonies of Saimiri sciureus (n=6-9). The third injection was preceded by administration of chlorpromazine (0.25, 0.5, 1.0 mg/kg, p.o.), haloperidol (0.25, 0.5 mg/kg, p.o.) or physostigmine (0.04, 0.08 mg/kg, i.m.). At the time of peak effect, an uninformed observer, using the focal animal technique, measured the frequency and duration of all social behavioral elements that the drugged monkey initiated or was recipient of (genital display, displacing, grasping, huddling) via a computer-based keyboard system. In addition, the level of motor activity was assessed and rapid head movements were counted as the most prominent stereotypy. We found that d-amphetamine and cocaine severely decreased all social interactions and increased stereotyped head movements. Chlorpromazine, haloperidol and physostigmine blocked the cocaine- and amphetamine-induced stereotypies and changes in motor activity. Haloperidol attenuated social withdrawal due to amphetamine, but produced dyskinesia in cocaine-treated monkeys. Physostigmine increased social behavior that was reduced by cocaine, but less so in amphetamine-treated animals. At doses which blocked stereotyped behavior, chlorpromazine-treated monkeys were sedated and haloperidol-treated monkeys showed dyskinesia. In a second series of experiments, we focussed on agonistic behavior in resident-intruder confrontations and on affiliative behavior toward familiar group members. We employed low doses of haloperidol (0.05, 0.1 mg/kg), chlorpromazine (0.25, 0.5 mg/kg) and physostigmine (0.03, 0.06 mg/kg) which avoided side-effects that interfered with social behavior. Our data provide evidence that psychostimulant-induced changes in primate social behavior are highly sensitive to the action of antipsychotic drugs and may be based on different mechanisms than those for motor stereotypies.

(This research was supported by USPHS research grant DA-1502).

2238 METHADONE EFFECTS ON TWO TYPES OF BEHAVIOR: RELATION TO CATECHOLAMINES. Lawrence D. Middaugh and Larry W. Simpson*. Depts. of Biochemistry and of Psychiatry and Behavioral Science, Med. Univ. of So. Car., Chas., S.C. 29403.

Methadone elevates locomotor activity of C-57 mice at doses up to 7.0 mg/kg. Higher doses of the drug first elevate, then reduce and again elevate activity across time after injection. In DBA mice, however, methadone only reduces activity regardless of the time after injection or the dose of methadone. For both strains of mice, however, the drug is disruptive to lever responding maintained by fixed ratio schedules of reinforcement but has a greater effect on DBA mice. The lack of consistent strain differences in the effects of methadone on locomotor activity vs. the lever response suggests the possibility that the effects on the two behaviors are mediated by separate neural systems.

In subsequent studies we found that the elevated activity noted under some doses and post-injection times for C-57 mice was dependent upon adequate stores of catecholamine since α-methyltyrosine (AMT), although not altering activity itself at the dose used, attenuated the methadone induced elevation of activity. Similar reductions in catecholamine concentrations (approximately 30%), however, had no effect on methadone induced reductions in locomotor activity observed in DBA mice and at higher doses for C-57 mice; or on drug induced disruption of lever responding for either strain. The requirement of catecholamine for methadone induced elevations in activity implicates one of the dopaminergic or noradrenergic tracts in mediating this drug response. The absence of this requirement for the reduction of locomotor activity or disruption of lever pressing following injections of methadone is consistent with the hypothesis that locomotor activity increases are mediated by neural systems different from those mediating the locomotor activity or lever press reductions. (Supported by Grant # DA 01750)

2239 INSENSITIVITY OF THE DORSAL RAPHE TO THE DISCRIMINATIVE STIMULUS PROPERTIES OF LSD. D.J. Minnema*, G. Krynock* and J. Rosecrans. Dept. of Pharmacol., Med. Col. of Va., Richmond, VA 23298.

Previous studies have strongly suggested that the dorsal raphe may be a major site of LSD action. To examine this possibility fourteen male Sprague-Dawley rats were trained to discriminate 96 µg/kg LSD from saline on a VI-15 second schedule of reinforcement for sweetened milk using a two lever operant paradigm. Operant sessions were 15 minutes long. The accuracy of LSD discrimination was determined during a 2.5 minute extinction period which was presented every fourth day preceding the training session. Once reliable discrimination was obtained (i.e. > 80% drug-lever responding with LSD, < 20% drug-lever responding with saline), chronic, indwelling guide cannulas for microinjection were stereotaxically implanted into the area of the dorsal raphe. After reestablishment of reliable LSD discrimination, various doses of LSD were administered via a 31 gauge needle into the dorsal raphe. The LSD was dissolved in artificial CSF and delivered in volumes not exceeding 0.5 µls. Extremely high doses of LSD (∿ 50 µg/kg) administered to the dorsal raphe were needed to produce generalization (∿ 80%) to the drug-lever. A time course study indicated that the duration of LSD generalization of the centrally administered drug closely mimicked the duration of peripherally administered drug. These results suggest that the dorsal raphe is relatively insensitive to the discriminative stimulus properties of LSD when the drug is applied directly at this site. Furthermore, the time-course data suggest that the LSD may be rapidly diffusing away from the site of injection and acting directly, or through the circulation, at other sites in the brain. (Supported by U.S.P.H.S. grant #DA-00296-05).

2240 NORADRENERGIC RESPONSIVENESS IN RAT PINEAL GLAND AFTER SINGLE OR REPEATED ADMINISTRATION OF DESMETHYLIMIPRAMINE. John A. Moyer, Alan Frazer*, Benjamin Weiss, Louise H. Greenberg and Joe Mendels*. Univ. of Pennsylvania Sch. Med., Pennsylvania Med. College, and V.A. Hospital, Philadelphia, Pa. 19104.

The effect of single and repeated desmethylimipramine (DMI) treatment on catecholamine-stimulated production of adenosine 3',5'-monophosphate (cyclic AMP) in rat pineal gland was studied both in vivo and in vitro. Male Sprague-Dawley rats were injected with DMI (10mg/kg, i.p.) either once as a single injection or twice daily for 5 days. Control rats received saline injections for corresponding time periods. Pineal gland concentrations of cyclic AMP were stimulated by the intraperitoneal administration of either isoproterenol (ISO) or norepinephrine (NE) (in vivo experiments) or by incubating glands with different concentrations of ISO or NE.

Acute DMI treatment increased the ability of NE injected in vivo to stimulate pineal cyclic AMP; repeated administration of DMI completely prevented the stimulatory effect of NE or ISO on pineal cyclic AMP in vivo. In pineal glands obtained from rats given DMI for 5 days, the stimulation of cyclic AMP produced by NE or ISO added in vitro was significantly reduced, even at concentrations of the agonists producing maximal stimulation of cyclic AMP.

Acute treatment with DMI had no significant effect on [^{3}H] dihydroalprenolol binding in pineal glands, whereas chronic treatment significantly reduced [^{3}H] dihydroalprenolol binding. Furthermore, when the enzyme adenylate cyclase was measured, it was found that repeated DMI treatment had no significant effect either on basal or NaF-stimulated enzyme activity, but NE-stimulated activity was reduced.

Repeated DMI treatment reduces the ability of ISO or NE administered either in vivo or in vitro to elevate the concentration of cyclic AMP in pineal glands. This effect is due, at least in part, to a decreased number of ß-adrenergic receptors and reduced NE-stimulated adenylate cyclase activity in pineals exposed to repeated DMI treatment. (Supported by research funds from the V.A. and NIMH grants MH-14654, MH-29094, MH-27289 and MH-30096.)

2241 REINFORCING EFFECTS OF INTRAVENOUS AND INTRACRANIAL OPIATES REVEALED BY A PLACE PREFERENCE PARADIGM. R.F. Mucha* and D. Van der Kooy (SPON: M.A. Linseman). Departments of Pharmacology and Anatomy, University of Toronto and Addiction Research Foundation, Toronto, Ontario, Canada. M5S 2S1

The bases of opiate reinforcement are still not known; its neuroanatomy, its relation to other opiate effects, and the role of tolerance and physical dependence in its production remain poorly understood. Since this deficiency largely reflects the lack of suitable paradigms for studying these aspects of opiate reinforcement, the present study demonstrated the suitability of the place preference paradigm for such a purpose. It was based on the demonstration that rats prefer environments in which they received i.p. morphine over other environments (Reid et al., Soc. Neurosci. Abt. 1978, 4, 501). Intravenous (i.v.) administration, the route most commonly used to produce reinforcement, was initially studied. Male Wistar rats were placed in one of two distinct environments: a black 40x40x37 cm box with a Plexiglas floor or a similar white box with a sawdust floor. Through implanted cannulae, each rat then received 0, 0.5 or 10 mg/kg morphine -SO_4, infused over 2 min. These training trials lasted 30 min. and occurred daily for 4 days. Noninfusion training trials were also given each day in the other box. Morphine infusions produced hypoactivity in all the rats. In the 10 mg/kg rats immobility and analgesia were noted at the end of the training trials. On day 5 the rats were tested by providing simultaneous access to both treatment environments for 15 min. These were found at the ends of a 90x40x37 cm test box, separated by a 10x40x37 cm neutral area with grey walls and grid floor. The 10 mg/kg group displayed a clear preference for the environment in which they received morphine, spending 3 times as much time on this side of the test box compared to the side associated with the noninfusion.

Intracranial administration of opiates was studied next. Morphine was administered into the lateral hypothalamus (0.5 ug in 0.5 ul, bilaterally over 2 min with 30 gauge inner cannulae) and rats were trained as above in the place preference paradigm. In contrast to i.v., hypothalamic morphine produced marked hyperactivity, "in vacuo" mouth movements, and frantic grooming. On the test day clear evidence of a positive reinforcing effect was seen. These data indicate that analgesia and immobility are not necessary for opiate reinforcement. Thus, the place preference paradigm may be a powerful tool for exploring the central motivating properties of opiates because of its ability to detect reinforcing effects in a few discrete drug exposures. We are currently using this paradigm to explore the reinforcing effects of other intracranial sites and will report some of the data.

2242 BEHAVIOURAL SUPERSENSITIVITY TO DOPAMINE-MIMETICS AFTER REPEATED AGONIST OR ANTAGONIST TREATMENT. INVOLVEMENT OF AUTORECEPTORS. Pavel Muller, Torgny H. Svensson and Arvid Carlsson*. Dept. of Pharmacol., University of Göteborg, S-400 33 Göteborg, Sweden.

Long-term treatment with both dopamine agonists and antagonists induces augmented behavioural responses to dopamine mimetics. This phenomenon has been attributed to sensitization of postsynaptic dopamine receptors. However, the postsynaptic receptor changes after long-term neuroleptic treatment develop much later than the behavioural sensitization for which they were proposed to account. Thus, an enhanced response to apomorphine was reported already 24 hours after single injections of neuroleptics. In addition, long-term amphetamine treatment does not appear to produce any postsynaptic receptor supersensitivity. We report here the results of behavioural and electrophysiological experiments designed to study the function of dopamine autoreceptors in rats following single or repeated agonist or antagonist treatment. Our experiments indicate that 24 hours after a single injection of haloperidol (0.5-5 mg/kg) the autoreceptor response to exogenously administered apomorphine is reduced, probably due to blockade of these receptors by residual haloperidol. Long-term amphetamine treatment (5 mg/kg daily for 2 weeks, 48 h withdrawal) induced desensitization of the autoreceptors, while leaving the postsynaptic dopamine receptors apparently unaffected. The blockade of the autoreceptors by haloperidol or their desensitization after chronic amphetamine treatment implies a reduced "presynaptic" inhibition of dopamine neurons by exogenously administered dopamine-mimetics. Thus, more dopamine will be available to stimulate the postsynaptic receptors together with the exogenous agonist. Consequently, an augmented behavioural response to exogenously administered dopamine-mimetics such as apomorphine may be the outcome of a reduced dopamine autoreceptor function.

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2243 SYSTEMIC OR INTRACEREBRAL ADMINISTRATION OF d-AMPHETAMINE DECREASES ACTIVITY IN HYPERACTIVE, SPONTANEOUSLY HYPERTENSIVE RATS. Richard E. Musty, Michael M. Myers*, Janice A. Forgays* and Edith D. Hendley. Depts. Psychology and Physiology & Biophysics, Univ. Vermont, Burlington, VT 05405.

A number of recent studies have shown that the spontaneously hypertensive rat (SHR), a strain derived from the normotensive Wistar Kyoto rat (WKy), is hyperactive as well as hypertensive. Increased locomotor activity in the open field test was found in both young (4 - 5 wk) and older (9 - 13 wk), male and female SHR (Myers et al., Fed. Proc. 36, 1977). We now report that both peripheral and central injections of d-amphetamine decrease locomotor activity in the SHR.

In the first study 10 - 12 wk old SHR and WKy rats were injected with d-amphetamine (5 mg/kg i.p.), and after 45 min were tested for 5 min in an open field. We found that while the WKy gave the expected increase in locomotor activity, SHR demonstrated a significant reduction in activity. In a second experiment SHR and WKy rats were given systemic d-amphetamine in dosages of 1.0, 2.0 or 5.0 mg/kg i.p., and then tested in an automated activity box for 1 h following injection. As in the previous study, WKy increased while SHR decreased their activity in a dose-dependent fashion and at all doses tested.

In the next experiment we sought to localize a central nervous system structure involved in these effects of d-amphetamine. The nucleus accumbens is known to be important in the control of spontaneous locomotor activity, and it is known that d-amphetamine injection into this area increases locomotor activity (van Rossum et al., Adv. Biochem. Psychopharm. 16:201, 1977). Female SHR and WKy rats were bilaterally implanted with cannulae in the n. accumbens, and 1 wk later were infused with either saline (1 µl) or d-amphetamine in saline (10µg in 1 µl), bilaterally in the n. accumbens. Results indicated that following saline infusion, activity of the SHR was significantly greater than the WKy. However, after infusion of d-amphetamine, WKy rats increased their activity while SHR showed a dramatic decrease in activity.

These data suggest that the SHR may provide an animal model for examining the paradoxical responses to d-amphetamine found in hyperactive children.

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2244 DIFFERENTIAL OPIATE EFFECTS ON INTRACRANIAL SELF-STIMULATION THRESHOLDS IN SUBSTANTIA NIGRA (A-9) AND VENTRAL TEGMENTUM (A-10). Jules M. Nazzaro*, Thomas F. Seeger* and Eliot L. Gardner. Depts. of Psychiatry, Neuroscience and Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, New York 10461.

In order to differentiate the roles of the nigro-striatal and mesolimbic-mesocortical dopamine systems in the action of opiates on dopaminergically mediated intracranial self-stimulation (ICSS), the effects of chronic morphine and acute naloxone on ICSS was tested in rats with electrode placements in the substantia nigra pars compacta (A-9) and the ventral tegmentum (A-10). To control for non-selective alterations in motor activity, arousal level or coordination, a rate-independent threshold reward titration paradigm for ICSS was utilized.

At the doses used, rats with electrodes in A-9 showed a large increase in ICSS threshold following both acute and chronic morphine treatment. Little or no tolerance to this threshold elevating effect of morphine was observed with 5 days of chronic administration. The threshold elevation was not reversed by naloxone given 0.5 hour after the final morphine treatment. In contrast, acute morphine significantly lowered self-stimulation threshold in rats with A-10 placements. Tolerance to this facilitatory effect was evident with chronic administration. Naloxone attenuated the lowering of threshold caused by opiate administration in these A-10 animals. Also, acute naloxone, administered prior to initiation of the morphine regimen, had no effect on basal self-stimulation threshold in either brain locus.

The present data suggest a specificity of action of opiates on different brain systems subserving reward and reinforcement. These findings also suggest that the mesolimbic-mesocortical system may constitute one focal area mediating the rewarding properties of morphine. (Supported, in part, by USPHS grant DA 01560.)

2245 NALOXONE REVERSIBILITY OF ETHANOL'S EFFECTS ON SEXUAL BEHAVIOR OF THE FEMALE SYRIAN HAMSTER. Jeffrey L. Nelson*, Nancy L. Ostrowski, Ralph G. Noble*, and Larry D. Reid. Dept. of Psychol., Rensselaer Polytechnic Institute, Troy, NY 12181.

This study evaluated the potential for naloxone to reverse ethanol's suppression of female sexual reflexes. The subjects were 24 ovariectomized, estrogen and progesterone-primed female hamsters, randomly assigned to 4 groups. Each group received two injections: (a) ethanol and naloxone, (b) ethanol and saline, (c) saline and naloxone, or (d) saline and saline while measurements of sexual behavior were made before and after each injection. Each subject's sexual reflexes were tested immediately before and again 10 min after a subcutaneous (sc) injection of ethanol (1.5 ml/kg in 10% solution) or physiological saline (15 ml/kg). Sexual reflexes, as measured by lateral displacement (the amount of lateral movement obtained when a tactile stimulus is applied to a female's perineal region while in a lordotic posture), were reduced about 50% with this dose of ethanol [saline vs ethanol, $t(22) = 7.47$, $p < .001$].

Following the 2nd test, i.e., the test for ethanol's effects, each subject received a sc-injection of naloxone hydrochloride (4 mg/kg) or physiological saline (1 ml/kg). Then they were again tested at 5 min and 20 min after this 2nd injection. Comparing subjects' difference scores (found by subtracting a subject's baseline score from the score in question) between groups getting naloxone or saline after ethanol yielded a $t(10)$ for the measurement 5 min after 2nd injection = 2.03, $p < .05$; $t(10)$ for 20 min after 2nd injection = 3.32, $p < .01$. The difference scores for subjects receiving ethanol and then naloxone were (a) under ethanol = -26.8, (b) under ethanol and naloxone, 5 min after injection = -8.5, and (c) under ethanol and naloxone, 20 min after injection = -4.5 compared to the same measurements of subjects receiving ethanol and then saline of -29.8, -29.2, and -32.3, respectively. Hamsters not receiving ethanol typically showed scores at or above baseline with injections and multiple measurements.

These results suggest the possibility of opioid involvement in ethanol's capability to modify female sexual behavior. A preliminary test with males provides some support for the idea that the effects may not be limited to female sexual behavior. (Supported by NSF grant BNS 78-17860.

2246 BEHAVIORAL COMPENSATION, MUSCARINIC RECEPTORS AND TOLERANCE DEVELOPMENT TO PHYSOSTIGMINE. David H. Overstreet* and Grant D. Schiller* (SPON: Tim Schallert). School of Biological Sciences, Flinders University, Bedford Park, South Australia 5042 Australia.

The contribution of behavioral compensation to tolerance development to physostigmine (0.5 mg/kg once daily) in rats was assessed by comparing the rates of tolerance development to physostigmine's suppressant effects on operant behavior (responding for water reward on an FR5 schedule) in groups treated chronically either before or after the operant session. The groups exhibited comparable rates of tolerance development to physostigmine, indicating that behavioral compensation does not plan a major role.

Challenges with the muscarinic receptor agonist, pilocarpine, revealed that the operant responding of the physostigmine-tolerant and control groups was suppressed to comparable degrees, suggesting that a decrease in the sensitivity of muscarinic receptors does not play a role in tolerance development to physostigmine. Binding assays with the specific muscarinic antagonist, ^{3}H-Quinuclidinyl benzilate, revealed similar densities of muscarinic binding sites in the tolerant and control groups, suggesting that a change in muscarinic receptor properties is also not involved in tolerance development to physostigmine.

These findings suggest that behavioral compensation and alterations in muscarinic receptors are unlikely mechanisms underlying tolerance to physostigmine. Therefore, mechanisms underlying tolerance to carbamate anticholinesterases appear to be different from those underlying tolerance to organophosphate anticholinesterases (See Schiller, Life Sciences 24, 1159-1164, 1979). (supported by a grant from the Australian Research Grants Committee to D.H. Overstreet and USPHS grants to H.I. Yamamura).

2247 CENTRAL CYCLIC NUCLEOTIDE SYSTEMS: MODIFICATION BY DIHYDROXY CHLORPROMAZINES. Gene C. Palmer, S. Jo Palmer* and Albert A. Manian*. Dept. Pharmacol., Univ. So. Ala. Col. Med., Mobile, AL 36688 and the Psychopharmacol. Res. Br., NIMH, Rockville, MD 20857.

Chlorpromazine (CPZ) is used primarily to treat symptoms of psychosis. The central action of CPZ is thought to be due to an antagonism of dopamine (DA) and norepinephrine neurotransmitter actions via a blockade of postsynaptic receptors coupled to the adenylate cyclase systems. A large number of metabolites of CPZ have been shown to possess varying degrees of pharmacological activity toward central adenylate cyclase systems. One such group, the dihydroxy CPZs, especially 7,8-dioxo- and 7,8-diOH- and 3,7,8-triOH-CPZs display potent antagonism of various components of the adenylate cyclase receptor complex, i.e., basal, receptor and fluoride-sensitive catalytic sites. Our interest has been to understand the molecular action of this highly active group. The kinetic profile of 7,8-diOH-CPZ inhibition of basal or DA activated adenylate cyclase (homogenates of rat frontal cortex) appeared to be noncompetitive with respect to ATP concentration. The elevation by Mg^{++} of basal, GTP and DA components of adenylate cyclase were all antagonized by 7,8-diOH-CPZ, an event not overcome by adding excess Mg^{++}. Increasing the concentration of Ca^{++} elevated basal activity but reduced stimulation by DA and GTP analogs. 7,8-diOH-CPZ did not influence enzyme activity by increasing the content of Ca^{++}. Alternatively in recent work, a number of diOH analogs (3,8-diOH; 3,7-diOH; 6,9-diOH; and 7,9-diOH) appeared to elevate both basal and DA-activation of adenylate cyclase. These compounds were subsequently shown to inhibit cyclic AMP-dependent phosphodiesterase (high Km, low Km and the high Km-Ca^{++}-dependent activator form). The latter enzyme subtype was especially susceptible to inhibition. The findings indicate a complex series of interactions by diOH-CPZs at synaptic sites involving central catecholamine mechanisms. As to how these effects influence the therapeutic or adverse profile of the parent compound in patients remains an unanswered question. (Supported by the Epilepsy Foundation of America).

2248 KANAMYCIN EFFECTS ON EXPLORATION, AGGRESSION, AND SEIZURE SUSCEPTIBILITY IN MICE. V. P. Perdue*, W. W. Eastman*, K. M. Michels*, E. A. Fink*, P. J. Donovick and R. G. Burright*. Dept. Psych., SUNY Binghamton, Binghamton, N.Y. 13901.

Kanamycin is an antibiotic which has effects similar to streptomycin. It shares broad range antimicrobial properties of streptomycin and related compounds and has similar side effects including ototoxicity and nephrotoxicity. Both cochlear and vestibular functions may be impaired. Loss in high frequency perception is the most common auditory side effect. The present investigation examined the behavior of adult mice as a consequence of the administration of kanamycin.

Singly housed adult male Binghamton heterogeneous stock (HET) mice, were injected daily for twenty days, continuing until the mice were approximately 70 days of age when behavioral testing began. Mice received either 400 mg/kg of kanamycin sulfate (supplied by Dr. M. J. Weinstein, Schering Corporation) or equal volume saline injections. The two consecutive days of behavioral testing included home cage observations, open field exploration measures, and aggression testing. For aggression testing, pairs of either kanamycin or saline injected mice were separated from each other by a barrier in a single cage for two days. On the third day the barrier was removed and agonistic behavior observed for 30 minutes. Most mice fought and latency to contact and fight was approximately equal for the two treatment groups. However, kanamycin mice fought much more frequently during the first 15 minutes of the test period. The rate of fighting per minute was less in the saline control group and distributed more evenly throughout the entire 30 minute test period.

In their home cage kanamycin treated mice were less active under these conditions than their control counterparts. However, when tested in the open field for 3 minutes, kanamycin treated mice were more active than their control counterparts. The introduction of a flashing light during the second minute of testing had differential effects on the two groups. Finally, frequency and duration of transcorneally induced seizures was enhanced by kanamycin.

These results suggest that prolonged administration of kanamycin affect a wide range of behaviors. We are currently examining the effects of kanamycin injection in preweanling animals and their ability to recover from such trauma. We are also in the process of attempting to differentiate the auditory consequences of kanamycin injections from its other undesirable side effects.

2249 ANTICHOLINERGIC ACTIVITY OF IMIPRAMINE ANALOGS AT MUSCARINIC RECEPTORS OF CULTURED MOUSE NEUROBLASTOMA CELLS. Ronald C. Petersen* and Elliott Richelson (SPON: B. Westmoreland) Mayo Medical School, Depts. of Psychiatry and of Pharmacology, Mayo Fdn., Rochester, MN 55901

The interaction of tricyclic antidepressants with neurotransmitter receptors may explain their therapeutic and/or adverse pharmacological effects. Previously, we studied a number of these drugs for their ability to antagonize muscarinic acetylcholine and histamine H_1 receptors. To obtain more definitive structure-activity information for the anticholinergic activity of tricyclic antidepressants, we studied imipramine and several of its analogs for their ability to inhibit muscarinic receptor-mediated cyclic GMP (cGMP) synthesis by mouse neuroblastoma cells (clone N1E-115) with the use of an assay technique described in detail elsewhere.[1] For these experiments different groups of cells were incubated with increasing concentrations of antagonist (i.e., the imipramine analogs) for 30 min in a shaker bath at 37° prior to stimulation with carbamylcholine (250 µM) for 30 sec. The concentration of antagonist required for half-maximal inhibition of cGMP synthesis was determined and the inhibition constant (K_i) was calculated assuming competitive inhibition at the muscarinic receptor and using a K_A = 200 µM for carbamylcholine (E. Richelson, unpublished data). The K_i's were essentially identical to the equilibrium dissociation constants (K_B) as determined by the dose-ratio method for the compounds which were tested by both techniques. The rank order of potency of these drugs as muscarinic antagonists was as follows: imipramine = 3-chlorimipramine > desmethylimipramine > 3-chloro-2-hydroxyimipramine ≥ 2-hydroxyimipramine >> didesmethylimipramine. The most potent drugs, imipramine and 3-chlorimipramine, were about 30 times more potent than the least potent compound, didesmethylimipramine. These results suggest the following structure-activity relationships for imipramine and its analogs: 1) The type of amine on the side chain is predictive of antimuscarinic activity with tertiary > secondary > primary; 2) 2-hydroxyl ring substitution markedly reduces activity of these compounds; and 3) 3-chloro and N-oxide substitutions have no effect on antimuscarinic activity. These structure-activity relationships for imipramine and its analogs at the muscarinic receptor may have important implications for predicting some side effects of these drugs in vivo. (Supported by Mayo Foundation and USPHS Grants MH 27692 & DA 1490)

[1]Richelson, E., Prendergast, F.G. and Divinetz-Romero, S. Muscarinic receptor-mediated cyclic GMP formation by cultured nerve cells: Ionic dependence and effects of local anesthetics. Biochem. Pharmacol., 27:2034-2048, 1978.

2250 THE EFFECT OF AMITRIPTYLINE ON BRAIN ETHANOL CONCENTRATIONS. S. Preskorn, and P. Alward* Dept. Psy., Sch. Med., KUMC, Kansas City, KS 66103

Tricyclic antidepressants (TCA) are known to interact with ethanol. However, these interactions are unpredictable. Both enhancement and antagonism have been reported. Recently TCA have been shown to increase the cerebral extraction fraction of diffusion-limited substances such as water and ethanol (Preskorn and Hartman, Biol. Psychiat. 14:235, 1979). For amitriptyline (AMI) this increase results from an augmentation in capillary permeability (Preskorn et al., Neuroscience Abstracts 5:500, 1978).

Based upon these findings, it was hypothesized that the AMI-induced enhancement of permeability would increase brain ethanol concentrations if ethanol is administered in conjunction with AMI. To test this hypothesis, rats were given intraperitoneally (i.p.) either: (I) ethanol alone (2gm/kg) or (II) ethanol plus AMI (62.5µmols/kg). Animals were decapitated at 5',15',30',45', and 60'. The brain and plasma ethanol concentrations were determined by gas chromatography.

The results are as follows. The absolute plasma ethanol concentrations did not differ between the two groups, indicating that AMI did not alter intraperitoneal absorption of ethanol. However, AMI did increase the brain:plasma concentration ratio at 5',15' and 30' (Table I, $0<0.001$). Ratios at 45' and 60' were lower than at 30' but still elevated above the control condition.

	Brain:Plasma Ethanol Concentration Ratio			
Time	Ethanol Alone	(n)	Ethanol + AMI	(n)
5'	0.72±0.03	(3)	0.96±0.00	(3)
15'	0.75±0.04	(4)	0.90±0.03	(6)
30'	0.77±0.02	(7)	0.93±0.01	(6)
45'&60'	0.74±0.02	(3)	0.84±0.05	(3)

Thus, AMI increases not only the diffusion of alcohol across the cerebral capillary but also results in a net influx of ethanol into the brain. This new observation has both clinical and basic neuroscience implications with regard to understanding drug interactions such as TCA and ethanol.

2251 BEHAVIORAL EFFECTS OF CHRONIC EARLY LEAD EXPOSURE IN MONKEYS. Deborah C. Rice* (SPON: V. G. Laties). Dept. Health and Welfare, Health Protection Branch, Tunney's Pasture, Ottawa, Ontario, Canada.

Monkeys (Macaca fascicularis) dosed orally from birth with 500 ug/kg/day of lead as lead acetate showed deficits compared to controls on a series of 20 form discrimination reversals at 2-3 years of age. There was no difference between groups in the effect of "overtraining" trials introduced between reversals. Treated monkeys also behaved differently than controls during initial sessions of a multiple fixed interval (FI) 8 min. - time out schedule of reinforcement, with lead exposure producing increased FI response rates, a "bursting" pattern of FI responding, and increased time out responding. Subtle differences in the pattern of FI responding persisted throughout the course of the study as evidenced by differences in inter-response time distribution. Blood lead levels at the time of behavioral testing were 20-40 ug/dl, which are comparable to lead levels of many children.

2252 THE EFFECT OF 6-OHDA LESIONS OF THE NUCLEUS ACCUMBENS ON COCAINE FACILITATION OF INTRACRANIAL SELF STIMULATION AND SELF ADMINISTRATION. D.C.S. Roberts, G.F. Koob, H.C. Fibiger, M.E. Corcoran, and F.E. Bloom. (SPON: R.Nick Hogan) A.V. Davis Ctr. for Behav. Neurobiology, Salk Inst., La Jolla, CA 92037 and Div. of Neurological Sciences, Univ. of British Columbia, Vancouver, Canada.

The effect of bilateral infusions of 6-hydroxydopamine (6-OHDA) into the n.accumbens was assessed on two measures of cocaine reinforcement: intravenous self-administration and facilitation of intracranial self-stimulation (ICSS).

Male Wistar rats were prepared with intravenous cannulae and trained to self administer cocaine at a dose of 0.75 mg/kg/inj. Injections were delivered with each depression of a lever during a daily 3 hr session. After self injection of cocaine had stabilized each rat received bilateral infusions of 6OHDA into the n.accumbens. All rats (N=15) showed an initial abstinence from cocaine self-administration. However, many rats showed a gradual recovery of cocaine intake. The number of post lesion days required to reach 50% recovery of pre-lesion cocaine intake correlated (r=0.81) with dopamine (DA) content remaining in the n.accumbens. The same rats which failed to respond for cocaine continued to self administer apomorphine (0.6 mg/kg/inj.) at pre lesion rates.

In an effort to establish if these lesions would also block the facilitation of ICSS from cocaine, 16 rats were prepared with electrodes in the posterior hypothalamus. Each day rats were allowed to press a lever for intracranial electrical stimulation and were tested for 5 min at eight current levels in a descending series. Half of the animals received bilateral injection of 6-OHDA (8ug/2ul) into the n.accumbens while the remaining half received vehicle injections (saline containing ascorbic acid, 0.1mg/ml). Twelve days following the lesion the animals were injected with cocaine (10mg/kg) 15 min prior to the test session. In the control animals, cocaine produced a significant shift to the left of the rate/intensity function. By contrast, the 6-OHDA group showed an attenuation of this cocaine facilitation of ICSS. These results add further support to the hypothesis that mesolimbic dopamine serves a critical role in cocaine reinforcement.

2253 AMPHETAMINE SENSITIZATION, STRESS AND GENETIC HYPERTENSION. N. Rowland, A.J. Eichler, S.M. Antelman, J. Shipley, D.Kocan and L. DeGiovanni. (SPON: J.R. Jennings). Western Psychiatric Institute & Clinic, Univ. of Pittsburgh, PA 15260.

Animals are known to exhibit sensitization with repeated amphetamine (AM) administration: in particular, single doses of AM can cause enhanced stereotyped behavior to a subsequent dose several days later. In man, AM psychosis closely resembles some forms of schizophrenia, a disease whose etiology is a combination of genetic factors and environmental stress.

In the first set of experiments, we have investigated whether stress and AM may act upon the same neural substrate(s) involved in sensitization in rats. We have already shown (Neurosci. Abstr. #1544, 1978) that repeated application of a mild stress, tail pressure (TP) produces an enhanced stereotyped response to a single dose of AM. We now report the converse experiment; a single dose of AM can produce a long-lasting "sensitization" of TP-induced eating and gnawing. That is, when tested up to 30 days after the AM injection, rats show a smaller haloperidol (HALO; 0.4 mg/kg)-induced suppression of TP behaviors than do vehicle injected controls. Similarly, a single session of footshock stress produced an enhanced AM stereotypy response two weeks later and a decreased efficacy of HALO in the TP test. To identify brain structures which might be associated with sensitization, we examined the effect of another stressor, chronic self-stimulation, which offers the advantage of site specificity, on the ability of AM later to induce stereotyped sniffing and anorexia. Nucleus accumbens and medial frontal cortex self-stimulators (but not rats with A-9 placements) showed a significant enhancement of each of these effects of AM when tested 24 hr after the last stimulation day. Since we have previously reported that food deprivation stress can produce similar sensitization, it appears that a wide variety of stress can induce the effect.

In a second experimental series we have investigated the behavioral effects of AM in spontaneously hypertensive rats (SHR) which are known to show enhanced behavioral and adrenomedullary responses to stress and therefore may be thought of as a genetic or naturally stressed preparation. We report that single doses of AM produce more intense and prolonged stereotyped behavior in SHR rats than in either WKY or Sprague Dawley (SD) controls. HALO was less effective in blocking TP behavior in SHRs than in controls, but was more effective in inducing catalepsy in these animals. This last finding suggests that the level of activation of the testing situation is an important variable in assessing the reactivity of brain catecholaminergic systems.

2254 EFFECTS OF CHRONIC ADMINISTRATION OF MORPHINE ON THRESHOLDS FOR ESCAPE BEHAVIOR MAINTAINED BY INTRACRANIAL STIMULATION. Stephen Sasson*, Howard S. Wheeling*, and Conan Kornetsky. Laboratory of Behavioral Pharmacology, Boston University School of Medicine, 80 E. Concord St., Boston, MA. 02118

It has been previously demonstrated that acute doses of morphine lower the threshold for intracranial self-stimulation (ICSS) and raise the threshold for escape from stimulation to "aversive" areas of the brain (Marcus & Kornetsky, Psychopharm., 38:1, 1974). Work in our laboratory has also shown that tolerance does not develop to the threshold lowering effect of morphine on ICSS (Esposito & Kornetsky, Science, 195:189, 1977). The present study is an attempt to determine whether or not tolerance develops to the threshold raising effect of morphine for escape from aversive brain stimulation. Six male albino rats were implanted with bipolar electrodes aimed at the mesencephalic reticular formation (RF). They were then trained to turn a wheel manipulandum in order to escape RF stimulation. Thresholds were determined by means of a modification of the psychophysical method of limits. After training and prior to the initiation of daily morphine injections, data were collected for at least 5 days in which saline was administered. Animals were given daily doses of morphine (4-20 mg/kg, s.c.) for 14 to 40 days. Starting with the first day and every 2 to 3 days thereafter, thresholds were determined before and 25 min after a test dose of morphine. The dose of morphine selected as the test dose initially raised the threshold at least 2 standard deviations above the mean saline level for the respective animal. Results indicate that tolerance does develop to the threshold raising effect of morphine; however, this loss of threshold raising effect is often contaminated by the disruption of behavior caused by abstinence. These results show that the phenomenon of tolerance to the antinociceptive effect of morphine is observable whether peripherally or centrally applied noxious stimuli are utilized. (Supported by NIDA Grant DA 00377, Biomedical Research Support Grant at Boston University School of Medicine, and Research Scientist Awardee MH 1759 - CK)

2255 ^{3}H-SEROTONIN RECEPTOR BINDING IN RAT BRAIN: DIFFERENTIAL EFFECTS OF MONOAMINE OXIDASE INHIBITORS AND SEROTONIN RE-UPTAKE INHIBITORS Daniel D. Savage*, Alan Frazer*, and Joe Mendels* (SPON: G.B. Koelle). Dept. Pharmacol., Univ. of Penn. and Veterans Administration Hospital, Phila., Pa. 19104.

The effect of repeated administration of monoamine oxidase inhibitors and serotonin reuptake inhibitors on 5-hydroxytryptamine (serotonin; 5HT) concentration and serotonin receptors in rat cerebral cortex and pons was examined. Serotonin concentrations were measured by the fluorometric technique of Curzon and Green (Brit. J. Pharmacol. 39:653, 1970). The receptor binding assay of Bennett and Snyder (Mole. Pharm. 12:373, 1976) was used to measure serotonin receptors.

While the administration of amitriptyline (10mg/kg, bid, for 16 days) caused no significant alteration either in the concentration of serotonin or the binding of ^{3}H-5HT in the brain regions examined, injection of the monoamine oxidase inhibitor tranylcypromine (5mg/kg/day) for an equivalent period of time caused a significant reduction in ^{3}H-5HT binding, both in cerebral cortex and pons.

At no time up to 16 days of administration, did either of the serotonin reuptake inhibitors fluoxetine (10mg/kg, bid) or chlorimipramine (10mg/kg, bid) change ^{3}H-5HT binding in comparison with that measured in control rats. In contrast, a significant reduction in ^{3}H-5HT binding and an increase in the concentration of serotonin was measured in cerebral cortex and pons 24 hours after single injection of the monoamine oxidase inhibitor nialamide (40mg/kg). This effect persisted up to 16 days of treatment with nialamide.

Treatment with the relatively selective A type monoamine oxidase inhibitor clorgyline (1.0mg/kg/day, for 4 days) resulted in a significant increase in brain serotonin and a reduction in ^{3}H-5HT binding, while treatment with the B type inhibitors deprenyl (1.0mg/kg/day, 4 days) or pargyline (0.5mg/kg/day, 4 days) resulted in no change either in binding or brain serotonin concentrations.

Analysis by the method of Scatchard of the changes seen in cerebral cortex due to clorgyline treatment for four days indicated an increase in the apparent binding constant (K_D) with no change in the maximum specific binding capacity (B_{max}). Treatment of rats with nialamide for 16 days resulted both in an increase in the K_D value and a decrease in B_{max}.

In conclusion, chronic treatment of rats with serotonin reuptake inhibitors has no effect on ^{3}H-5HT binding, while type A monoamine oxidase inhibitors caused significant elevations in brain serotonin levels and decreased ^{3}H-5HT binding. (Supported by Research Funds from the Veterans Administration, NIMH Grant 29094, and USPHSGM 07302).

2256 ACUTE EFFECTS OF NEUROLEPTICS ON BRAIN SELF-STIMULATION CURRENT THRESHOLDS IN RATS. Gerald J. Schaefer and Richard P. Michael, Dept. Psychiatry, School of Medicine, Emory University and Georgia Mental Health Institute, Atlanta, GA 30322.

Rats were implanted with stimulating electrodes in the medial forebrain bundle and trained in a brain self-stimulation procedure. Each response on a conventional lever produced an electrical stimulus that was initially set at a suprathreshold intensity. Every fifth lever-press reduced the stimulation current by 5 μAmp and animals could reset the current to its initial intensity by pressing an omnidirectional lever (reset lever). The average current intensity at which the animal pressed the reset lever was defined as the reinforcement threshold. After thresholds stabilized, dose-response curves were obtained for five neuroleptic drugs, each representing a different chemical family of antipsychotic compounds. Haloperidol (0.01-0.10 mg/kg) and loxapine (0.03-0.56 mg/kg) produced dose-dependent increases in reinforcement thresholds which were accompanied by reductions in response rates (behavioral performance). Chlorpromazine (0.10-3.0 mg/kg) did not produce significant changes in reinforcement thresholds, but did produce dose-dependent reductions in response rates. Behavioral disruption (when animals failed to make any resets) occurred at the two highest doses (1.75 and 3.0 mg/kg). Although dose-dependent reductions in response rates also occurred with pimozide (0.1-1.75 mg/kg), an increase in threshold only occurred at the highest dose. The 1.75 mg/kg dose also produced behavioral disruption. Clozapine produced increases in reinforcement thresholds from 0.10-1.75 mg/kg without significant changes in response rates. When 3.0 mg/kg of clozapine was administered, a marked disruption of behavior occurred. Thus, neuroleptics differ with respect to their effects on reinforcement thresholds and on behavioral performance. The three patterns were: (1) an increase in reinforcement thresholds with a corresponding decrease in response rates (haloperidol and loxapine), (2) no changes in reinforcement thresholds, but a marked, dose-dependent decrease in response rates (chlorpromazine and pimozide), and (3) an increase in reinforcement thresholds without any changes in response rates (clozapine).

A distinction can, therefore, be made between the effects of neuroleptics on central reinforcement thresholds and on motor behavior which helps us interpret the relation between chemical and clinical potency.

2257 DIFFERENTIAL EFFECTS OF ANTIANXIETY AND ANTIPSYCHOTIC DRUGS ON TWO DISTINCT SUBSYSTEMS OF AROUSAL. T. Schallert, C. H. Wang*, S. Hsiao* & I.Q. Whishaw. Depts. of Psychology, Univ. Illinois, Champaign, IL 61820, Univ. Arizona, Tucson, AZ and Univ. Lethbridge, Alberta Canada.

So-called active states of consciousness such as arousal, alertness, and wakefulness traditionally have been defined, in large part, by the presence of cerebral activation (i.e., desynchronized neocortical EEG activity), movement or an upright standing posture, open eyes, and behavioral responsiveness to sensory stimuli. Recently Vanderwolf and others have shown that there are two pharmacologically distinct inputs to the cortex, each one of which is capable of producing cerebral activation, and each has different relations to behavior. One is active during immobility and the other is active during "voluntary" movement. The present study was designed to determine whether antianxiety drugs (e.g., chlordiazepoxide) could affect the immobility-related system without influencing the movement-related system. To do this, we developed a technique that involves the use of an antipsychotic drug (haloperidol), which we found could centrally inactivate the movement-related system so that the immobility-related system can be studied in isolation (that is, without permitting the animal to stay "aroused" simply because movement occurs; agents such as curare that stop movement peripherally are not useful in this regard because although the animal cannot overtly move, it can still "try" to move, which activates the movement-related input to the cortex). To measure arousal, or the lack of it, during immobility, we recorded the EEG and behavioral indices such as posture, eye closure, and reactivity to sensory stimuli. The primary results were that low doses of antianxiety drugs exert profound deactivating effects on behavior during immobility, while having no effect on behavior or EEG when the animal, without the influence of haloperidol, freely moves around. Clinical implications will be discussed.

2258 THE BEHAVIORAL PHARMACOLOGY OF 5-METHOXY N,N-DIMETHYLTRYPTAMINE (5-MeODMT) IN PRIMATE SOCIAL COLONIES. R.F. Schlemmer, Jr., N. Narasimhachari, C.B. Tyler, & J.M. Davis, Illinois State Psychiatric Institute, Chicago, IL 60612.

5-MeODMT is a hallucinogenic substance which has been postulated as an endogenous psychotogen in humans. We have previously reported that 5-MeODMT induces abnormal behavior & significantly alters normal affiliative behavior when administered to selected members of primate social colonies (Comm. Psychopharmacol. 1:105, 1977). The present studies were designed to examine the role of certain neurotransmitter systems in the mediation of 5-MeODMT-induced behavior in monkeys. All expts. were performed using stable social colonies of 4-5 adult Stumptail macaques. Each expt. began with at least a 5-day baseline observation followed by administration of 5-MeODMT 0.25 mg/kg to 2-3 monkeys daily for 1 wk. Then the agent to be studied was given both alone & concomitantly with 5-MeODMT for 1 wk. All drugs & saline were given i.m. (except phenelzine). Drugs tested in the study included: the serotonin (5-HT) antagonists, cinanserin (CINAN) 5 mg/kg & metergoline (METER) 0.3 mg/kg, the dopamine (DA) antagonists, haloperidol (HALO) 0.15 mg/kg & trifluoperazine (TRIFLUO) 0.04 mg/kg, methiothepin (METHIO) 0.15 mg/kg, a 5-HT & DA antagonist, the central cholinesterase inhibitor, physostigmine (PHYSO) 0.06 mg/kg + methscopolamine 0.01 mg/kg, the anticholinergic, atropine (ATROP) 0.2 mg/kg, the antihistamine, promethazine (PROMETH) 4 mg/kg, the sedatives, pentobarbital (PENTO) 7.5 & 10 mg/kg & diazepam (DIAZ) 1 mg/kg, & the MAO inhibitor, phenelzine (PHEN) 4 mg/kg given n.g. All expts. were conducted using a cross-over design so that each monkey in the colony received each treatment. Behavioral observation by a "blind" observer began 5 min. after 5-MeODMT or saline injection & lasted for 1 hr. daily. 5-MeODMT-induced abnormal behavior - body shakes & limb jerks - was antagonized by CINAN, METER, METHIO, HALO, TRIFLUO, & PHYSO. However, 5-MeODMT-induced increase in submissive gestures given by treated monkeys was antagonized by only HALO, TRIFLUO, & METHIO. METER was the only agent tested which at least partially reversed 5-MeODMT-induced social withdrawal & decreased social grooming. On the other hand, PENTO, DIAZ, PROMETH, & ATROP - agents which all possess sedative properties - did not return any 5-MeODMT-induced behavioral change to baseline levels. Inhibition of MAO by PHEN significantly potentiated 5-MeODMT-induced body shakes, limb jerks, increased submissiveness, & reduction in social grooming. These results suggest that the major behavioral changes induced in monkeys by 5-MeODMT are mediated via 5-HT & DA systems. In addition, cholinergic systems may also play a modulatory role in the mediation of 5-MeODMT-induced abnormal behavior in monkeys.

2259 REVERSAL OF DOPAMINERGIC BEHAVIORAL SUPERSENSITIVITY: EFFECTS OF CHRONIC L-DOPA AND LITHIUM. Thomas F. Seeger* and Eliot L. Gardner, Depts. of Pharmacology, Psychiatry, and Neuroscience, Albert Einstein College of Medicine, Bronx, New York 10461.

We have previously demonstrated that chronic haloperidol treatment (21 days at 1 mg/kg/day) causes an increase in intracranial self-stimulation (ICSS) rate in rats implanted with electrodes in the ventral tegmental nucleus. This increase persists for three weeks at full magnitude, before declining to baseline rates at four weeks after the termination of haloperidol treatment. This long-lasting period of supersensitivity allows for the testing of pharmacological agents which may modify or reverse the neuroleptic-induced increase in ICSS rate. In the first experiment, 31 rats which demonstrated ICSS at high rates were given three consecutive tests to establish a baseline rate. 16 of the rats were then given three weeks of haloperidol injections. The rats were retested at 3, 5 and 7 days after haloperidol withdrawal, and at this time demonstrated an average 50% increase in ICSS rate. After the test on day 7, 8 of these rats were given 100 mg/kg L-DOPA plus 20 mg/kg Carbidopa daily for the next seven days. These rats showed a progressive decline in ICSS rate, leveling off at 120% of baseline rate, and persisting at this level for one week after the last L-DOPA treatment. The 8 rats which received haloperidol, but not L-DOPA, maintained their supersensitive response rate (50% of baseline) during this period. 5 rats given L-DOPA and Carbidopa for one week without previous haloperidol treatment showed no change in ICSS rate either during or after the L-DOPA treatment. 10 control animals given saline injections for three weeks also showed no significant changes. These results indicate that chronic L-DOPA treatment can cause a reversal of neuroleptic-induced behavioral supersensitivity, at a dosage level which does not by itself cause subsensitivity (i.e. a decrease in ICSS rate). In the second experiment, 8 rats were given daily lithium carbonate in their food (1600 mg/kg of powdered chow) concurrently with three weeks of haloperidol injections (1 mg/kg/day). In this group, a small but significant increase in ICSS rate was seen at 3 and 5 days after the last haloperidol injection, with the rate declining to baseline levels at day 7. This result suggests that lithium treatment attenuates both the magnitude and time course of neuroleptic-induced supersensitivity. The current results demonstrate the reversal of supersensitivity in a behavior mediated by the mesolimbic dopamine system, and agree with the findings of other investigators who have used behaviors mediated by the nigro-striatal dopamine system. They suggest the possible usefulness of pharmacological desensitization in disease states which may involve abnormal receptor sensitivity, such as tardive dyskinesia and schizophrenia. (Supported, in part, by USPHS research grant DA-02089).

2260 PLATELET MAO IN HYPERACTIVE BOYS TREATED WITH D-AMPHETAMINE. Walid O. Shekim, Leonard G. Davis, Javaid Javaid, Eric G. Brunngraber, and Lucy Fikes*. Dept. of Psychiatry, Sch. Med., Univ. Missouri, Columbia, MO 65212 and Illinois State Psychiatric Instit., Chicago, IL (Dr. Javaid).

The monoamines are thought to play an important role in the pathophysiology of hyperactivity in children. Recent studies are suggesting a role for serotonin, norepinephrine (NE), and dopamine (DA) in the etiology of childhood hyperactivity. We recently have presented data suggesting possible involvement of NE and DA in the etiology of hyperactivity (Shekim et al., Am. J. Psychiat., 134:1276, 1977; Shekim et al., Sci. Proc. Am. Psychiat Assn., 132, in press, 1979). We found that urinary 3-methoxy-4-hydroxyphenylglycol (MHPG), the main metabolite of CNS NE was significantly lower in hyperactive boys than controls. We also found that MHPG correlated negatively with urinary homovanillic acid (HVA), the main metabolite of DA. The administration of d-amphetamine significantly decreased MHPG excretion.

Hyperactive boys were admitted to a Clinical Research Center (CRC) after being free from medications for at least two weeks. They were placed on VMA and serotonin free diet from day 1 of admission. Bloods were drawn in the A.M. while still in bed and fasting on the 4th and 5th day of admission for assay of MAO. They were discharged and then placed on d-amphetamine 0.5 mg/kilogram body weight divided over two doses daily for two weeks, and were readmitted at the end of the two weeks to the CRC. They were placed on VMA and serotonin free diet from day 1 of admission and bloods were drawn on the 4th and 5th day in a similar fashion to the first admission. MAO was assayed according to the method of Murphy et al (Biochem. Med. 16, p. 254, 1976). Platelet MAO levels were lower at baseline than after treatment with d-amphetamine. The administration of d-amphetamine increased platelet MAO values in 8 children of the first 9 studied. These findings are discussed in terms of the relationship of MAO to hyperactivity and urinary MHPG levels as well as the catecholamine hypothesis of hyperactivity in children.

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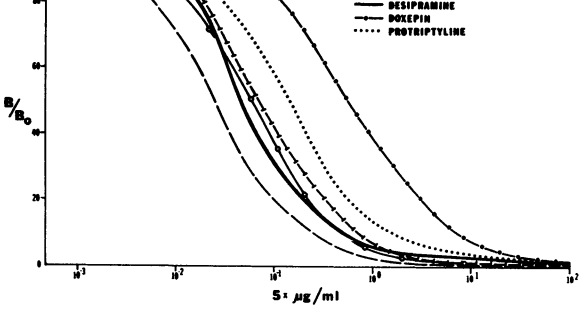
2261 TRICYCLIC ANTIDEPRESSANT DETERMINATIONS BY RADIOIMMUNOASSAY William F. Shivers* and Charles J. Hannan, Jr. (SPON: Gerald O. Carrier) Department of Psychiatry and Clinical Investigation Service, Eisenhower Army Medical Center, Ft. Gordon, GA 30905.

An antiserum was produced in rabbits which was found to be adequate to determine plasma levels of all the clinically available tricyclic antidepressants (TCA). This tool provides a means of titrating the proper TCA plasma levels in depressed patients and maximizes the chances of a successful treatment. Antigen was prepared by conjugating desipramine to bovine serum albumin (BSA) via succinic anhydride and a carbodiimide reaction. The conjugate, after purification by dialysis, was shown to contain desipramine by UV and fluorometric spectroscopy. Six rabbits were innoculated subcutaneously with a divided dose of 2 mg conjugate in a slurry with Freund's complete adjuvant. After eleven weeks a booster injection was given to each rabbit. One week later animals were exsanguinated by cardiac puncture and the serum collected. Diluting with phosphate buffered saline (PBS), pH 7.5, the optimum concentration of antiserum was found to be 1:300 with our assay conditions, which are as follows in the order they are added: 0.2 ml 0.05 M EDTA in PBS with 1:400 normal rabbit serum; 0.015 ml ^{3}H-imipramine, 2.5 uCi/ml; 0.01 ml unknown or standard; 0.2 ml antibody, 1:300 dilution; 0.5 ml PBS with 1% BSA. This was incubated at 4°C for 2 hours with appropriate control tubes and a second antibody used to separate the free from the bound fraction.



2262 EFFECT OF SOME AMPHETAMINE ANALOGUES IN RATS TRAINED TO DISCRIMINATE LSD. Peter B. Silverman and Beng T. Ho. Tex. Res. Institute of Mental Sciences, 1300 Moursund, Houston, TX 77030.

Adult, male Sprague-Dawley rats, food deprived to about 80% of their free feeding weight, were trained to bar press in a two-lever operant box on a fixed ratio (FR) 20 schedule for food reinforcement. Fifteen minutes prior to daily, 20-minute training session, animals were injected i.p. with 75µg/kg LSD (as the free base) or 1.0 ml/kg saline. In those sessions following LSD administration, reinforcement was provided for operating one lever, while in sessions following saline, reinforcement was provided for operating the other lever. Responses on the incorrect lever were counted, but had no consequence. Acquisition of the discrimination between LSD and saline was assessed by determining the number of responses on the lever paired with LSD, out of the first 20 responses (i.e., prior to any reinforcement).

When subjects had acquired the discrimination, and performance was stable, test sessions were interposed among training sessions. Fifteen minutes prior to a test session, subjects were injected with a dose of (+)-, (-)- or (±)-2,5-dimethoxy-4-methylamphetamine (DOM, "STP"), (±)-2,5-dimethoxy-4-bromoamphetamine (DOB), (±)-2,4,5-trimethoxyamphetamine (TMA-2) or (+)-amphetamine. The number of responses on the lever paired with LSD, out of the first 20 total responses, was recorded. A dose response curve for each compound was generated in this manner. With the exception of (+)-amphetamine, all the compounds showed generalization with LSD, i.e., an average of ≥16 of the first 20 responses were emitted on the LSD lever. The order of potency for generalization with LSD was found to be DOB > (-)-DOM > (±)-DOM > (+)-DOM > TMA-2. This order parallels psychotomimetic potency in human beings, with the exception of (+)-DOM which reportedly has neither stimulant nor psychotomimetic activity in man (Shulgin, J. Pharm. Pharmac. 25, 1973). Treatment with the serotonin antagonist, cinanserin, prior to (+)-DOM or (-)-DOM completely blocked the generalization of these compounds with LSD, showing that the two isomers produce their LSD-like effect via the same mechanism. The finding of (+)-DOM generalization with LSD opens to further question the nature of the "hallucinogenic stimulus" in rats.

2263 EFFECTS OF AGE AND CHRONIC NEUROLEPTICS ON RECEPTOR BINDING IN RAT BRAIN. Robert C. Smith, Chandra H. Misra, Harnath Shelat*, Behav. Neurochem.,Tex. Inst. of Men. Sci., Houston, TX 77030

Older-age is associated with changes in catecholamine neurons and neurotransmitters and their receptors. Tardive dyskinesia, one long-term toxic effect seen after chronic administration of neuroleptics to man is much more prevalent in older-age humans. To investigate whether receptor changes may be involved in these phenomenon, we are studying the effects of age and chronic neuroleptic drugs on receptor binding in rat brain. Fisher 344 rats, treated chronically with fluphenazine or saline, sacrificed 9-10 after termination of chronic injections, were used in all experiments. Receptor binding for dopaminergic, adrenergic, and cholinergic receptors was assayed using tritiated spiperone, DHA, WB-4101, DHE, and QNB using established techniques. Our preliminary results show a significant decrease in specific binding of DHA, and WB-4101 in the cortex of old age rats, a significant decrease in spiperione binding in the striatum of old age rats. Kinetic analysis showed a decrease in B_{max} but no change in K_d in the spiperone binding in the striatum of old rats. Specific binding of DHE in the cortex and QNB in the hippocampus showed a curvilinear pattern in relation to age. Prior treatment with chronic neuroleptics increased spiperone binding in the striatum, with a greater relative effect in 10 month as compared to 4 month old rats. Chronic neuroleptics slightly increased DHA binding in the cortex of young rats, but significantly decreased DHA binding in the cortex of old rats. Further kinetic studies of adrenergic and dopaminergic binding, and the interaction of age and chronic neuroleptics on cholinergic vs dopaminergic binding are currently being pursued.

2264 EFFECTS OF CHLORPROMAZINE ON ESCAPE AND AVOIDANCE RESPONSES: A CLOSER LOOK. W. Spirduso, L. Abraham, and M. Wolf*. Dept. of Physical & Health Ed., Univ. of Texas, Austin, TX 78712.

Chlorpromazine (CPZ) has been reported to decrease or abolish avoidance responses, while not influencing escape responses of rats in a shuttle box. Closer analysis reveals that rigorous time constraints placed on rats trained to release a bar from a learned position yield three basic responses; Escape Responses (ER) to the unconditioned stimulus (2.5 mA shock), Avoidance Responses (AR) to a conditioned stimulus (light and buzzer), and Orienting Responses (OR) to both the conditioned and unconditioned stimuli. In this study the effects of CPZ on ER, OR, and AR were analyzed using a highly trained response approaching the animal's speed capacity.

Holtzman rats (15) were trained over a 6 day period to depress and hold a lever and to release it when the auditory/visual stimulus was presented. Minimal latency responses were obtained by reducing the CS-UCS interval in stages from 1000 to 200 msec. Following training, animals were pretested over 50 trials at decreasing intervals, then were injected with CPZ (Thorazine, 3.5 mg/kg). After 40 minutes the animals were retested, ER and AR latencies were determined by bar release, while the latency of the movement initiation was detected by high speed cinematographic analysis. Film analysis also provided characteristics of the movement patterns for ER, AR, and OR.

Avoidance responses increased during training to 60($\pm$ 10)% occurrences by the 6th day for all rats. CPZ produced increased decrements in percent avoidance with decreased CS-UCS intervals, and at the 200 msec. interval only 4 animals were capable of avoiding. Contrary to previous reports, CPZ significantly delayed escape responses. CPZ avoidance latencies were slower only at the least constrained CS-UCS interval of 1000 msec. and at the shortest interval for those few animals capable of avoiding. The most striking of the qualitative differences between ERs and ARs was that the initial component of the ER was a total body jump, while the initial component of the AR was an independent movement of the head and/or forelimbs. A major effect of CPZ on the movement pattern was a change in starting position, which may have been related to the CPZ-induced tendency to begin the AR with a postural adjustment. Finally, film analysis confirmed that CPZ delayed the onset of the AR and expanded the temporal aspects of the movement pattern.

These results show that when escape and avoidance behaviors (a) are initiated at speed capacity, (b) start from a learned and maintained ready position, and (c) are cinematographically analyzed, CPZ produces results that deviate from those previously reported. (Supported by University Research Institute, UT Austin)

2265 EFFECTS OF NALOXONE ON FEEDING AND DRINKING IN RATS. J. M. Stapleton, M. D. Lind*, J. R. Quinan*, T. L. Foley*, M. F. Wu*, and L. D. Reid. Dept. of Psychol., Rensselaer Polytechnic Institute, Troy, NY 12181.

Naloxone decreases certain appetitive behaviors (Holtzman, J. Pharm.Exp.Ther., 1974). Such decreases may be due to naloxone's modification of endorphinergic systems or these effects may be nonspecific, e.g., due to illness-producing effects. This presentation summarizes results of several studies of the effects of naloxone on feeding and drinking, one of which addresses the issue of specificity.

Naloxone reduces water consumption in fluid-deprived rats, using doses as low as 0.5 mg/kg, subcutaneously (sc). Furthermore, naloxone produces decreases in drinking induced by injection of hypertonic saline solution (5 ml/rat of 1.5 M NaCl). Naloxone also probably produces some malaise in rats since it can sustain a conditioned taste aversion (CTA) (LeBlanc & Cappell, Pharmacol.Biochem.Behav., 1975). Since there are stable individual differences in extent of CTA and naloxone's suppression of drinking, we reasoned that if the reductions in drinking were due to the nonspecific illness-producing effects indexed by the CTA-test, we would find a significant positive correlation between these two measures. A group of 19 male rats were tested for CTA using naloxone and subsequently tested for naloxone suppression of drinking. There was no reliable relationship between the two measures, rho = -.03. These results are concordant with the results of Frenk and Rogers (Behav.& Neural Biol., in press) who found that lithium chloride produces a strong CTA but does not suppress drinking, when given under the same testing parameters as naloxone.

Naloxone also produces a decrease in consumption of a 10% sucrose solution by nondeprived rats. A summary of dose-response relationships for this effect will be presented for both systemic and intracerebroventricular administration of naloxone. Doses smaller than 2 mg/kg sc appear to be effective. Also, naloxone reduces the intake of sucrose solution in rats deprived of food and water for 22 h/day.

Diazepam and sodium pentobarbital both induce feeding in rats. Naloxone, in doses as low as 0.25 mg/kg sc blocks feeding induced by diazepam (25 mg/kg, intragastrically) while it does not affect diazepam-induced sedation. Naloxone does not completely block feeding induced by sodium pentobarbital (5 mg/kg sc) in doses up to 10 mg/kg sc.

These results suggest that naloxone may have relatively specific effects on only certain ingestive behaviors and support a hypothesis of endorphinergic modulation of certain appetitive behaviors.

2266 ANTIDEPRESSANTS ATTENUATE A CONDITIONED IMPAIRMENT OF PERFORMANCE. L. K. Stefany*, J. K. Saelens and M. Roffman. Research Dept., Pharmaceuticals Div., CIBA-GEIGY Corp., Summit, NJ 07901

Seligman and Beagley (J. Comp. Physio. Psych. 88: 534, 1975) demonstrated that rats previously exposed to inescapable shock were impaired in their ability to learn a simple shock-escape task. These findings have been confirmed using a modification of their "learned helplessness" model. Further it has been found that this conditioned impairment of performance (CIP) can be attenuated by certain psychoactive drugs, particularly antidepressants.

During conditioning sessions, male, Sprague-Dawley rats (170-200 g) were exposed to 80 trials of inescapable footshock (1.7 mA, 30 sec, VI = 1 min) in an operant chamber either without a lever or with an inoperable lever. Twenty-four hr following the conditioning session the lever was inserted or activated and the animals were tested for 40 trials (1.7 mA, 60 sec, VI = 1 min) for their ability to learn to escape footshock by lever pressing. For the first 10 trials a single lever press terminated the shock (FR 1). For the remaining 30 trials, two responses were required to terminate the shock (FR 2). Rats subjected to the conditioning procedure on day 1 exhibited a CIP on day 2 when compared to those not subjected to this procedure. The presence of an inoperable lever (as opposed to no lever) during the conditioning procedure reduced the variation among animals in second-day performance and thus further differentiated between these two groups.

Intraperitoneal administration of d-amphetamine (1 mg/kg), desipramine (15 mg/kg), imipramine (30 mg/kg), iprindole (30 mg/kg) and fluvoxamine (10 mg/kg) prior to testing on day 2 attenuated the CIP in studies where the lever was not present during conditioning on day 1. In subsequent experiments where the lever was present but inoperable on day 1, iprindole (30 mg/kg), mianserin (30 mg/kg) and welbatrin (30 mg/kg) also attenuated the CIP. Diazepam (10 mg/kg) and scopolamine (1 mg/kg) failed to attenuate the CIP in both paradigms.

The CIP model appears to be sensitive to a wide range of antidepressants, of diverse mechanisms of action, which are useful in the treatment of affective disorders. The results tentatively suggest that drugs capable of facilitating monoaminergic transmission attenuate the CIP. Since monoamines have been implicated in affective disorders, the CIP may be an animal model of depression and is useful in detecting new antidepressants.

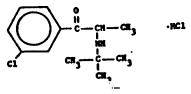
2267 SUPPRESSION OF PROLACTIN SECRETION IN MAN AND RAT BY BUPROPION HCL (WELLBATRIN®), A NOVEL ANTIDEPRESSANT. Warren Stern, Judith Rogers*, Victor Fang* and Herbert Meltzer. Burroughs Wellcome Co., Research Triangle Park, NC 27709 and Univ. Chicago Sch. Med., Chicago, IL 60637.

Bupropion HCl, a non-tricyclic compound with antidepressant properties in man, was evaluated for effects on plasma prolactin (PRL) and growth hormone (GH) levels in normal human subjects, and for effects on plasma PRL levels in a series of pharmacological studies in normal rats. Single oral doses of 50 or 200 mg of bupropion given to 6 male and 12 female normal volunteers produced a marked suppression (up to 70% decrease) of plasma PRL levels for at least 4 hours (see Table). At the end of 24 hours plasma PRL levels were still below normal values. At one hour after drug administration there was a +.56 correlation ($p < .05$) between bupropion plasma levels and the percentage decrease in PRL levels. On the other hand, GH only showed small and erratic changes in plasma levels. In the rat, single doses of 10 or 25 mg/kg, i.p., of bupropion failed to lower PRL levels. However, bupropion at 25 mg/kg, i.p., significantly decreased PRL in rats in which baseline PRL levels were elevated by pretreatment with alpha-methyltyrosine (AMT at 150 mg/kg, i.p.), 5-hydroxtryptophan (5-HPT at 100 mg/kg) or quipazine (10 mg/kg; see Table). Overall, the results in man and rat are consistent with the view that bupropion has significant dopamine agonistic properties. Whether bupropion is a directly or indirectly acting dopaminergic agonist cannot be determined from the present results.

Mean ± S.E. ng/ml Prolactin in Plasma

Humans	Hr.: 0	1	2	4
Males (200 mg)	14 ± 3	4 ± .4	4 ± .7	4 ± .3
Females (200 mg)	24 ± 1	7 ± 1	10 ± 5	6 ± 1

Rats	Saline plus:	Bupropion plus:
AMT	37 ± 5	16 ± 5
Quipazine	25 ± 3	4 ± 1
5-HTP	22 ± 5	8 ± 5

bupropion: 

2268 LOCUS COERULEUS LESIONS DIFFERENTIALLY ALTERS MORPHINE'S EFFECTS ON SELF-STIMULATION BEHAVIOR IN RATS. Ann Tempel*, Steven J. Ellman, Robert Pierro*, Douglas Ocheret*, and Solomon S. Steiner. Behavioral Physiology Lab., Dept. Psych., The City College of New York, New York, N.Y. 10031.

Drugs of abuse have been shown to modulate mechanisms of reward through their action on catecholaminergic systems. The following experiment was designed to examine the effect of chronic morphine administration on self-stimulation (ICSS) in the rat after discrete lesions to the locus coeruleus (LC).

Rats were stereotaxically implanted with 2 pairs of bipolar stainless steel electrodes aimed at one of the following combinations: fields of forel (ff) & crus cerebri (cc); medial forebrain bundle (mfb) & ff; mfb & cc. Stimulation consisted of biphasic rectangular pulse-pairs. Each bipolar electrode was used in a monopolar fashion, with each tip serving as cathode to a cortical screw anode. After response rates stabilized, each animal received 7 days saline, 7 days morphine, 1 day morphine + naloxone and 6 days post-drug saline. Morphine doses were 1.25 and 2.5 mg/kg injected sc 40 min before the beginning of the ICSS session. Naloxone dose was 1 mg/kg. Rats were then given d- and l-isomers of amphetamine alternated in an ABBA series. All doses were 1 mg/kg. Acute LC lesions were then made unilaterally in all animals. Following the lesion, rats were run for 21 days of saline to allow for complete fiber degeneration and neuroheumoral depletion. ICSS drug paradigms post-lesion were identical to the pre-lesion paradigm.

It was found that some ICSS sites which had in common that they were both equally potentiated by the d- and l-isomers of amphetamine and also showed a facilitation under morphine, no longer were facilitated by morphine administration after the LC lesion. Conversely, sites which showed a greater facilitation to d- rather than the l-isomer of amphetamine and also showed a facilitation under morphine, maintained their morphine facilitation, which was naloxone reversible, after the LC lesion.

Relevance of this data to the effect that opiate centers modulate catecholamine effects on ICSS will be discussed.

2269 SOLUBILIZATION OF THE BENZODIAZEPINE BINDING SITE FROM MAMMALIAN BRAIN. John W. Thomas, M.Ayub Khan Yousufi*, and John F. Tallman. Biological Psychiatry Branch, NIMH, Bethesda, MD 20205

The high-affinity binding site for benzodiazepines in mammalian brain has been solubilized using a non-ionic detergent. In a single step approximately 60% of the binding sites are solubilized, while maintaining a high-affinity for [^{3}H]diazepam (K_D of 11 nM as compared to 4 nM for the membrane-bound binding sites). The order of potency of benzodiazepines in inhibiting [^{3}H]diazepam binding is identical for the solubilized and membrane-bound binding sites, and both the solubilized and membrane-bound binding sites have a higher affinity for [^{3}H]diazepam at 4°C than 37°C. However, γ-aminobutyric acid which enhanced [^{3}H]diazepam binding to membrane fractions was without effect on the solubilized binding site.

The molecular weight of the solubilized binding site was determined by gel filtration on Sephadex G-200. The calculated molecular weight of the solubilized binding site was 220,000 daltons; however, this may represent an overestimate, since it is not known how many molecules of detergent or other proteins remain associated with the receptor.

Several other factors which might influence the stability of the receptors were examined. Neither sulfhydryl groups nor carbohydrate moieties seem critical for binding, but both urea and guanidine-HCl were capable of totally inhibiting binding, and this inhibition was partly reversible.

2270 AGONIST POTENCIES AT THE DOPAMINE RECEPTORS. M. Titeler and P. Seeman, Department of Pharmacology, University of Toronto, Toronto, Ontario M5S 1A8.

A series of ergot derivatives have been assayed for their ability to compete for the specific binding, in calf caudate homogenates, of ^{3}H-spiperone, a dopamine receptor antagonist (1), ^{3}H-dihydroergocryptine (^{3}H-DHEC) a dopamine receptor agonist (2,3), and ^{3}H-dopamine, the neurotransmitter. Bromocryptine has been shown to be a potent dopamine receptor agonist in vivo (2) and in vitro (3), and this drug demonstrates a 10-20 fold higher affinity for the ^{3}H-spiperone and ^{3}H-DHEC specific binding sites than for the ^{3}H-dopamine binding site. Dihydroergocryptine has also been shown to be a potent dopamine receptor agonist in vivo (1) and in vitro (2) and this drug demonstrates a 16-40 fold higher affinity for ^{3}H-spiperone and ^{3}H-DHEC specific binding sites than for ^{3}H-dopamine sites. These data along with other data on location and function of these sites indicate that ^{3}H-DHEC and ^{3}H-spiperone label the same site, the post-synaptic dopamine receptor, and 3H-dopamine labels another dopamine neuronal structure, possibly the dopamine autoreceptor (4). These data cannot possibly be interpreted in terms of the two-state model of the dopamine receptor, as has been suggested (5).

	IC_{50}		
	^{3}H-Spiperone	^{3}H-DHEC*	^{3}H-Dopamine
bromocryptine	35	15	300
dihydroergocryptine	5	1.5	80
8-isobromocryptine	600	200	6000
α-ergocryptine	10	3	45
ergocristine	10	5	70
β-ergoptine	20	10	60
β-ergosine	60	25	60
ergocornine	7	5	50
ergotamine	65	60	15

*in the presence of 10^{-7}M phentolamine

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2271 ACUTE AND CHRONIC TOLERANCE TO A BEHAVIORAL EFFECT OF NICOTINE IN RATS. Glenn Daniel Todd* and John A. Dougherty* (SPON: Ralph E. Miller). VA Medical Center, Depts. Pharmacology and Psychiatry, Univ. of Kentucky, Lexington, Ky. 40507.

Acute and chronic tolerance to the behavioral suppressant effects of nicotine were examined by injecting the drug twice daily for several weeks in male Wistar albino rats. The daily interdosing intervals were 1,2,4, or 8 hours, with a different group of rats used at each dosing interval. Each i.p. nicotine injection was followed immediately by a 30-minute session of water-reinforced lever pressing according to a fixed-ratio 50 schedule. The rats were maintained at 80% of their free-feeding weight by giving supplemental water access after the second daily session. The overall response rate after saline pretreatment was used as a predrug baseline with which to compare the effects of nicotine dosing.

The first nicotine injection(200 ug/kg, BASE) decreased overall response rate by about 50% in all four groups. However, the second nicotine injection, 1,2,4, or 8 hours later on the same day had no significant behavioral effect. On the second day this pattern was repeated, with nicotine again decreasing the responding after the first, but not the second injection. Over nine consecutive days, the response rates after the first daily drug injection gradually recovered to within predrug ranges, while the behavior after the second injection remained unchanged or slightly stimulated when compared to controls. There were no differences between the 1,2,4, or 8 hour interval groups either on the initial effect, the response to the second daily injection, or the rate of tolerance development over nine days.

Analysis of the behavioral pattern showed that an initial period of no responding after the first daily drug injection was primarily responsible for the lowered overall response rate. After the initial pause, responding was abruptly resumed at the predrug rate. This pause was unrelated to motor impairment. The pause gradually decreased in duration over the nine day period and was not apparent after any of the second daily injections.

A subsequent dose increase to 350 ug/kg(BASE) twice a day reinstated the initial suppression with the development of tolerance over nine days again following the same pattern as seen with the lower dose of nicotine.

These results indicate that a rapid tolerance occurs to the suppressant effects of nicotine within one hour and persists up to eight hours, but not for 16 to 23 hours. A more gradual tolerance also occurs after 6 to 9 days of injections. This biphasic development of tolerance suggests that more than one process may be involved.

2272 ASCORBATE ATTENUATES THE L-DOPA SUPPRESSION OF RAT PLASMA PROLACTIN AND LH LEVELS. L. C. Tolbert, T. N. Thomas, L. D. Middaugh, J. G. Ondo*[O], and J. W. Zemp, Neurosci. Prog., Univ. Alabama in Birmingham, B'ham, AL., 35294. Depts of Biochem., Psych. and [O]Physiol., Med. Univ. of South Carolina, Charleston, S.C., 29403.

We have previously reported that ascorbate inhibits dopamine-sensitive adenylate cylase without an effect on norepinephrine-sensitive adenylate cyclase in vitro. Additional studies indicated that intraperitoneal (IP) injections of pharmacologic doses of ascorbic acid elevate levels of ascorbate in the brains of experimental animals in a time and dose-related manner and that this elevation modified dopamine-mediated behaviors without an effect on norepinephrine-mediated behaviors. Subsequently, we demonstrated that ascorbate increased the relative turnover of dopamine, in vivo, without affecting the turnover of norepinephrine. In the present study, experiments were designed to test whether ascorbate could influence the suppression of prolactin secretion normally produced by L-Dopa as well as the suppression of LH reported by some people.

Male rats were first injected with RO4-4602/1, a peripheral decarboxylase inhibitor. Animals were then given L-Dopa (100 mg/kg IP). Thirty minutes before decapitation animals were injected with ascorbate (1 or 2 g/kg IP) or vehicle. Plasma prolactin and LH levels were determined by radioimmunoassay from the trunk blood of these animals.

L-Dopa produced a significant reduction in both prolactin and LH levels. Ascorbate, by itself, had no effect on prolactin or LH levels. Ascorbate (2 g/kg) significantly attenuated the L-Dopa induced suppression of prolactin and LH levels ($p < .02$). A smaller dose of ascorbate had a lesser, non-significant, effect.

This study indicates that ascorbate can alter certain neuroendocrine responses mediated by dopamine and is compatible with our previous demonstrations of the ability of pharmacologic doses of ascorbate to antagonize CNS dopaminergic activity.

2273 DISCRIMINATION OF PURE NARCOTIC ANTAGONISTS BY PIGEONS. Valentino, R.J.,* Herling, S.* and Woods, J.H. Dept. of Pharmacology, Univ. of Mich., Ann Arbor, Mi. 48109

Pigeons were trained to discriminate an intramuscular injection of naltrexone (NTX; 32 or 56 mg/kg) from saline by reinforcement of responses on one of two keys. Following NTX administration, 20 consecutive responses on the appropriate key resulted in 4 sec access to grain, while responses on the other key were reinforced when saline was administered. During test sessions, 20 consecutive responses on either key resulted in grain delivery. Naloxone generalized to NTX in 4 out of 5 pigeons and was equipotent to NTX. Administration of behaviorally active doses of cyclazocine, amphetamine, and ethylketazocine did not result in drug-appropriate responding. Quaternary NTX produced saline-appropriate responding in 3 out of 5 birds. One bird generalized completely to quaternary NTX, while another responded only partially on the drug-appropriate key. UM-1046 (3-cyclopropylmethyl-1,2,3,4,5,6-hexahydro-8-hydroxy-6-methyl-3-benzazocine) produces a syndrome resembling the narcotic abstinence syndrome in narcotic-naive monkeys. In the isolated guinea-pig ileum preparation, UM-1046 produces a contraction resembling the contraction produced by naloxone in ileal preparations which have been exposed to morphine (Valentino and Smith, Fed. Proc. 38:587, 1979). The administration of UM-1046 to naltrexone-trained pigeons produced complete NTX-appropriate responding in 2 of the animals and partial generalization in two others. UM-979 ((-)-5,9-dimethyl-2-(3-furylmethyl)-2'-hydroxy-6,7-benzomorphan), a drug with narcotic-antagonist properties, produced results similar to the administration of UM-1046. These data indicate that naltrexone has discriminative effects in nondependent animals which may be distinguished from those of other narcotic agonists and mixed narcotic agonist-antagonists. Characterization of narcotic antagonists using drug discrimination procedures may be useful in determining the effects of these drugs in both the naive and narcotic-dependent state. (Supported by NIDA grants DA 00154 and 00254)

2274 STIMULATION OF SYNAPTOSOMAL TYROSINE HYDROXYLASE ACTIVITY BY PHENCYCLIDINE. Thomas W. Vickroy* and Kenneth M. Johnson. Dept. of Pharmacol. and Toxicol., Univ. of Texas Med. Branch, Galveston, TX 77550.

Phencyclidine(PCP) is a CNS active agent which possesses psychomotor-stimulant properties in rodents and sedative-hypnotic, hallucinogenic, and anesthetic properties in primates. Like d-amphetamine, PCP produces increased locomotor activity, stereotypic behavior, and ipsilateral turning in rats with unilateral lesions of the nigro-striatal pathway. Each of these PCP effects can be inhibited by haloperidol. These data strongly suggest that dopamine(DA) is involved in the mediation of these effects.

The purpose of this study is to examine the effects of PCP on tyrosine hydroxylase(TH), the rate-limiting enzyme in the synthesis of DA. A crude synaptosomal homogenate was prepared by homogenizing pooled striata from untreated male Sprague-Dawley rats in 20 vol. of 0.32 M sucrose. The synaptosomal tissue was collected by centrifugation and resuspended in 20 vol. of either 37 C or ice-cold physiological phosphate buffer(pH 6.8) containing o-benzylhydroxylamine(134 uM), an inhibitor of DOPA decarboxylase. Either saline or PCP was added and preincubated for 20 min. The reaction was initiated by the addition of tyrosine(20 uM) containing 1 uCi L-(3,5-^{3}H)-tyrosine and was stopped after 20 min. by the addition of glacial acetic acid. Tritium-labelled water, which was formed following hydroxylation of L-(3,5-^{3}H)-tyrosine by TH, was separated from tritium-labelled tyrosine and its metabolites by ion-exchange chromatography and quantified by liquid scintillation spectrophotometry. TH activity assayed in this manner was completely inhibited by 3-iodotyrosine(2 uM).

PCP was observed to stimulate TH activity in a dose-related manner over the range of 0.1 uM to 100 uM with maximal stimulation(100%) occurring at 10 uM. On the other hand, PCP had no effect on the uptake of ^{3}H-tyrosine at these concentrations. However, little or no stimulation of TH activity was observed at any PCP concentration tested when the synaptosomal preparation was allowed to preincubated for 20 min. at 37 C, suggesting that PCP may be acting indirectly via the release of DA which normally inhibits TH. These effects are similar to those previously reported for d-amphetamine on synaptosomal TH activity.

2275 PRODUCTION OF PHYSICAL DEPENDENCE IN THE ALCOHOL-PREFERRING AND -NONPREFERRING LINES OF RAT. Marshall B. Waller, William J. McBride, Lawrence Lumeng* and Ting-Kai Li.* Institute of Psychiatric Research and Depts. of Psych., Biochem. and Medicine, Indiana Univ. School of Medicine and VA Medical Center, Indianapolis, IN 46223.

One of the criteria for an animal model of alcoholism is that ethanol (EtOH) consumption ultimately leads to physical dependence, manifest upon withdrawal by the appearance of abnormal behavioral and physical signs. We have raised by selective breeding and free-choice testing with 10% EtOH and water, two lines of rats, the alcohol-preferring (P) and -nonpreferring (NP) lines. This study examines the effect of caloric restriction and flavoring of 10% EtOH solution upon EtOH intake and whether chronic free-choice drinking can produce physical dependence in these lines of rats. In the first experiment, both P and NP rats were weight-reduced to 80% of their free-feeding weight and given free-choice between water and a 10% EtOH solution flavored with saccharin, 0.125 g%, and NaCl, 1 g%. EtOH consumption increased from 7 to 14 g/kg/d in the P rats and from 1 to 12 g/kg/d in the NP rats. After 8 weeks, alcohol was discontinued and the animals were tested for withdrawal. Physical signs, e.g., tremor, wet dog shakes, teeth chattering, Straub tail, were observed in 8 of 9 rats of the P-line. Sound from a bell (100 db) induced seizures in 3 animals while 2 others exhibited hyperreactivity to this stimulus. Physical signs were seen in all NP animals. The bell induced seizure in one animal and two showed increased sensitivity to the sound for up to 72 hours post-withdrawal. These signs appeared within the first day. Subsequently, behavioral impairment in the runway test appeared in 7 of 9 P animals and 7 of 8 NP animals. Head-poke and rearing activity were depressed at 24-72 hours post-withdrawal in 7 of 9 P rats and 6 of 8 NP animals tested. In the open field, one P animal exhibited hypoactivity while 7 animals became hyperactive. By contrast, 7 NP animals were hypoactive and only one was hyperactive in this test. In a second experiment, 8 rats of the P-line were given food ad libitum and the free-choice drinking of unflavored 10% EtOH and water for 13 weeks. Ethanol intake remained constant in 3 animals while 5 exhibited a progressive rise in alcohol consumption from 5 to 9 g/kg/d. When alcohol was discontinued, physical signs of withdrawal appeared in 6 of the animals on the first day. Disrupted behavior was exhibited by at least 5 of 8 animals in the open field, head-poke and rearing activity, or the runway test. These data indicate that chronic EtOH consumption, with or without food restriction and flavor additives in the EtOH solution, can produce physical dependence in the selectively bred, alcohol-preferring line of rats. (Supported by USPHS Grant No. AA003243).

2276 BRAIN SEROTONIN, BODY WEIGHT AND ISOLATION-INDUCED AGGRESSION IN MICE ON A TRYPTOPHAN-FREE DIET. James K. Walters, Maria Lavooy* and Robert Posch.* Biology Department, William Paterson College of New Jersey, Wayne, NJ 07470.

Diets lacking the essential amino acid, L-tryptophan, significantly deplete brain serotonin (5-HT) in rats and have been shown to influence pain sensitivity, acoustic startle, sexual behavior and mouse killing. No previous studies of the effects of tryptophan-free (TF) diets in mice have been reported. One mouse behavior thought to involve 5-HT mechanisms is aggression induced by prolonged isolation. Isolation-induced aggression can be decreased by depleting brain 5-HT with raphe lesions or p-chlorophenylalanine or by administering 5-HT receptor blockers. The present study determined the effects of a TF diet on isolation-induced aggression in two different strains of mice.

Forty Swiss-Webster (SW) and 48 SJL/J mice were isolated in plastic cages. Each mouse had powdered lab chow and water freely available. Two weeks later half of the mice from each strain were randomly switched to a powdered TF or tryptophan control (TC) diet. After 12 days, pairs of mice from the same strain and diet condition were placed in single divided cages for two more days. The divider and food cups were then removed for a 15 minute test period during which latency to fight, fight duration, tail lash duration and chase duration were recorded. Brains from five pairs of mice in each strain and diet condition were removed after testing and assayed for brain 5-HT concentration.

The TF diet significantly ($p < .01$) depleted brain 5-HT in both SW (34%) and SJL (45%) mice. The TF diet produced a 17% decrease in mean body weight for SJL mice after two weeks, and a 26% decrease for SW mice. The TC diet reduced SJL body weights by only 3% and SW weights by 17%. Significant effects on body weights were found for Diet ($p < .001$), Strain ($p < .001$) and Diet X Strain interaction ($p < .05$). Despite the significant brain 5-HT depletions, there were no significant effects of diet on any of the behavioral measures for either strain of mice.

This study shows that a TF diet depletes brain 5-HT in mice but also produces considerable weight loss over a two week period. Strain differences in acceptance of the diet were evident from the differential weight losses. The degree of brain 5-HT depletion which resulted from eating the TF diet may not have been sufficient to reduce isolation-induced aggression. Such was the case for acoustic startle in rats which could only be affected by tube feeding the TF diet (Walters, Davis & Sheard, Psychopharmacology, 1979, in press).

This study was supported by a William Paterson College Summer Research Fellowship.

2277 A BEHAVIORAL MODEL OF EARLY ENCEPHALOPATHY IN THE END-TO-SIDE PORTACAVAL SHUNTED RAT. John D. Warbritton, III, Mark A. Geyer, Bengt Jeppsson* and Josef E. Fischer*. Dept. of Psychiatry, Univ. Calif. San Diego, La Jolla, CA, Dept. of Surgery, Harvard Medical School, Boston, MA, and Dept. of Surgery, Univ. Cincinnati Medical Center, Cincinnati, OH 45267.

Although models of hepatic failure in large and small animals abound, models of early hepatic encephalopathy (HE) are difficult to create. A rat with an end-to-side portacaval shunt (PCS) is an extensively used experimental animal for characterization of biochemical alterations following diversion of portal flow from the liver, but few behavioral abnormalities have been detected. Biochemical changes observed in rats after PCS include increased plasma and brain levels of the serotonin (5-HT) precursor tryptophan, with increased brain serotonin and its metabolite 5-hydroxyindoleacetic acid (5-HIAA). Accumulating evidence indicates that serotonin may be involved in the modulation of the startle response in the rat. In the following study, the magnitude of startle responses to both tactile and auditory stimuli were shown to be abnormally decreased in chronic PCS rats and suggest that such studies may be used to advantage in the study of HE. End-to-side PCS were carried out in groups of 20 rats by a non-suture technique. Rats were studied at weekly intervals 4 and 5 weeks following PCS utilizing a tactile startle stimulus, an air puff administered through a solenoid activated valve, and auditory startle consisting of 110 dB tones. The results suggest that animals with end-to-side portacaval shunts exhibit decreased responsivity to both tactile and auditory stimuli. The simplicity of these tests, when carried out under reproducible experimental circumstances , suggest that they may be used to assess the effects of pharmacological manipulation of PCS rats in the treatment of hepatic encephalopathy.

2278 ALTERATIONS IN STEREOTYPY AND LOCOMOTOR ACTIVITY IN THE RAT WITH MULTIPLE DAILY INJECTIONS OF d-AMPHETAMINE. Susan B. Weinberger* and David S. Segal. Dept. Psychiatry, Sch. Med., UCSD, La Jolla, CA 92093.

Repeated single daily injections of d-amphetamine in the rat have been reported previously to produce a progressive augmentation in stereotypy and locomotion (Segal and Mandell, 1974; Segal, 1975).

However, since amphetamine-induced psychosis in humans is typically associated with shorter intervals between successive amphetamine administrations, we have extended our previous studies by characterizing the progressive behavioral changes associated with multiple daily amphetamine injections.

Male Wistar rats, housed individually in sound-attentuated chambers, received 30 successive s.c. injections of either d-amphetamine sulfate (2.5 mg/kg of free base) or saline at 4 hr intervals, followed 4 hr later in all animals by 2.5 mg/kg d-amphetamine. Locomotor activity was monitored continuously with the use of a Nova 1200 computer; stereotyped behaviors (sniffing, repetitive movements, and oral stereotypies) were assessed at 10 min intervals for 3 hr after the last amphetamine injection.

Multiple daily amphetamine injections produced a progressive decrease in latency to onset of the stereotypy phase and an increase in intensity of the stereotyped behaviors. These alterations in the amphetamine response pattern are similar to those produced by repeated single daily administration of amphetamine. In addition, the durations of both the stereotypy and the post-stereotypy hyperactivity periods were markedly shortened with multiple daily injections. When the animals were retested with the same dose of amphetamine 5-8 days after discontinuation of chronic treatment, the time of offset of these latter two phases had recovered to acute amphetamine treatment values, whereas the more rapid onset of stereotypy persisted.

These data suggest that the pattern of enhanced behavioral responsiveness produced by both single and multiple daily amphetamine injections might represent an animal model for the schizophrenia-like behavioral syndrome that develops with repeated amphetamine administration in humans.

2279 DRUG TOLERANCE WITHOUT PRIOR DRUG TREATMENT: INDUCTION OF BEHAVIORAL TOLERANCE TO DIAZEPAM BY A BEHAVIORAL TREATMENT. J.R. Wenger* and S.C. Woods* (SPON: N.J. Kenney). Dept. Psychol., Univ. Wash., Seattle, WA 98195

Learning theory can explain drug tolerance by proposing compensatory skill-learning as the mechanism underlying behavioral tolerance to drugs, i.e., behavioral tolerance is the result of learning to compensate behaviorally for drug-induced impairment of behavior. This suggests that animals given the opportunity to learn to cope with other, non-pharmacologically-induced but otherwise similar, impairments of behavior would be tolerant to drug-induced behavioral impairment. The present experiment tested and confirmed this prediction. Drug-naive rats, previously trained to walk on a treadmill in order to avoid footshock, were spun daily for 3-min at 120 rpm to render them dizzy and then immediately made to walk on the treadmill. Their performance improved over a 10-day treatment period suggesting that they were becoming tolerant to the effects of the rotation treatment. They were then tested on the treadmill after an injection of diazepam (9 mg/kg ip), an ordinarily disabling dose, and found to be behaviorally tolerant relative to appropriate control animals. Since this was the first time these animals had ever received drugs, classical mechanisms of tolerance were eliminated as potential explanations. Hence, these data support the learning theory interpretation of behavioral tolerance to drugs.

2280 INTRACRANIAL DETECTION THRESHOLDS FOR ELECTRICAL STIMULATION: EFFECTS OF CHLORPROMAZINE. Howard S. Wheeling*, and Conan Kornetsky. Laboratory of Behavioral Pharmacology, Boston University School of Medicine, 80 E. Concord St., Boston, MA. 02118 (SPON: Steven Matthysse)

Detection thresholds for intracranial electrical stimulation to the rat were determined by means of a modified psychophysical method of constant stimuli. Animals were implanted with two bipolar electrodes, one aimed at either the mesencephalic reticular formation (RF), a negatively reinforcing site, or the caudate nucleus (CN), a positively reinforcing site, and the second electrode aimed at the medial forebrain bundle (MFB). Non contingent delivery of a 0.5 sec train of current (0-40 μA, 40-60 Hz) to either the RF or the CN (S1) signalled the availability of a contingent reinforcing stimulus (S2) to the MFB. Intensities of MFB stimulation were selected that would maintain responding to detectable intensities of S1. Detection thresholds were computed from the presence or absence of a response to S1, the intensity of which was randomly varied in equally spaced steps over a predetermined range. Latency to respond and intertrial responding served as measures of drug effects unrelated to threshold changes. The current levels employed here are lower than those we find necessary to maintain escape from RF stimulation. Chlorpromazine causes dose-related (0.125 - 2 mg/kg, s.c.) increases of detection thresholds. Minimally effective doses for raising detection threshold are generally lower than those we find are necessary for elevating self-stimulation thresholds. The chlorpromazine induced elevation of intracranial stimulation detection threshold may be related to the attentional disruption observed in both animals and man to peripherally signalled, go-no-go tasks after drug administration.

(Supported by NIMH Grant MH 12568, Biomedical Research Support Grant at Boston University School of Medicine, and Research Scientist Awardee MH 1759 - CK)

2281 FENFLURAMINE AS A DISCRIMINATIVE STIMULUS IN RATS. Francis J. White and James B. Appel. Behavioral Pharmacology Lab., Dept. Psych., University of South Carolina, Columbia, SC 29208 USA

In a two-lever, water-reinforced drug discrimination task male albino rats (n = 15) were trained to discriminate 1.0 mg/kg of fenfluramine (FF) from saline. Within 16 sessions all animals reached a criterion of at least 85% correct responding. Generalization (transfer) testing, conducted during extinction, revealed an orderly increase in per cent FF responding following doses of .25, .50 and .75 mg/kg FF. Three serotonin agonists, quipazine (QP), LSD and MK 212 were also administered during transfer tests. Each of these compounds showed dose-related generalization to the FF cue: responding on the FF-appropriate lever reached 82% following a dose of 2.0 mg/kg of QP; 66% FF responding occurred following .16 mg/kg of LSD and 88% FF responding was observed after 1.0 mg/kg of MK 212. Thus, at the doses tested, the order of transfer potency was MK 212 > QP > LSD. These results confirm reports that FF exerts its behavioral effects primarily by interacting with the serotonergic neuronal system, although this interaction may be indirect (Ann. NY Acad. Sci. 305:222, 1978).

2282 DISINHIBITION OF PREDATORY ATTACK IN KITTENS BY OXAZEPAM. David L. Wolgin and Susan Servidio.* Dept. Psychol., Florida Atlantic Univ., Boca Raton, Fla. 33431.

The development of predatory attack in kittens was analyzed. Film analysis revealed that at each stage of development, attack consists of reflexive components of approach and withdrawal. Components of approach include orientation of the head and body, extension of the head and upper torso, approach involving the whole body, trapping with the forepaws and/or seizing with the mouth, and biting. The sequential appearance of these components in ontogeny appears to depend, at least in part, on the ability to maintain an adequate level of endogenous arousal. Similarly, once development is complete, the recruitment of components that appear late in ontogeny (seizing with the mouth, biting) appears to require reafferent activation from the repetition of components that develop earlier ("warm up").

Components of withdrawal include retraction of the head and upper torso, withdrawal involving the whole body, and swiping. These components inhibit seizing with the mouth and biting, and produce a more playful or defensive pattern of attack. They are more prominent in kittens tested with live prey (vs dead prey), in satiated kittens (vs food deprived kittens), and in naive kittens (vs experienced kittens).

Oxazepam in doses of 1 and 3 mg/kg greatly facilitates attack in both experienced and naive kittens by reducing components of withdrawal and increasing the intensity of attack. Unlike muscarinic cholinergic drugs, however, oxazepam does not facilitate reflexive biting to touch of the lip. These results suggest that oxazepam disinhibits predatory attack.

2283 AN ECONOMICAL SCREEN FOR PHENETHYLAMINE TYPE HALLUCINOGENS: MOUSE EAR SCRATCHING. G.K.W. Yim, T.E. Prah*, W.R. Pfister*, and D.E. Nichols[1]. Dept. Pharmacology, Toxicology and Med. Chem-Pharmacog.[1], Purdue University, W. Lafayette, IN 47907.

Stereoselectivity for the (-) isomers of DOM and of its cyclopropyl analog in eliciting either mouse ear scratching or cat limb flicks was observed in our earlier study in which single doses of the compounds were tested (NIDA Research Monograph 22: 70-81, 1978). THis study was aimed at further evaluating mouse ear scratching as an animal model for predicting hallucinogenic activity. Doses calculated for eliciting 100 scratching episodes in 30 min were: 0.7 mg/kg for (-) DOM; 1.2 g/kg for (+) DOM; 7.0 mg/kg for escaline and 24 mg/kg for mescaline. Head twitches, but no ear scratches were observed with either (+) DOM or quipazine. Thus the mouse ear scratch response was stereoselective and ranked the phenethylamine-type compounds in order of observed human potency. Phenycylidine induced a low level of scratching activity. Both LSD (0.1-125 ug/kg) and psilocybin (1.5 and 6 mg/kg) failed to elicit the scratching response. Though not sensitive to tryptamine-type compounds, the mouse ear scratch response may be an inexpensive and convient initial test for predicting potential hallucinogenic activity of phenthylamines. (Supported in part by USPHS Grant DA0191601 and a Purdue Research Foundation Fellowship (to W.R.P.).

2284 ABSTINENCE FROM L-ALPHA-ACETYLMETHADOL (LAAM), NOR-LAAM AND DINOR-LAAM IN DEPENDENT RATS: EEG AND BEHAVIORAL CORRELATES. Gerald A. Young, George F. Steinfels and Naim Khazan. Dept. of Pharmacol. and Toxicol., Univ. of Maryland Sch. of Pharmacy, Balto., MD 21201

We have previously studied and compared EEG and behavioral correlates during self-administration of LAAM, nor-LAAM and dinor-LAAM in dependent rats (Young et al, J. Pharmacol. exp. Ther., in press). The results demonstrated that the pharmacodynamic profile of LAAM differed from those of its two N-demethylated metabolites. LAAM disrupted behavior the least during self-administration. In the present report we extended these studies to a comparison of EEG and behavioral correlates during abstinence from LAAM, nor-LAAM and dinor-LAAM in dependent rats.

Adult female Sprague-Dawley rats were prepared with chronic intravenous cannulas and cortical and temporalis muscle electrodes. They were made tolerant to and physically dependent on morphine by a series of automatic hourly injections that progressively increased in dose. Each rat was then trained to lever press in order to self-administer morphine (10 mg/kg/inj) on an FR-20 schedule of reinforcement. Upon stabilization of morphine self-administration, LAAM, nor-LAAM or dinor-LAAM, each at a dose of 1 mg/kg, was substituted for morphine for an additional one to two weeks, and continuous EEG and EMG recordings were collected. Saline was then substituted for each of these narcotics which, thus, precipitated abstinence.

Upon abstinence from nor-LAAM, REM sleep was severely suppressed from the 18th through 28th hrs. In contrast, during abstinence from LAAM and dinor-LAAM, REM sleep was moderately suppressed for two days. Increases in lever pressing during abstinence from nor-LAAM occurred earlier and was more prolonged than for LAAM and dinor-LAAM. The incidence of head shakes peaked earlier and was higher for nor-LAAM and dinor-LAAM during abstinence than for LAAM.

These results demonstrated further pharmacodynamic differences between LAAM and its two N-demethylated metabolites. Our findings suggest that in dependent rats abstinence from LAAM was least severe when compared with abstinence from nor-LAAM and dinor-LAAM. (Supported by NIDA Grant DA 01050.)

2285 Chronic Amphetamine Treatment and Intracranial Self-Stimulation: Response Sesnsitization and Depression. Robert M. Zacharko* and Larry Kokkinidis* (Spon. T.B.Wishart). Dept. Psych., University of Calgary, Calgary, Alberta, and Dept. Psych., University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

Long-term amphetamine treatment results in tolerance to some drug-induced behaviors, (e.g., anorexia), whereas other drug-induced behaviors do not undergo tolerance (e.g., stereotypy). In fact, following chronic exposure to amphetamine, drug-induced stereotypies are exacerbated. Although the enhanced stereotypies following chronic amphetamine treatment may involve several factors, it appears that the increased neuronal efficacy of dopamine (DA) transmission induced by chronic exposure to drug plays a primary role in this respect. In the present study we examined the effects of chronic amphetamine treatment on intracranial self-stimulation (ICSS) from the substantia nigra in order to determine whether chronic drug treatment would modify ICSS and whether these changes parallel those observed with stereotypy. Baseline rates of responding were determined at a standard current intensity of 30 uA, 100 Hz biphasic square wave for all animals (n=40). Following baseline testing one-half of the surgically prepared animals received intraperitoneal (i.p.) injections of d-amphetamine sulphate (7.5 mg/kg) twice daily for 5 consecutive days. The remaining half were chronically treated with saline. On test day (Day 6) the animals were subdivided further such that half the animals in each group were tested with a test dose of 0.3 mg/kg d-amphetamine sulphate while the other half received saline. This chronic drug treatment/test sequence was continued for 5 sessions (30 days). Rats chronically treated with saline and tested with d-amphetamine (0.3 mg/kg) showed comparable self-stimulation rates relative to animals chronically treated and tested with saline on all test days with the exception of test day 4. Among rats chronically treated with the test dose of d-amphetamine a significant facilitation of self-stimulation relative to the remaining groups was observed on four of the five test days. In contrast, rats chronically treated with amphetamine and tested with saline showed a depression of self-stimulation rates relative to control animals Thus,within the same self-stimulation paradigm both response depression and sensitization were observed as a consequence of long-term amphetamine administration. It appears that when animals are tested with a low dose of amphetamine, which ordinarily has no behavioral consequences, ICSS responding is enhanced (sensitization), presumably due to a hyperactive dopamine system (receptor supersensitivity) resulting from chronic exposure to the drug. In the absence of the drug on test day the dopamine system remains hypoactive and consequently a depression of ICSS is observed.

2286 SPECIFIC [^{3}H] PHENCYCLIDINE BINDING IN THE RAT CENTRAL NERVOUS SYSTEM. Stephen R. Zukin* and R. Suzanne Zukin* (SPON: W. T. Norton). Department of Psychiatry, Downstate Medical Center, Brooklyn, N.Y. 11203 and Department of Biochemistry, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

Phencyclidine (PCP), a leading drug of abuse, has anesthetic, analgesic, cardiovascular and psychotomimetic effects. In order to investigate the mechanisms of CNS actions of PCP, we studied binding of [^{3}H] PCP to rat brain membrane preparations. [^{3}H] PCP binds specifically and with high affinity (K_D = 1.5 x 10^{-7} M at pH 7.4) to a saturable class of binding sites. Specific binding constitutes approximately 70% of total binding at 0° and 33% of total binding at 37° (at 10^{-8} M [^{3}H] PCP). This binding can be displaced by nonradioactive PCP and a series of ten PCP analogs with relative potencies that closely parallel those determined in vivo in mouse rotarod ($p < 0.005$) and rat drug discrimination ($p < 0.001$) tests. Muscarinic cholinergic ligands inhibit [^{3}H] PCP binding, but only at high concentrations (IC_{50}s = 10^{-4} - 10^{-3} M) and in rank order at variance with that determined for their binding to muscarinic receptor sites or for their pharmacological potencies. Other centrally acting drugs including opiates are unable to displace specifically bound [^{3}H] PCP at > 10^{-4} M concentrations. In subcellular fractionation experiments, [^{3}H] PCP binding is most enriched in the crude synaptosomal membrane fraction (3.3 times higher than in the mitochondria-myelin fraction; 5.1 times higher than in the whole brain homogenate). In regional binding distribution studies, [^{3}H] PCP binding is about three times higher in hippocampus (region of highest density) than in cervical spinal cord (region of lowest density); intermediate levels are found in hypothalamus > caudate nucleus > frontal cortex > cerebellum > medulla/pons, amygdala. Binding of [^{3}H] PCP to some peripheral tissues does occur but displacement of this binding by PCP analogs fails to correlate with the CNS binding or with pharmacological properties. Trypsin, pronase, N-ethylmaleimide, iodoacetamide, acidic pH, high temperature, and calcium reduce specific [^{3}H] PCP binding. Thus, PCP and its analogs may exert their CNS effects via binding to specific CNS receptor sites.

PSYCHOPHYSICS

2287 ROLE OF POSITION SENSE IN DIRECTION DETECTION ON THE SKIN. D.A. Dreyer, G.H. Duncan*, and C.L. Wong*. Dental Research Center; Dept. of Oral Surgery, School of Dentistry; Dept. of Physiology, School of Medicine; Neurobiology Program, University of North Carolina, Chapel Hill, N.C. 27514.

Somesthetic discriminatory capacity is generally recognized to be optimal when the stimulating object is moving parallel to the skin's surface. An experimental model of this mode of stimulation is the ability to correctly identify the direction of a brush stroke across the skin. Previous studies with this model have shown that the accuracy of direction discrimination improves with increasing length of skin contacted by the brush. This may be due to the increased surface area of stimulus contact or to the increasing separation of the extreme points of stimulus contact. The present study investigates these two possibilities.

Discrimination of brush stroke direction was assessed in normal human subjects for stimuli applied to the ventral forearm. The independent variables studied were the velocity of brush movement and the area of skin contacted by the brush. A servomotor moved the brush at any of nine velocities ranging from 0.5 to 250 cm/sec; velocity and direction of movement were randomized within each block of 180 trials. Area of skin contacted by the brush was controlled by plates with 0.5 cm wide apertures of different lengths. These apertures consisted of either a single slot (1-6 cm long) or two short slots split by a solid midportion. Thus the 4 cm. split aperture, for example, was identical to the 4 cm. continuous aperture except for the central 3.5 cm. which shielded the skin from brush contact. The subjects' reports of perceived brush direction were elicited by a forced-choice procedure.

The results of the present study indicate that, at slow brush velocities the ability to identify the stimulus direction was comparable for the continuous and split apertures, although the amount of skin exposed to the stimulus in the split apertures was from 50-91% less than that exposed in a continuous aperture of comparable length. At faster velocities, discriminative ability fell off sharply with both aperture types, but the performance decline with the split aperture was much more pronounced. These results are interpreted as evidence for the importance of position detection in correctly identifying the direction of a moving stimulus. Although the total area of stimulus contact remained constant for all split-aperture plates, direction discriminability increased with increasing distance between the two apertures.

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2288 NON-ANALGESIC EFFECTS OF NITROUS OXIDE ON SOMESTHETIC SENSITIVITY AND PERCEPTION OF SENSITIVITY. G.H. Duncan*, D.A. Dreyer, and J.M. Gregg* (SPON: J.S. Hanker). Dental Research Center; Oral Surgery, School of Dentistry; Dept. of Physiology, School of Medicine; Neurobiology Program, UNC, Chapel Hill, N.C. 27514

Subanesthetic levels of nitrous oxide (N_2O) have been shown to produce impairments in auditory and visual acuity, psychmotor performance, short-term memory, and reaction time. A single study involving stationary somatosensory stimuli reported that 25% N_2O impaired warmth and pressure thresholds in three subjects. It is generally acknowledged that the capacity to identify an object is optimal when the object and skin are in tangential motion relative to each other (identification of a coin by passing the fingers across its surface). This study investigates the effects of N_2O on somesthesia as measured by an experimental model of tangential stimulus movement.

Ten volunteers were each tested under two conditions -- 100% O_2 and 30% N_2O/70% O_2. All sessions were otherwise identical and consisted of 100 brush strokes presented to the thenar eminance. A servomotor moved the brush at any of 5 velocities ranging from .4 to 150 cm/sec.; velocity and direction of movement (either proximal to distal or the reverse) were randomized within each session. The area of skin contacted by the brush was defined by a rectangular aperture 0.5 cm. wide and 1.5 cm. long. The subject reported the perceived direction of brush movement past the aperture by pushing a switch in the appropriate direction. Following each session, the subject estimated his performance and his level of sedation on visual analogue scales.

Subjective reports of sedation levels ranged from 35-90% during the N_2O sessions as compared to 0-30% during the O_2 sessions. Nine of the ten subjects reported that 30% N_2O significantly altered their ability to detect brush direction. Analysis of the actual performance data revealed no significant differences in the subjects' ability to differentiate brush directions at any of the 5 velocities. Thus at 30% N_2O, which resulted in moderate sedation and altered preception of performance, actual discriminability on this somesthetic test was not greatly effected. These results are consistent with previous reports of subjective increases and objective decreases in auditory acuity with N_2O sedation. The previous report of decreased pressure sensitivity due to N_2O used a stationary threshold measure, whereas discrimination of brush stroke direction involves temporal and spatial evaluation of a suprathreshold moving stimulus. Performance in this integrative task may not be as sensitive to the effects of N_2O as the simple identification of pressure thresholds. Supported by DE 02668, RR 05333, DE 07018, DE 00011 and the Alfred P. Sloan Foundation.

2289 SPATIAL SUMMATION IN THE COLD SENSE. Joseph C. Stevens. John B. Pierce Fndn. Lab. and Yale Univ., New Haven, CT 06519.

This study revealed that the level of cold sensation that a person experiences depends almost as much on the areal extent of skin stimulated as on the amount of skin cooling. Under the right circumstances a person cannot tell how large is the area of stimulation, because area registers not as apparent area, but as level of cold sensation. These findings were obtained through the use of a set of stimulators composed of cold copper disks (2 to 20 cm^2) surrounded by a ring of styrofoam of such size as to make the total area of contact a constant 24 cm^2. Groups of subjects made magnitude estimates of cold sensation level (C) aroused by various amounts of skin cooling (ϕ) and various areas (A). Most of the results come from the forearm, with secondary results from the face and the back. Subjects almost never came to realize that the thermal area was being varied. Also, it turned out that, for any constant A, cold grows approximately as a power function of ϕ (average exponent = 1.07) and that given any constant ϕ, cold grows as a power function of A (exponent = 0.82). A single equation applies to all the results, namely: $C = k\phi^{1.07}\, cA^{0.82}$. For any constant C (termed K) we can write the equation $K = \phi A^{0.82/1.07}$ or $K = \phi A^{0.77}$, showing how level and area trade to preserve constant cold sensation. This equation shows that the cold sense summates well (but imperfectly) the neural effects of stimulation over wide areas. However, the amount of cold experienced tends toward an asymptote when A and ϕ together become large enough. This latter result suggests that the "ceiling" on cold sensation is imposed by a neural mechanism more central than the cold receptors. The findings concerning cold contrast with earlier findings concerning spatial summation of warmth (Stevens et al., Physiol. & Behav., 13, 825, 1974). For warmth, the rules of summation are more complex. For the absolute threshold, area and level trade according to a nearly perfect reciprocity. As the criterion level of warmth is progressively increased, area counts less and less relative to level. At the threshold of pain, area counts little or none at all, i.e., there is negligible spatial summation. The areal dimensions of pure warmth stimulation, as for cold, are very difficult to perceive. The available evidence so far suggests that the basic features of spatial summation in both thermal senses take shape in the central nervous system.

2290 ADAPTATION LEVEL IN NORMALS & SCHIZOPHRENICS TESTED BY A PRODUCTION OF EQUAL TIME INTERVALS METHOD. George A. Vroulis. Behavioral Neurochemistry Section, Texas Research Institute of Mental Sciences, Medical Center, Houston, TX 77030.

According to the concept of adaptation as it was developed by Helson, adaptation level represents the zero or origin to which gradients of stimulation are referable. The steeper the gradient the greater the impact of the stimulus on the organism and the greater the response to it. In summary the level of adaptation is the pooled effect of (a) focal stimuli, (b) background stimuli and (c) residual stimuli.

When normal subjects are instructed to produce equal time interval, (TI), by counting 4 beats in 20 consecutive trials, their performance is described by a sawtooth function. (The TI decreases progressively during the first 3 or 4 trials but it increases abruptly during the 4th or the 5th trial. This pattern is repeated throughout the 20 trials).

The progressive decrease of the TI is attributed to the residual stimulation of the previous trial, (focal and background stimuli are constant), while the sudden decrease of TI (or increase of speech rate) every 3 or 4 trials is a self-corrective response of the subject's awareness of "mispronounciation" as the rate of speech increases.

The above mentioned task will be given to inpatient schizophrenics before and during the administration of antipsychotic medication. The slope of the progressive decrease of the TI will be used as a measure of adaptation level and may prove a helpfull behavioral rating device when correlated to the blood level measurement of various antipsychotic medications.

In addition subjects will be scored on: "Reaction Time", "Finger Tapping Speed", "Brief Psychiatric Rating Scale" and "Modified New Haven Schizophrenia Scale".

2291 SEMANTIC FUNCTIONAL MEASUREMENT OF PAIN: INTEGRATING PERCEPTION & LANGUAGE. P.J. Wolskee* and R.H. Gracely* (SPON: G.J. Bennett). Neurobiology & Anesthesiology Br., NIDR, NIH, Bethesda, MD 20205.

This study used Functional Measurement (FM) scaling procedures to demonstrate that subjects can scale and integrate pain that is produced by an electrical tooth pulp stimulus and symbolized by a word. Unlike conventional psychophysical scaling methods, FM includes a testable integration criterion that must be met before the scale is accepted. This method does not require physical measures of stimulus intensity and can produce direct scales of sensory input of non-metric stimuli such as words or pain experience. We required subjects to respond to the average of a metric and a non-metric stimulus set: 1) painful electrical stimuli (metric stimulus) applied to the tooth pulp (1 sec trains of 100 Hz, 1 msec monophasic monopolar pulses) and 2) verbal descriptors (non-metric stimulus) of sensory intensity (weak, mild, moderate, strong, intense) and unpleasantness (annoying, uncomfortable, dreadful, horrible, agonizing) that symbolize different dimensions of the pain experience. Eighteen subjects received all possible pairs of 1) 5 tooth pulp stimuli ranging in equal log steps from pain threshold to tolerance, plus 2) 5 descriptors of sensory intensity or unpleasantness twice each for a total of 50 stimulus pairs. Each subject rated the average sensory intensity or unpleasantness of each tooth pulp-word pair by squeezing a hand dynamometer. The ability to average the perceptions and the validation of both the task and resultant scales can be tested by a statistical test (2 way ANOVA). A nonsignificant interaction term validates the test and resultant interval scales of both stimulus sets. Statistical analyses of sensory intensity responses showed significant main effects of tooth pulp stimuli ($F(4,36) = 12.87$ $p<0.0001$) and words ($F(4,16) = 25.20$ $p<0.0001$) and nonsignificant interaction ($F(16,144) = 1.11$ $p<0.3483$). Analyses of the unpleasantness responses also showed significant main effects of tooth pulp stimuli ($F(4,28) = 4.84$ $p<0.0043$) and words ($F(4,16) = 12.99$ $p<0.0001$) but the interaction was only marginally nonsignificant ($F(16,112) = 1.73$ $p<0.0508$). This result shows that subjects can use the intensity of a sensation or a word that symbolizes sensory intensity interchangeably in an averaging scaling task, supporting the use of verbal descriptors of sensory intensity in the assessment of pain. Unpleasantness scaling shows that these word scales are not integrated with actual stimuli as easily as sensory words. We conclude that subjects can integrate the pain produced by a tooth pulp stimulus with pain symbolized by a word and that this integration varies with the type of words used. FM methods may provide a new and promising tool for the assessment of pain experience.

REGENERATION

2292 THE CONTROL OF REGENERATION SPECIFICITY IN EARTHWORM GIANT AXONS
Stewart C. Birse* and George D. Bittner, (Spon:H.M. Eisenberg) Dept. of Zoology, Univ. of Texas, Austin, Texas 78712

We have previously reported that transected and ablated giant axons in the earthworm ventral nerve cord (VNC) regenerate by growing neuronal processes which arise from the severed stumps of the giant axons, traverse the lesion site and contact a giant axon on the opposite side of the lesion (Birse and Bittner, 1976,1977, 1978). Furthermore, the specificity of giant axon reconnection was very high in that every physiological recording from a giant axon which demonstrated regeneration, also showed that the appropriate giant axons had reconnected and that no inappropriate functional contacts were made.

To examine the morphological basis for the high degree of regeneration specificity, we injected Lucifer Yellow CH intracellularly into a giant axon in 22 animals with VNC transections and 15 animals with VNC ablations. The injections filled neuronal processes, many of which crossed the lesion site and established appropriate contacts on the opposite side of the lesion, as we have previously reported. However, some neuronal processes grew for 1-2 VNC segments beyond the lesion or grew away from the lesion site. In some cases, the neuronal processes of an injected giant axon appeared in close proximity to an inappropriate giant axon. We also observed that as postoperative time increased, regenerating giant axons produced greater numbers of axonal processes.

Our observation of occasional misdirected axonal outgrowth is at variance with our observation of highly specific functional reconnection. This paradox might be resolved, however, if some mechanism were to insure that only the correct contact were permitted to establish a functional connection. The probability of making a correct contact is increased by extending many processes from a regenerating giant axon. If only the correct contact established function, regeneration specificity would be very high, even when the control of outgrowth was not always directed appropriately. The high degree of regeneration specificity we observed in the earthworm, then, might be the result of a highly selective mechanism for establishing functional contacts, not a highly directed growth mechanism.

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2293 THE MECHANISM OF AXONAL REGENERATION IN CRAYFISH MOTOR NEURONS.
George D. Bittner, Michael S. Bouton*. Dept. of Zoology, Univ. of Texas, Austin, TX 78712.

Previous studies have shown that the distal segment of a severed crayfish motor axon may survive for 100-250 days following separation from its cell body. Regeneration of the severed motor axon occurs by reconnection of the proximal stump with the surviving distal stump via mechanisms as yet unknown (Hoy, et. al., Sci., 1967; Kennedy and Bittner, Cell Tiss. Res., 1974). Axonal regeneration in these animals has been associated with the presence of multiple profiles assumed to arise from the severed proximal stumps and to grow within the glial sheaths of surviving distal segments (Nordlander and Singer, Z. Zellforsch, 1972). Similar phenomena have been reported in the leech (Van Essen and Jansen, Cold Spring Harbor Symposia, 1976; Frank, et al., J. Comp. Neurol. 1975; Carbonetto and Muller, Nature, 1977) and the earthworm (Birse and Bittner, Brain Res., 1976).

Several theories have been proposed to account for regeneration in these various preparations:

1) The proximal stump grows out for a greater or lesser distance and morphologically fuses or makes electrotonic connections with the surviving distal stump. A new set of synapses are never formed (Hoy, et.al., Sci., 1967; Bittner and Kennedy, Cell Tiss. Res., 1974; Frank, et. al., J. Comp. Neurol., 1975).

2) The proximal stump grows out as described above, but eventually reaches the target tissue and makes a new set of synapses. The original distal stump may or may not degenerate (Nordlander and Singer, Z. Zellforsch, 1972; Carbonetto and Muller, Nature, 1977).

We have initiated a series of experiments to determine more precisely the mechanism of regeneration in crayfish motor neurons. We are examining the distal stumps of severed motor axons at various times up to 400 days after regeneration. In reconnected cases, we have found more multiple profiles near the target muscle at 400 days than at 50 days after regeneration is first noted. We are injecting the proximal and distal stumps of regenerated motor axons to determine the origin and specificity of these multiple profiles.

2294 THE EFFECT OF A REPEATED OPTIC NERVE INJURY ON THE RETINAL GANGLION CELL RESPONSE IN THE NEWT (Notophthalmus viridescens).
T. O. Brock, III and J. E. Turner, Jr, Dept. of Anat., Bowman Gray Sch. of Med. of Wake Forest Univ., Winston-Salem, NC 27103

Optic axon outgrowth from the lesioned newt retinal ganglion cell (RGC) can be enhanced by administering a second optic nerve lesion proximal to and 4 days after the initial lesion. The newt RGC responds after a single or double lesion of its axon with a visible time course of chromatin changes that corresponds to an increase in cell activity. These chromatin pattern changes progress in a linear fashion from a homogeneous to a heterogeneous state with over 50% of the cells demonstrating prominent changes by 14 days after lesion. The nuclear reactivity plateaus until day 21 and then decreases to control values by 90 days after lesion. A series of experiments was designed to ascertain if a second optic nerve lesion administered 21 days after the initial lesion would maintain the cells in an extended period of heightened activity. In these experiments all animals received an initial optic nerve crush and then were divided into single lesion (control) and double lesion (experimental) groups. Controls were sacrificed at various times between 24 to 90 days after lesion. Experimental animals received a second crush just proximal to the initial lesion at day 21 and were sacrificed at various times between 3 to 69 days after second lesion. In each case the total survival time was the same in control and experimental groups. Results indicate that the second optic nerve lesion maintains the number of RG cells demonstrating prominent chromatin changes for periods of time twice as long as those receiving a single lesion. EM morphometric analysis of cells in the RGC layer during this extended plateau period revealed that perikaryal area, nucleolar area and number and, the perikaryal to nuclear ratio were significantly greater than in controls of the same survival period.

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2295

Withdrawn by Author

2296 RATES OF GROWTH AND COMPOSITION OF AXONALLY TRANSPORTED PROTEINS IN REGENERATING OLFACTORY NERVE. P. Cancalon and J. S. Elam. Dept. Biol. Sci., FSU, Tallahassee, FL., 32306.

Previous ultrastructural and biochemical studies of the olfactory nerve have indicated that axonal injury causes total degeneration of the mature neurons followed by replacement with new neuronal cells arising from undifferentiated mucosal cells. (Graziadei, P.P.C. and Graziadei, G.A., in Neuronal Plasticity, 1978). Intra-axonal transport of 35S methionine labeled protein was used to study regeneration in the unusually long garfish olfactory nerve.

Results indicate that nerves crushed 1.5 cm from the cell bodies produce three distinct populations of regenerating fibers. The first traverses the crush site one week postoperative and progresses along the nerve at a rate of 5.8 ± 0.3 mm/day for the leading fibers of the group and 4.9 ± 0.2 mm/day for the majority of axons. The second group of fibers traverses the crush site after 2 weeks postcrush and advances for 60 days at the constant rate of 2.1 ± 0.1 mm/day for the leading fibers and 1.9 ± 0.1 mm/day for the bulk of the axons. The velocities subsequently fall to 1.6 ± 0.2 and 0.8 ± 0.1 mm/day for the leading and main population of fibers respectively. Electron microscopy of the proximal and distal regions of a regenerating nerve at 100 days indicates that each of the two most rapid phases of growing fibers represents between 2 to 5% of the original population of axons. They both are typically characterized by small fascicles of axons surrounded by glial cells and large amounts of collagenous material. The leading fibers of a third group of regenerating axons traverse the crush site after 4 weeks and advance at a constant rate of 0.8 ± 0.2 mm/day. The fibers appear to represent 50-70% of the original axon populations.

Analysis of fast axonal transport of radioactive proteins in the most rapidly growing phase of regenerating fibers indicates that the rate of transport is unaffected by regeneration. However preliminary calculations indicate that this phase may contain as much as 8 to 16 times more radioactivity per fiber that unoperated controls. Subcellular and polypeptide analysis were also performed on material accumulating at the tip of the most rapidly growing fibers. Although there was general similarity to results in intact nerves, a shift of radioactivity from higher to lower density membranous fractions was observed. This may reflect a relatively lower proportion of protein in immature nerve cell membrane (Pfenniger and Bunge, J. Cell Biol. 63:180, 1974). Small but significant differences were also observed in the molecular weight distribution of transported polypeptides. (Supported by the National Paraplegia Foundation and NIH grant NS 11456).

2297 MORPHOLOGY OF LAMINA IX NEURONS OF THE RAT THORACIC SPINAL CORD FROM DAY 14 OF GESTATION TO ADULTHOOD. John P. Cummings* and Dennis J. Stelzner. Department of Anatomy, SUNY-Upstate Medical Center, Syracuse, New York 13210.

Following rat spinal hemisection, Bernstein and Bernstein(Experimental Neurology 30:336-351) proposed that neurons proximal to the lesion "de-differentiate" to a more primitive state in which the neuron is once again capable of accepting synapses. From day 10 to day 90 following hemisection motor neurons were observed to have decreased dendritic fields, loss of dendritic branches and an increased number of dendritic varicosities along the entire length of their dendrites. The current study was undertaken to study the maturation of lamina IX neurons in the rat thoracic spinal cord from day 14 of gestation to adulthood so that the stages of differentiation of lamina IX neurons could be compared with the stages of "de-differentiation" described by Bernstein and Bernstein.

From day E-14 to day E-18 the primary dendrites of lamina IX neurons appear to be adult in number. However, they are short in length and have varicosities of uneven diameter. Hair-like structures protrude from these varicosities. From day E-18 until postnatal day 2 the more proximal parts of the dendrites retain these varicosities, and, in addition are invested with long hair-like processes up to 5μm in length. These structures are also occasionally found on the cell body while the more distal dendrites retain the morphology found earlier. Between postnatal days 2 and 12 the secondary and tertiary dendrites develop dendritic spines which are greater in number but shorter in length than those found earlier on the primary dendrites. The primary dendrites at this stage have a much smoother appearance and shorter spines than are found on the more distal dendrites. Beginning with postnatal day 12 there is a gradual shortening and reduction of dendritic spines on all parts of the neuron accompanied by a reduction in the diameter of the dendritic varicosities until the adult morphology of lamina IX neurons is obtained between postnatal days 24 and 30.

Thus immature lamina IX neurons resemble the "de-differentiated" cells reported by Bernstein and Bernstein in certain respects, but the stages of maturation are not directly comparable with the stages of "de-differentiation" that they found. Possibly the correlation would be more exact if lamina IX neurons were studied over a larger number of intervals following spinal cord injury. Such a study is in progress comparing neurons after spinal transection in the newborn and weanling rat. (Supported by Grant NS-14096)

2298 ENDOGENOUS LECTINS IN COCKROACH MUSCLES-CANDIDATE RECOGNITION MACROMOLECULES. Jeffrey L. Denburg. Dept. Zoology, Univ. of Iowa, Iowa City, IA 52242.

One biochemical model for the intercellular recognition process between motor neurons and muscles involves lectin-like macromolecules that may function as intercellular cross-bridges. The coxal depressor muscles of the cockroach Periplaneta americana, offer an ideal system for testing this hypothesis. They are innervated by identified motor neurons in the thoracic ganglia which upon crushing their axons regenerate until the original innervation pattern is reformed (Pearson and Bradley, Brain Res. 47: 492, 1972). This pattern consists of muscles 178, 179 innervated by a single motor neuron D_f, muscles 177d,e innervated by a single motor neuron D_s and muscles 177d',e' which are a homogeneous population of fibers innervated by both D_f and D_s (Pearson and Iles, JEB 54: 215, 1971).

Lectins have been detected in homogenates of each of the cockroach muscles using the microtiter assay for agglutination of human red blood cells. The lectin in muscles 178, 179 has a carbohydrate binding site of different specificity from that in muscles 177d,e. This is determined by the ability of sugars to inhibit hemagglutination. Galacturonic acid (1mM) can inhibit the lectin of muscles 178, 179 by 50%. At least 10mM concentration of this sugar is needed to produce the same amount of inhibition of lectin from muscles 177d,e. These muscles on the other hand are preferentially inhibited by amino sugars. These muscle hemagglutinins are insoluble and may be localized to the basement membrane as inferred from the inability to solubilize them with non-ionic detergents. They are heat labile and inactivated by treatment with proteolytic enzymes.

Evidence for the role of such macromolecules in intercellular recognition comes from the following observations: 1) Correlation between presence in muscle of lectin with particular sugar specificity and innervation by particular identified motor neuron; 2) Denervation of muscles results in a lowering of levels of hemagglutinin which recover as reinnervation occurs; and 3) Detection in neuronal extracts of soluble, heat stable, protease insensitive macromolecules that inhibit hemagglutination by muscle macromolecules.

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2299 ENHANCEMENT OF AXONAL OUTGROWTH BY A PRIOR ("CONDITIONING") LESION: EFFECT OF LESION INTERVAL. D. Louise Edwards*, Roberta M. Alpert*, Peter G. Mandelson*, Irvine G. McQuarrie, and Bernice Grafstein. Dept. Physiol., Cornell University Medical College, New York, NY 10021

The rate of outgrowth of goldfish optic axons is enhanced by a prior lesion of the same axons (I.G. McQuarrie & B. Grafstein, Soc. Neurosci. Abstr. 4: 533, 1978). This enhancement was found to be maximal when the "testing" lesion from which outgrowth is measured (crushing the optic nerve) was preceded by a "conditioning" lesion (cutting the optic tract) 14 days earlier. Lesion intervals of 7 and 21 days were less effective. These results were obtained by measuring the time required for recovery of visual function following the testing lesion. Recovery of function was defined as the appearance of a startle response to light after a 20 minute period of dark adaptation. For other indicators of visual function, e.g. the dorsal light reflex and food localizing behavior, which recover more slowly than the startle response, the recovery times did not vary with the interval between testing and conditioning lesions.

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2300 REGENERATION OF SPECIFIC NEUROMUSCULAR CONNECTIONS IN THE CRAYFISH. Pamela Ely* and Samuel J. Velez. Department of Biological Sciences, Dartmouth College, Hanover, N.H. 03755

In the projection of six motoneurons to a flat sheet of 40 muscle fibers, the superficial flexor muscle of the crayfish Procambarus clarkii, the probability that a given axon innervates a given muscle fiber is a simple function of the position of the fiber in the muscle sheet (Velez & Wyman; J. of Neurophys. 41: 75-96). The specificity of these connections makes this a very suitable system in which to study the regeneration of connections between identifiable cells. The third superficial root was cut as the nerve crossed between some muscle fibers, leaving the proximal stump anchored to the muscle and some neuromuscular junctions intact. The presence of these few remaining junctions facilitated nerve regeneration; in their absence, with an unattached nerve, degeneration of the proximal stump occurred. The animals were allowed to recover, and analysis was performed on isolated abdomens on a weekly basis by recording the spontaneous activity of the nerve with an extracellular oil electrode and measuring junction potentials (jp's) with 3M KCl microelectrodes. Regeneration was first found in animals 2-3 weeks post-op. The small axons (1, 2 and 3) regenerated first, the large axons (4 and 6) regenerated later. By 5-6 weeks the entire plane of the muscle was innervated by at least one of the axons (usually a small one) though there were 'holes' in the map, i.e., some muscle fibers innervated by certain axons in controls were not innervated in regenerated animals. JP's of 10-20 mV in size, giant relative to controls (1-5 mV in size), were invariably found on a few muscle fibers in regenerating animals. The region of these giant jp's varied with the post-operative age of the animals. Methylene blue staining revealed that the region of giant jp's corresponded to the area of the growing tip of the nerve. It was further noted that the regenerated nerve was initially a single process; branching was rare until relatively late in the course of regeneration. The regenerated nerve frequently ran ventral to the plane of the muscle fibers, in contrast to controls where the nerve always ran dorsal to the plane of the muscle fibers. Regeneration was complete, and comparable in connection specificity to control animals, by 9-10 weeks.

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2301 RESTITUTION OF LONG DESCENDING TRACTS AND NORMAL BEHAVIOR FOLLOWING SPINAL CORD TRANSECTION IN ANURA. Cynthia J. Forehand* and Paul B. Farel (Spon: D.L. McIlwain). Neurobiol. Prog. and Dept. Physiol., Sch. Med., Univ. N. Carolina, Chapel Hill, NC 27514.

Midthoracic spinal cord transection in bullfrog tadpoles produces behavioral deficits that persist until metamorphosis, regardless of the developmental stage at which the transection is performed. In contrast, operated tadpoles allowed to go through metamorphosis are, as juvenile frogs, behaviorally indistinguishable from unoperated animals. Similarly, we have, in horseradish peroxidase (HRP) studies, found no evidence of long tract regeneration in tadpole; however long descending fibers are clearly demonstrable in previously transected, metamorphosed animals.

Following transection of tadpole spinal cord, gross anatomical continuity of the cord is restored in 1-2 weeks. For periods up to 60 days postoperative, however, righting reflexes are sluggish, responses are difficult to evoke by visual stimuli, and there is little swimming in the absence of stimulation. Injection of HRP into the lumbar enlargement of the previously operated tadpole reveals heavy labeling of fibers caudal to the thoracic transection site but no fibers bridging this region are labeled. While in the unoperated tadpole, cell bodies in the vestibular nuclei and brainstem reticular formation are consistently found, cells in these areas are not labeled in the previously transected tadpoles. Electrophysiological evidence indicates functional connection between the previously severed halves in tadpole stages may be mediated by short axons of propriospinal neurons crossing the gap.

Transected tadpoles held through metamorphosis showed the same pattern of behavioral deficits before completion of metamorphosis. Following the 2-3 week metamorphic period, these animals showed behavioral recovery such that they were indistinguishable from unoperated controls in their walking, leaping and swimming. Righting reflexes were brisk, and scratch reflexes were properly directed and of normal threshold. HRP injection into the lumbar enlargement labeled fibers in the ventral funiculus that traversed the transection site. Somata were labeled in the vestibular nuclei and throughout the reticular formation as far rostral as the midbrain.

Two possible explanations of the differences between tadpoles and juvenile frogs can be considered. First, the stimulus necessary for regeneration of long tracts is present only during metamorphosis. Alternatively, different brainstem neurons might project to lumbar regions in tadpole and frog. During metamorphosis these new descending connections could develop in both unoperated and previously transected tadpoles to form the substrate of normal behavior in both groups of animals.

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2302 SYNAPTIC RECEPTORS IN THE DEGENERATING AND REGENERATING VISUAL PATHWAY. Andrew Francis* and Nisson Schechter. Department of Neurology, Cornell University Medical College, New York, 10021 and Department of Psychiatry and the Long Island Research Institute, SUNY, Stony Brook, New York 11794.

After optic nerve crush, regeneration and apparent specific reconnection of optic axons occurs at the optic tectum in goldfish. Critical to an understanding of the molecular basis of reconnection is an analysis of the disconnection and reconnection processes at the synaptic level. We have been employing established synaptic receptor ligands in the effort to follow changes in the number, concentrations, and type of synaptic molecules during the disconnection and reconnection process, focusing on the cholinergic system. Nicotinic-cholinergic receptor activity was estimated by the binding of alphabungarotoxin (aBuTx) and muscarinic-cholinergic activity by quinuclidinyl benzilidate (QNB) binding. After optic nerve crush in goldfish, there is an initial rapid loss, followed by a later recovery of aBuTx binding which correlates with regeneration of the optic nerve; after eye removal, which produces a permanent disconnection of the optic tectum, the same rapid loss in aBuTx binding occurs, but is permanent (Brain Res., 166, 57). In parallel experiments, analysis of QNB binding indicated that there were no changes in binding activity of goldfish optic tectum during optic nerve degeneration. We have also observed the same pattern of rapid loss in aBuTx activity with stable QNB activity after similar disconnections of the retinotectal system in developing and adult birds, as well as in the retino-collocullar pathway in rats. In these systems, we have not observed loss of choline acetyltransferase activity, although in the goldfish system, there are small, consistent losses in activity which recover as optic nerve reconnection proceeds (in press). These results suggest that there may be some important biological significance to the plasticity of nicotinic-cholinergic receptor activity in these systems, which is in contrast to the marked stability of the muscarinic-cholinergic activity. It would appear that the nicotinic receptors are closely involved in the primary retinotectal synapse, possibly presynaptic or postsynaptic at the retinotectal terminals. The muscarinic receptors may be in another independent tectal system of afferents of intrinsic neurons. Therefore, nicotinic-cholinergic receptor activity may represent a useful and selective biochemical probe for retinotectal synapses and their dynamics.

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2303 THE EFFECT OF N^6,O^2-DIBUTYRYL ADENOSINE 3',5'-MONOPHOSPHATE ON THE DEGENERATION AND REGENERATION OF CRUSH-LESIONED RAT SCIATIC NERVES. M. R. Gershenbaum* and F. J. Roisen* (SPON: S. Rosner). Dept. of Anatomy, Rutgers Medical School-CMDNJ, Piscataway, NJ 08854.

The ability of N^6,O^2-dibutyryl adenosine 3',5'-monophosphate [$(But)_2$cAMP] to stimulate nerve development in vitro prompted us to study the effects of the nucleotide on the in vivo degeneration and regeneration of crushed rat sciatic nerves. The animals received daily intramuscular injections of either $(But)_2$cAMP (50mg/Kg) or 0.9% saline after operation and were tested daily for the return of sensorimotor function (SMF). An index of SMF was obtained by placing the foot of the lesioned limb over the aperture of a shutter-controlled high intensity light box. The time required for foot withdrawal was recorded to the nearest second. $(But)_2$cAMP and saline-treated rats showed little difference in the rate of return of SMF for 10 days, but by day 12, the response times for the nucleotide-treated animals diverged from those in the saline-treated group. Nucleotide-treated animals appeared to be completely recovered by day 16, while saline treated rats did not exhibit full recovery until day 26. To investigate the morphological changes that accompanied this functional recovery, rats were sacrificed 3, 10, 18, and 32 days following crush and their nerves prepared for transmission and scanning electron microscopy. These observations, fiber counts and measurements of fiber size indicated that $(But)_2$cAMP accelerated the initial processes of Wallerian degeneration and enhanced the rates of regeneration and maturation of the peripheral nerve fibers. In general, the crushed nerves of nucleotide treated animals appeared 7-8 days more advanced in their degenerative and regenerative processes than the saline controls. Previous workers who found no effect of $(But)_2$cAMP on nerve regeneration probably did not allow enough time to pass for these effects to express themselves, since morphological and physiological differences between regenerating nerves of nucleotide and saline treated rats appeared only after 8-10 days post lesion.

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2304 OBSERVATIONS ON THE CENTRAL CANAL AREA OF YOUNG RATS FOLLOWING SPINAL CORD INJURY. Shirley A. Gilmore and Jane E. Leiting*. Dept. Anat., Univ. Arkansas Med. Sci., Little Rock, AR 72201.

Ependymal proliferation plays an important role in regenerative processes in the spinal cords of some vertebrates. Proliferative capacities of ependyma in mammalian spinal cord in normal or injured states is poorly documented. There is little evidence that proliferation occurs later than the periods of normal marked developmental activity in the wall of the neurocele. Several years ago a study was undertaken to evaluate the responses of the immature rat spinal cord to a lesion in the lateral aspect of one hemisection. One of the observations was that the central canal and its surrounding wall had undergone marked changes in general appearance and orientation. Variations in thickness of the cellular wall suggested that proliferation had occurred. Because of the paucity of data regarding this area and its response to injury in the mammalian spinal cord, additional studies were undertaken.

Charles River CD^{R} rats, 3 to 4 weeks old, were used. Under Nembutal^{R} anesthesia a unilateral laminectomy was performed at T_{12} and L_1. A small wound was made with a microscalpel in the lateral or dorsolateral portion of the spinal cord; if the wound was shown later to include the central canal area, the material was not considered in this study. These animals and their sham-operated controls were perfused with 10% phosphate-buffered formalin from 2 days to 6 weeks post-operative. Spinal cords were embedded in paraffin and stained by a variety of histopathologic techniques. Since cellular proliferation was suspected, ^{3}H-thymidine was injected into some animals 2 hours prior to autopsy. Autoradiographs were prepared by the dipping technique.

The central canal area of the normal 3- or 4-week-old rat resembles that of the adult. The canal is usually round or oval with its long axis oriented dorsoventrally. The walls are composed of cells in pseudostratified arrangement usually with two, sometimes three, layers of nuclei. After injury the canal changes shape and orientation so that a flattened wall, as originally seen laterally, "faces" the lesion. The arrangement of the cells is no longer uniform. The lateral wall often appears to have only one cell layer, whereas the poles have an aggregate or clustering of nuclei. In general, the walls lose their uniformity in thickness and cellular arrangement. Preliminary autoradiographic findings show that at 2 days post-operative 5.75% of the cells are labeled as opposed to 0.21% labeling in the sham-operated or intact controls. Mitotic figures are also evident. By 5 days post-operative, 0.85% of the cells are labeled and by 7 days or more the proliferative activity is at the control level. (Supported by USPHS Grant NS 04761.)

2305 MORPHINE-ENHANCED REGENERATION OF CENTRAL NORADRENERGIC NERVES FOLLOWING 6-HYDROXYDOPA TREATMENT OF RAT PUPS. Craig T. Harston, Judy C. Hardin* and Richard M. Kostrzewa. Dept. of Pharmacology, College of Medicine, East Tennessee State University, Johnson City, Tennessee 37601.

The locus coeruleus which provides the noradrenergic innervation to the cerebellum is known to be innervated by serotonergic, substance p- and endorphin-containing neurons. Because of the possibility that neurosecretory substances from these nerves could serve as neuromodulators on the development of the locus coeruleus, we investigated the effect of morphine, an endorphinomimetic, on development of the noradrenergic innervation in cerebellum. Morphine sulfate (20 µg/g i.p.) was injected into intact and neurotoxin-treated (i.e., 6-hydroxydopa {6-OHDOPA} 60 µg/g i.p.) rat pups on the day of birth or three days after birth. Norepinephrine (NE) levels in the cerebellum, pons-medulla and hippocampus of these rats (8 weeks after birth) were assayed by either a spectrophotofluorometric or radiometric method. It was found that morphine sulfate potentiated the recovery of NE levels in the cerebellum and pons-medulla of the rats treated with 6-OHDOPA on the day of birth and in the pons-medulla of the rats treated with 6-OHDOPA on the third day after birth. No effect of morphine was found on the NE content of the hippocampus of control or 6-OHDOPA treated rats. The cerebellar effect was morphine dose-related (0.25 - 20. µg/g i.p.) in rats treated on the day of birth and was apparent by 14 days of age. The morphine-induced increase of NE in the cerebellum was corroborated by increased density of histofluorescent fibers (glyoxylic acid method) in the cerebellum of adult rats. These data show that acute morphine treatment potentiated the recovery of the noradrenergic system in the brain following neonatal damage with 6-OHDOPA. These results suggest that neurosecretory agents (e.g. endorphins) could modulate regeneration of damaged neural systems. (Supported by the National Foundation March of Dimes)

2306 COMPLEX ACTION POTENTIALS AFTER RECOVERY FROM NERVE LIGATION. Kenneth Horch. Dept. Physiol., Univ. Utah, Salt Lake City, UT 84108.

En passant, whole nerve recording of activity in Aα fibers in one branch of the femoral cutaneous nerve of a cat was made with a monopolar hook electrode 16 months after the branch had been ligated with 6-0 silk thread. At the time of the recording, there was no trace of the ligature and no sign of damage to the nerve when examined under 25x magnification. The nerve branch innervated 18 type I receptors (domes), each of which produced a typical continuous, but irregular train of discharges when stimulated with an 80 mg von Frey hair. The stimulus did not excite neurons other than those innervating the domes. For 17 of the domes, the action potentials appeared normal. For one, however, a complex action potential waveform was seen (see below). The spacing of these "complex action potentials" indicates that only one axon innervated the dome and was excited there by the mechanical stimulus. Speculation is invited as to whether this represents ephaptic coupling of large myelinated axons, or an oscillatory process in the membrane of a single axon.

Legend. Each figure was made by action potential-triggered superposition of 20 or more sweeps during sustained, mechanical stimulation of the dome. The waveform did not change with changes in tension on the nerve or changes in the firing rate of the axon. (A) Initial recording site, about 30 mm proximal to the dome. Sweep speed: 0.2 msec/div. (B) The electrode was moved distally to a point about 23 mm from the dome. Sweep: 0.5 msec/div. (C) The electrode was moved back to near the original recording site. Sweep: 0.5 msec/div.

2307 A STUDY OF NEURAL SOMATA REGENERATION - A TEST OF THE NEURONAL ADDITION HYPOTHESIS. C. E. Hulsebosch* and G. D. Bittner (SPON: T. Viancour). Dept. of Zoo., University of Texas, Austin, TX 78712.

One hypothesis which may account for the capacity of a species to regenerate neural somata is the neuron addition hypothesis (Hulsebosch, C. E. and G. D. Bittner. Soc. for Neurosc. Abst., 4: 532, 1978; Leonard, R. B., et al., J. Comp. Neurol. 179(1): 13-22, 1978). This hypothesis states that species which add CNS somata during ontogeny may be more likely to regenerate somata in response to injury than those species which have a constant or decreasing number of CNS somata.

To test the neuronal addition hypothesis, we selected two species of marine polychaetes with similar life styles; however, one species, *Nereis virens*, adds body segments (and consequently neural somata) and one species, *Clymenella torquata*, has a constant number of body segments. Using ultrastructural and histological techniques, we counted the number of neurons (as opposed to glial and connective cells) in a particular ganglion in mature individuals of each species. From these counts, we determined that neither species add neural somata to ganglia of the mid-body region. We then performed ganglionic ablations and determined that somata regeneration does not occur in *Nereis virens* allowed to survive for up to three months after lesioning. Conversely, neural somata did regenerate in *Clymenella torquata* within two weeks after ganglionic ablation. To test that nerve cells in the ablation site of *Clymenella* are a result of regeneration and not a result of somata rearrangement from adjacent uninjured regions, we counted the number of neurons in the ablation site and adjacent CNS regions of controls and regenerates. Our counts demonstrate that neural somata in the ablation site are a product of regeneration and not of rearrangement.

Our results in *Nereis virens* indicate that the addition of neural somata in one region of the neuraxis can not provide for somata regeneration in another region of the neuraxis. Furthermore, our results in *Clymenella torquata* indicate that CNS somata addition is not a necessary prerequisite for somata regeneration. Hence, our experiments demonstrate that the neuronal addition hypothesis alone can not account for CNS somata regeneration.

2308 FOREIGN NERVE INNERVATION OF A MUSCLE FIBER POPULATION IN THE CRAYFISH. William P. Hunt* and Samuel J. Velez. (SPON: H. L. Borison) Department of Biological Sciences, Dartmouth College, Hanover, New Hampshire 03755.

The six motoneurons that innervate the 40 muscle fibers of the superficial flexor muscle of the crayfish Procambarus clarkii can regenerate their connections within 9-10 weeks with a high degree of specificity (Ely & Velez, Neurosciences Abstracts, 1979). Experimental manipulation of this system during regeneration can give us insight on the processes that influence the formation of specific connections between identifiable cells. The third superficial root that innervates the left superficial flexor muscles of the third segment (to be referred to as 'the foreign nerve') was transplanted to the contralateral superficial flexor muscles of the same segment. In some experiments, the innervation of the recipient muscle was left intact, while in others the muscle was denervated. Animals were left to recuperate, and analysis was performed on a weekly basis by monitoring the spontaneous activity of the nerve in isolated abdomens with an extracellular oil electrode, stimulating the nerve with a suction electrode, and recording muscle junction potentials (jp's) with 3M KCl microelectrodes. Foreign nerve innervation occurs earlier in denervated muscle than in innervated muscle. In both cases, the small axons appear to grow first, followed by the large axons. Initial innervation spreads over the entire field leaving some 'holes' in the map, just as was observed when the original nerve reinnervates the muscle after it has been cut (Ely & Velez, ibid). The initial synapses made by the foreign nerve are characterized by gigantic jp's (10-20 mV in size when compared to 1-5 mV in size in control animals), little facilitation (when comparing jp sizes at 1 Hz and 10 Hz stimulation) and some misses in firing (no jp's produced even though the axon is firing). The presence of the original nerve in a muscle fiber seems to have no effect on the ability of the foreign nerve to make connections with that fiber as jp's produced by the foreign nerve could be larger than those produced by the original nerve on the same fiber. Innervation by the foreign nerve is complete across the muscle field for the first growing axons by 8 weeks, and the connectivity map appears to be the same as the map of the original nerve.

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2309 Association of Spermidine with Axonally Transported 4S RNA in Regenerating Optic Nerves of Goldfish. N.A. Ingoglia, P. Jaggard*, C. Perez* and J. Sturman. Dept. of Physiology and Neurosciences, New Jersey Medical School, Newark, N.J. 07103, and Dept. of Pathol. Neurobiol., Institute for Basic Research in Mental Retardation, Staten Island, N.Y. 10314

Recent experiments have shown that during regeneration of the optic nerves of goldfish, spermidine (Spd) is axonally transported (AT) in both an unbound TCA soluble, as well as a TCA insoluble form. We have proposed that TCA insoluble Spd is associated with AT 4S RNA. The present experiments were performed to test this hypothesis.

In one set of experiments, inhibition of retinal RNA synthesis by intraocular injections of 10μg of cordycepin, blocked the AT of both ^{3}H-RNA and ^{14}C-Spd by about 65% 6 and 14 days after injection. Intraocular injections of Vinblastine, (0.1, 0.5 or 1.0μg) an agent which interrupts AT of proteins, had no effect on retinal RNA synthesis nor on the amount of ^{14}C Spd incorporated into the TCA insoluble fraction of retinal extracts. However, the AT of both ^{3}H-RNA and ^{14}C-Spd was effected in a dose dependent fashion; the inhibition of both was approximately 80% at the higher dose. Further evidence for an association between AT 4S-RNA and Spd came from experiments in which regenerating optic axons were cut and allowed to degenerate 6 days after injection of ^{3}H-Spd into the eye. The loss of optic axons from the tectum 7 days after cutting the nerve resulted in an 86% loss of TCA insoluble Spd, indicating a largely intra-axonal locus for Spd. A similar loss of 4S RNA was found in identical experiments following injections of ^{3}H-uridine into the eye. Finally, experiments were performed in which ^{3}H-Spd was injected into both eyes of 12 fish whose optic nerves had been regenerating for 18 days. Six days later, fish were sacrificed and RNA was extracted from tectal homogenates by hot phenol and ethanol precipitation. The major stable RNA species were separated by SDS polyacrylamide disc gel electrophoresis and radioactivity was determined by extraction of 2.0 mm gel slices. Results showed co-migration of ^{3}H radioactivity only with the 4S RNA optical density peak, and not with 28S and 18S ribosomal RNA peaks, suggesting that Spd may be AT along with 4S RNA.

The data indicate that some Spd which is transported during regeneration of goldfish optic nerves is probably associated with 4S RNA, perhaps complexed in a manner similar to that described for Spd and bacterial tRNA.

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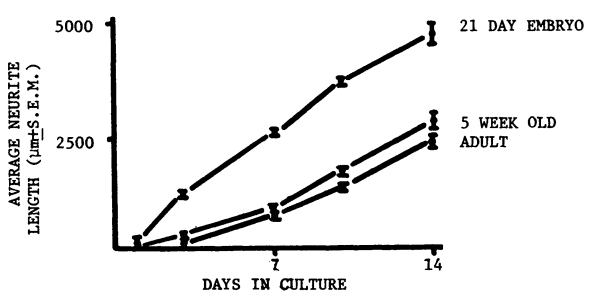
2310 COMPARISON OF GROWTH PATTERNS OF CULTURED SYMPATHETIC NEURONS FROM PERINATAL AND POSTNATAL RATS. M. Johnson and V. Argiro*. Dept. of Anat. & Neurobiol., Wash. Univ. Sch. Med., St. Louis, Missouri 63110.

Long term cultures of superior cervical ganglion (SCG) neurons can now be obtained from not only embryonic but also postnatal rats, including fully adult animals (Nature 267: 536, 1977). This allowed the study of the relative growth capacities of neurons from rats of different ages. Explants of the SCG from 21-day embryo and postnatal rats were grown in the same dish on an air-dried collagen substrate and fed either standard feed containing human placental serum and embryo extract or feed with added antimitotic agents to eliminate nonneuronal cells. All feeds contained NGF. The onset and rate of neuritic outgrowth were monitored sequentially by photography and the data analyzed by computer. Explants from perinatal rats exhibited vigorous neuritic outgrowth (by 6 hours) with an average growth rate of 35mm/d. The neuritic outgrowth from explants of adults was delayed in onset (56 hours) and lengthened at .19mm/d. The growth pattern of neurons from 5 week old rats was intermediate between the perinatal and adult (32 hours; .21mm/d). When antimitotic agents were present in the feed the rate of growth was slowed in all age groups. Further studies are in progress to determine whether antimitotic agents act directly on the neurons or by removing nonneuronal cells from the environment. This system will also allow the evaluation of other possible influences on regenerating neurites including that of NGF.



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2311 NON-SPECIFIC AXONAL GROWTH IN THE OPTIC TECTUM OF ADULT FROGS. Elliot I. Kaplan* and Carmine D. Clemente. Brain Research Institute and Department of Anatomy, UCLA School of Medicine, Los Angeles, California 90024.

The reestablishment of appropriate synaptic connections in regenerating CNS tissue is a requisite to functional recovery. That some form of recognition exists between growing axons and their potential target cells has been postulated in numerous studies of the retinotectal system of several lower vertebrates. Such interactions may be highly specific as reflected by the accurate retinotopic organization of regenerated ganglion cell terminals upon the dendritic fields of tectal neurons. However, studies of CNS plasticity following lesions frequently indicate that specificity of cellular interactions may not always prevail. While such a reorganization of synapses might compensate for lesion-induced deficits, it might also interfere with functional recovery.

In an effort to investigate neuronal non-specificity, experiments were performed to determine whether aberrant axonal growth and synapse formation can occur in the optic tectum of the adult frog, Rana pipiens. The right mandibular nerve was exposed and severed and its proximal stump inserted through a cranial opening overlying the right optic tectum. The dura-arachnoid was opened and in some animals the pia was pierced. The peripheral nerve was then placed onto the rostro-dorsal surface of the tectum with its longitudinal axis oriented parallel to the tectal surface. The skin was closed and operated animals placed in individual cages and maintained on a 12-hour light/dark cycle at 18-20° C for 2-12 weeks post-surgery (WPS). Light microscopic data are reported from 30 animals whose tecta have been stained to reveal nerve fibers by means of a reduced silver technique. Approximately 90% of these specimens are judged successful, the criteria being survival and growth of the grafted nerve, and the maintenance of its position.

By 2 WPS a connective interface between nerve and tectum, 350-450 μ in diameter, was established. Several regenerating peripheral fibers invariably deviated nearly 90° from the longitudinal orientation of the nerve and entered superficial layers 8 and 9 of the tectum. The growth reaction appeared most pronounced at 6 WPS and was greater in tecta with opened pia than in intact tecta. While most peripheral fiber growth was restricted to the outer layers of the tectum, some fibers occasionally penetrated as deep as the periventricular region. These observations demonstrate that a highly ordered CNS may support non-specific axonal growth and, perhaps, even encourage it under certain conditions. Present investigations are aimed at better elucidating the time course of the interaction, observing the possible trophic effects of controlled tectal denervation, and analyzing the fate of regenerated fiber terminals ultrastructurally.

2312 GANGLIONIC LOCALIZATION OF CELLS CONTRIBUTING TO ANOMOLOUS SYMPATHETIC INNERVATION IN TRANSECTED HIPPOCAMPUS. James D. Lindsey* and Rebekah Loy (SPON: W.T. Schlapfer). Dept. Neurosciences, University of California at San Diego, La Jolla, CA 92093.

Following transection of the fornix or anterior hippocampal formation (HF), large noradrenergic fibers grow into the neuropil predominantly in the ventral HF. These fibers concentrate their plexus in the infragranular hilus, the inner molecular layer of the dentate, and in stratum lucidum of CA3. There are three possible origins of these noradrenergic fibers: locus coeruleus (LC), other noradrenergic brainstem nuclei, or the superior cervical ganglia (SCG). The latter normally innervates extracerebral vessels in the lateral ventricle adjacent to the HF. To test the cellular origin of this anomolous innervation we injected 0.05 ul of 30% horseradish peroxidase (HRP) into the ventral HF of rats 30 to 60 days after transection. 48 hours later the brains as well as both SCG were processed for HRP using benzidene dihydrochloride as the substrate. We then analyzed the normal noradrenaline-containing brainstem nuclei as well as the SCG for evidence of transported reaction product.

When compared with controls, the number of labeled cells in the ipsilateral LC appears unaltered by anterior HF transection. However, the number of labeled cells in the contralateral LC is drastically reduced in transected animals. This reduction suggests that the primary path of this crossed projection is through the fornix and/or the cingulum bundle. The existence of an alternate decussating pathway(s) is suggested by the persistence of a very few labeled cells in the contralateral LC. Aside from the above described patterns in LC, no cells were labeled in the other known noradrenaline-containing nuclei of transected animals.

Examination of the SCG's revealed distinctly labeled cells in the ipsilateral SCG. These multipolar cells are located primarily in the rostral portion of the ganglion. Other work has shown the axons of cells in this region leave the ganglion via the internal carotid nerve. This nerve follows the internal carotid artery into the skull to innervate extracerebral arteries.

Thus, the large noradrenergic fibers innervating the ventral HF after anterior HF transection do not arise from central noradrenergic nuclei. Rather, these fibers arise from branching rami from the sympathetic innervation of the blood vessels serving the brain. This possibility is supported by the rostral position of the labeled SCG cells.

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2313 ANOMALOUS SYMPATHETIC TERMINALS ON HIPPOCAMPAL VASCULATURE FOLLOWING FIMBRIAL DAMAGE. Rebekah Loy and Robert Y. Moore. Dept. Neurosciences, Univ. Calif. San Diego, La Jolla, CA 92093

Following lesions of the septum or fimbria, anomalous, noradrenaline (NA)-containing axons grow into the hippocampal formation (HF). These fibers appear to originate from a normal sympathetic innervation of the longitudinal hippocampal arteries. Fluorescence histochemistry reveals that the large caliber NA-containing axons are confined to the arteries within the subarachnoid space of the lateral ventricle. Two weeks following a fimbrial lesion, fluorescent axons also accompany penetrating branches of the internal and external transverse hippocampal arteries into the HF at the obliterated hippocampal fissure and the fimbria-dentate fissure.

In order to determine the localization of any synaptic contacts formed by this anomalous sympathetic innervation, we have compared brains from normal rats and rats which 15-90 days previously had received an aspirated transection of the fimbria. Some animals of each group received an intraventricular injection of 5-hydroxydopamine (5-OHDA) 1 hr prior to perfusion, to produce small granular vesicles (SGV's), which have been shown to contain NA in central axons. The HF was sectioned transverse to its longitudinal axis, leaving intact the meninges, choroid plexus and vasculature within the lateral ventricle.

Electron microscopic examination of normal rat brains showed many varicosities containing SGV's adjacent to the basal laminae surrounding the smooth muscle of ventricular arteries. In lesioned brains similar SGV-containing varicosities appear immediately adjacent to the basal laminae of smooth muscle surrounding subpial arteries and arterioles. Other SGV-containing varicosities appear adjacent to the basal laminae of capillary pericytes or of endothelial cells directly. Similar varicosities are present within the neuropil as well, however these are most common in perivascular areas. No SGV-containing varicosities are present immediately adjacent to hippocampal vessels in normal brains not pre-treated with 5-OHDA, although a small number of such varicosities appear scattered in the neuropil of the dentate hilus.

In conclusion, normal sympathetic innervation of arteries supplying the HF is restricted to the extracerebral vessels of the lateral ventricle. Following a transection of the fimbria, which presumably damages a distal segment of this normal plexus, NA-containing axons leave the subarachnoid space and accompany penetrating arteries into the capillary bed of the hippocampus itself. Such anomalous innervation may have significant consequences for the regulation of cerebral blood flow and capillary permeability following CNS damage.

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2314 MEMBRANE PROPERTIES OF A REGENERATING AXON. H. Meiri*, M.E. Spira* and I. Parnas* (SPON: M. Devor) Neurobiology Unit, The Hebrew University of Jerusalem, Jerusalem, Israel.

Cobalt injections into Giant axons from the cockroach Periplaneta americana reveals that 8-10 days after cutting the axon, the proximal cut end regenerates and sprouts. The membrane properties of the regenerating tip of the severed axon (20-40 μmdiameter) were studied by conventional electrophysiological techniques. One hour after axotomy the membrane resting potential and input resistance are greatly decreased (-80mV to -10mV; and 4MΩ to 0.3 MΩ correspondingly) close to the cut end. The membrane potential and input resistance gradually recover and reach normal values on about the 8th day post-axotomy. During the recovery process the membrane properties are altered in the following sequence: (a) 1-4 hr post-axotomy the axon tip is permeable in a non-selective way to all ions. The spike generation mechanism seems to be normal as it is possible to evoke TTX sensitive spikes after hyperpolarization of the membrane; (b) 4-24 hr post-axotomy, the resting Na^+, K^+ and Ca^{++} conductances are increased (Na^+ from 0.03 to 0.55; K^+ from 0.2 to 0.32; Ca^{++} from close to 0-0.38 x 10^9 Se.) while Cl^- remains constant. During this period, the normal Na^+ dependent regenerative action potential could not be evoked even with strong hyperpolarization, and Ca^{++} dependent spikes (not sensitive to TTX and blocked by Co^{++}) appeared; (c) 60 hr post-axotomy the Ca^{++} dependent spikes disappear and there is a gradual recovery of the Na^{++}dependent spikes and resting conductances for Na^+, K^+, Ca^{++}. These changes are restricted to the regenerating tip of the axon (0.2-0.5 mm).

2315 AN AUTORADIOGRAPHIC STUDY OF EPENDYMAL CELL PROLIFERATION IN THE REGENERATING SPINAL CORD OF XENOPUS TADPOLES. M.E. Michel* and P.J. Reier, Dept. Anat., Sch. Med., Univ. of Maryland, Baltimore, MD. 21201.

Axonal elongation in the injured spinal cords of amphibian species has been shown recently to entail the development of a cellular substrate within the lesioned area of the cord. In Xenopus tadpoles, for example, neuritic outgrowth between the cut ends was associated with a concurrent emergence of ependymal cells derived from central canal diverticula within the rostral and caudal stumps (Michel and Reier, J. Neurocytol., In Press). The following autoradiographic study was undertaken in order to characterize the proliferative response of ependymal cells to spinal cord injury in this animal model. A 1 mm segment of the lumbar spinal cord was resected in Stage 54 tadpoles which were then divided into two groups. The first (G1) received a single intraperitoneal injection of ^{3}H-thymidine (5μCi/gm; Sp.Act. 70Ci/mM) at 5 days post-operatively (d.p.o.) and were sacrificed either 4 hours later or at 14 d.p.o. Tadpoles in the second group (G2) were injected at 3, 5, and 7 d.p.o. and were sacrificed at 14 and 21 d.p.o. Four hours after a single injection of thymidine at 5 d.p.o., numerous heavily-labeled ependymal cells were seen within the dorsal and ventral aspects of the ventricular layer immediately adjacent to the cut edge of the cord. In contrast fewer labeled cells were seen at more rostral and caudal levels; the majority of them were located within the alar aspect of the ventricular layer. A similar pattern of labeling was seen in normal spinal cords. By 14 d.p.o. (G1 and G2) the lesion zone was repopulated by ependymal cells. The majority of these cells were labeled; however, more silver grains overlaid nuclei of cells at the rostral and caudal cut ends. No significant change in thymidine labeling was observed at more rostral and caudal levels of the cord. At 21 d.p.o. (G2) a similar pattern of labeling was obtained, although fewer grains were seen above nuclei within the reconstituted segment than at 14 d.p.o. These results indicate that: (1) ependymal cell mitotic activity following injury to the cord increases above normal developmental levels; (2) the initial proliferative response is confined to the rostral and caudal stumps where even relatively inactive basal ependymal cells begin to divide; and (3) based upon the pattern of label dilution most ependymal proliferation is confined to the reconstituted segment.

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2316 SYMPATHETIC REGENERATION INTO THE HIPPOCAMPUS: EFFECT OF LESION SITE. Teresa A. Milner* and Rebekah Loy. (SPON: S. Barondes). Department of Neurosciences, University of California, San Diego, La Jolla, California 92093.

Regeneration of severed axonal projections and reestablishment of terminal connections are essential prerequisites for recovery of function in many neuronal systems. Previous experiments have examined reorganization of the hippocampus following selective deafferentation and have found that existing, undamaged afferent systems invade the partially deafferented area following entorhinal cortex lesions. Recent studies have found in addition to this intrinsic reorganization, that sympathetic fibers of the peripheral nervous system (PNS) appear to innervate the hippocampal dentate hilar area and stratum lucidum of CA3 following destruction of the fornix or anterior hippocampus. The pattern exhibited by these PNS fibers is selective and does not correspond to any other innervation patterns from hippocampal afferent projections. The present study investigates more fully the conditions necessary and sufficient to produce this anomalous growth of PNS fibers into the hippocampus following injury.

Various combinations of afferent and efferent pathways of the rat hippocampus were selectively destroyed. After 10 to 30 days, the brains of these animals were analyzed using the glyoxylic acid method of fluorescence histochemistry.

Anomalous growth occurs in the hippocampus following destruction of either septal or combined septal/commissural fibers. No growth follows disruption of commissural fibers alone, entorhinal cortex, or vasculature and cortex overlying the anterior hippocampus. Following combined entorhinal cortical lesions and septal/commissural fiber disruption, no anomalous hippocampal innervation was observed; however, a minimal but significant number of the peripheral fibers grow into the caudal part of the cerebral hemisphere around the lesioned area. Lesioning the perforant path along with septal/commissural fibers, without damaging the caudal cerebral vasculature, produced anomalous innervation of PNS fibers into the hippocampus. These findings suggest that anomalous innervation requires (1) damage to afferents at the rostral end of the hippocampus and (2) intact vascular innervation in the caudal end of the structure.
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We would like to thank Lauralee Butler for technical assistance.

2317 REGENERATION OF THE OPTIC NERVE AXONS AND THEIR TERMINALS IN GOLDFISH. Marion Murray and Wendy Battisti* Department of Anatomy, The Medical College of Pennsylvania, Philadelphia, PA 19129.

The optic axons of goldfish regenerate when crushed intraorbitally. They innervate the tectum and subserve visual function within 5-7 weeks post-crush. Previous studies have suggested that these regenerating axons branch repeatedly so that an excessive number of regenerating axonal sprouts enters the tectum and should then be potentially available to reinnervate tectal neurons.

In this study the right optic nerve of 6-8 inch goldfish was crushed within the orbit. Animals were allowed to survive from 6 days to 8 months, perfused, and the optic nerves, tracts and tecta prepared for EM. Observations on: 1) the number and size of regenerating axons, 2) the percent of them which are myelinated, 3) the amount of glial cytoplasm present in the nerve distal to the crush and in the stratum opticum, 4) the numbers and types of synapses and 5) the proportions and types of neural and non-neural elements present in the tectal neuropil (SFGS) during the course of regeneration suggest the following: The normal optic nerve is composed almost entirely of myelinated axons which are organized in fascicles. At 2 weeks post-operatively large numbers of glial cells are present between the fascicles of degenerating fibers. Within the fascicles the degenerating fibers and glial cells are located peripherally, while bundles of regenerating axons form the core of the fascicle. Estimates at this time show that the number of regenerating axon profiles increases over the normal number of axons. During the ensueing weeks and months the amount of debris and glial cytoplasm decreased markedly and the size of regenerating axons increases; the number of regenerating axons appears to remain considerably in excess of normal values. By 6 months postoperatively the optic nerve has begun to regain its preoperative appearance.

Regenerating axons have entered the tectum by 5-7 weeks post-operatively. The number of the unmyelinated axons in SO and SFGS is greatly encreased but the number of axon terminals making synapses in the SFGS is less than normal. By 6 months post-operatively the number of axons in the SFGS is still greater than normal but the number of axon terminals approaches normal levels. It appears that although an excess number of axonal sprouts are formed and grow into the tectum, they do not hyperinnervate the SFGS. This suggests that the tectal cells have the capacity to accept only a limited number of retinal connections.

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2318 SUBSTANTIA NIGRA GRAFTS REDUCE MOTOR ABNORMALITIES PRODUCED BY DESTRUCTION OF NIGRO-STRIATAL DOPAMINE SYSTEM. Mark J. Perlow, William J. Freed, Farouk Karoum, Barry J. Hoffer, Ake Seiger, Lars Olson and Richard Jed Wyatt. Lab. of Clin. Psychopharm., NIMH, St. Eliz. Hosp., Wash. D.C. 20032; Dept. Pharmacoloyg, Univ. of Colorado Med. School, Denver, Colo; Dept. Histology, Karolinska Institute, Stockholm, Sweden.

The principal pathology common to all forms of Parkinson's Disease is neuronal cell loss and gliosis in the zona compacta of the substantia nigra (SN). As a result of the loss of these cells, there is a decrease in the concentration of DA in the striatum and SN. Replacement for this missing DA is the conceptual backbone of current therapy. The peripheral administration of L-DOPA and dopaminomimetic agents unfortunately is only partially effective and is associated with side effects. Since the ideal therapy would be a circumscribed infusion of DA into DA-deficient areas of the brain, we wondered if it would be possible to achieve this result by grafting DA-producing cells into DA-deficient areas.

To test our idea, we used the rotating rat model of Parkinson's Disease. In this model the SN is lesioned unilaterally by 6-hydroxydopamine. Ipsilateral to the lesion there is a reduction in the concentration of DA in the striatum and an electrophysiological, biochemical and behavioral supersensitivity. The latter is manifest by contralateral rotation in response to the DA agonist, apomorphine. We hypothesized that if we were able to graft DA-containing and secreting cells from the SN to the DA-denervated striatum, there would be a reversal of these well described events.

Following the grafting of fetal rat SN (17-day gestation) to SN-lesioned adults rats, we found: (1) Histofluorescentally, all SN grafts survived and contained catecholamine (CA) and indoleamine producing cells. The CA containing cells had matured and sent axons into the brain tissue adjacent to the graft with heaviest innervation into the caudate nucleus. (2) Biochemically, all the grafts contain DA; the amount of DA in the lesioned caudate was increased in those animals with SN grafts, especially in areas adjacent to the graft. (3) Behaviorally, the apomorphine induced turning was reduced in SN grafted animals.

Except for the presence of lipofusin granules in the host caudate nucleus, the behavioral and microscopic picture observed at 8-9 months post-grafting was unchanged from that observed at 2 months post-grafting. There was no evidence of tissue rejection.

Current efforts are being undertaken to determine if similar grafting techniques can be used in the non-human primate.

2319 COMPETITION BETWEEN FOREIGN AND NATIVE REINNERVATION OF AUTONOMIC NEURONS. W. Proctor, B. Taylor* & S. Roper, Depts.Anatomy, Physiology, Univ.Colo. Health Sciences Ctr.,Denver, CO 80262

In some regions of the vertebrate nervous system, such as mammalian autonomic ganglia, damaged axons can regenerate and restore appropriate functional connections. On the other hand, regeneration elsewhere, for example motor innervation, is generally non-specific, and inappropriate connections are formed. We are investigating reinnervation of the parasympathetic cardiac ganglion of the frog to elucidate the cellular mechanisms underlying the reestablishment of appropriate synaptic connections after nervous tissue damage. We have shown (Neuroscience Abstracts 4, 533, 1978) that hypoglossal motor axons can synapse with denervated parasympathetic neurons in the heart. Here we report that when vagal preganglionic axons reinnervate the cardiac ganglion after hypoglossal innervation has been established, foreign (hypoglossal) innervation disappears.

The surgical operation for these experiments consisted of sectioning both vagi and suturing the left hypoglossus to the distal stump of the left vagus. Regenerating hypoglossal axons innervate parasympathetic neurons in the cardiac ganglion in about 8 weeks. After a further delay of a few weeks, we allowed axons from the right vagus nerve to reinnervate the ganglion. At intervals from 8-52 weeks after the operation, animals were killed and cardiac ganglia removed. Neurons were impaled with microelectrodes to record synaptic input from hypoglossal and/or regenerating vagal axons. Ganglia were subsequently fixed for light and electron microscopy.

Animals were divided into three groups corresponding to early (8-20 weeks), middle (21-30 weeks) and late (31-52 weeks) stages of reinnervation. The proportion of neurons receiving vagal innervation at these stages were 13±5%, 56±12%, and 76±17%, respectively. The proportion receiving hypoglossal innervation at the corresponding stages were 73±5%, 57±13%, and 14±10% (N=15 animals, values are means ± s.e.m.). These data show (1) regenerating vagal axons restore functional connections despite the presence of foreign innervation, and (2) hypoglossal innervation disappears concomitant with restoration of vagal inputs.

Electron microscopy show that vagal terminals reestablish synapses on the axon and cell body region of ganglion cells. These synapses were similar to those in ganglia from unoperated animals. Hypoglossal terminals, on the other hand, contacted only the axons of parasympathetic neurons. We speculate that the difference in the location of foreign and native synapses is fundamental to the observed selectivity in the reinnervation of cardiac ganglia, and that the glial cell which envelopes the ganglion cell body acts as a barrier to inappropriate terminals but not to native ones.

2320 ANALYSIS OF THE TECTAL PROTEINS IN THE VISUAL PATHWAY OF GOLDFISH BY 2D GEL ELECTROPHORESIS. Wolfgang Quitschke*, Andrew Francis*, and Nisson Schechter (SPON: I. Fand). Departments of Biochemistry and Psychiatry and the Long Island Research Institute, SUNY at Stony Brook, New York 11794; and (A.F.) Cornell University Medical College.

In goldfish, after optic nerve crush, regeneration and specific reconnection of the optic nerve to the tectum occurs. This paradigm is emerging as an important model system for the study of neuronal specificity. One critical aspect of this problem is the role of unique or specific proteins involved in the reconnection process. Right-sided enucleation or intraorbital optic nerve crush was performed on large (10-12 cm) common goldfish maintained on a standard diurnal cycle. Protein separation patterns were obtained from the affected (contralateral) as well as the intact (ipsilateral) tectum by two-dimensional isoelectric focusing-gel electrophoresis described by O'Farrell. After coomassie blue staining, analysis of the gel patterns revealed at least one protein, having a molecular weight of 57,000, whose presence paralleled optic innervation. This protein was lost from the tectum after enucleation, and appeared to be present in tecta after optic nerve regeneration and reconnection. Further analysis indicated that the protein could not be detected from whole brain preparations or spinal cord, forebrain, cerebellum, vagal lobes, or medulla. The protein was detectable only in the optic nerve and tectum of the goldfish. The protein was associated with a membrane fraction of the optic nerve and with both a membrane and soluble fraction of the tectum. With known standards it did not comigrate with either actin or tubulin. Studies are in progress to determine the site of synthesis and possible axonal transport phenomena of this protein.

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2321 REINNERVATION OF SCIATIC NERVE GRAFTS TO THE RAT SPINAL CORD. Peter M. Richardson, Ursula M. McGuinness* and Albert J. Aguayo. Department of Neurology and Neurosurgery, McGill University, Montreal, Canada.

In young adult female rats, a segment of the rat spinal cord was resected and replaced by an autologous sciatic nerve graft. Twenty-four animals were sacrificed from 10 days to 4 months later and the grafts and their junctions with the spinal cord were examined with the light and electron microscope. All grafts of more than 3 weeks duration were richly innervated with myelinated and unmyelinated axons even in 8 animals in which the spinal roots entering the pia at the graft site were avulsed together with their ganglia. At the border of the CNS tissue, dome-shaped structures were present, each filled with astrocytic processes and surrounded by a basal lamina. Myelinated and unmyelinated fibres frequently lay within these neuroglial domes. Occasional nodes of Ranvier were seen in which one heminode had peripheral myelin and the other had central myelin. In a further group of rats, horseradish peroxidase was injected into the spinal cord immediately adjacent to the graft (of 2 - 4 months duration). Two days later some neurons in the spinal cord on the other side of the graft were labelled with the enzyme. These results suggest that at least some axons of the central nervous system can grow into a peripheral nerve graft and be ensheathed by Schwann cells.

2322 AUTORADIOGRAPHIC DEMONSTRATION OF THE CENTRAL ORIGIN OF REGENERATING FIBERS INTO IRIS TISSUE IMPLANTS IN THE RAT MESENCEPHALON. A. R. Schonfeld* and R. Katzman. Albert Einstein College of Medicine, Bronx, N.Y. 10461.

Central monoaminergic fiber systems have the ability to sprout and regenerate in response to mechanically or chemically induced injury (e.g. Katzman, Bjorklund, Owman, Stenevi and West, Br. Res. 25:579, 1971). One model which has proven particularly suitable for demonstrating regeneration is that in which peripheral tissue, particularly the iris, is implanted into the brain or spinal cord (for a review see Svendgaard, Bjorklund and Stenevi, Adv. anat. embryol. Cell Biol. 51:7, 1975). In previous work, identification of some regenerating fibers as adrenergic has been established by histofluorescent, histochemical and ultrastructural techniques. The purpose of the present study is to demonstrate by histofluorescence and by autoradiography that axons projecting from the locus coeruleus are capable of regenerative growth into iris tissue implants placed into the caudal mesencephalon.

Briefly, heterologous iris implants were inserted in the caudal mesencephalon in the region of the dorsal tegmental bundle in female rats previously subjected to ipsilateral superior cervical sympathectomies. Previous histofluorescent work had shown that the implantation procedure severed the dorsal noradrenergic bundle (Katzman, Broida and Raine, Br. Res. 138:423, 1977). In order to observe regeneration of adrenergic fibers into in situ implants, half of the specimens were freeze dried and treated according to the histochemical fluorescence method of Falck and Hillarp. To establish the origin of these regenerating noradrenergic fibers, the remainder of the subjects received injections of ^{3}H-leucine into the ipsilateral locus coeruleus 2 days before sacrifice and the brains were prepared for autoradiographic analysis according to the method of Cowan, Gottlieb, Hendrickson, Price and Woolsey (Br. Res. 37:21, 1972).

We observed the presence of many bright green fibers running from the dorsal catecholaminergic tract and penetrating into the iris tissue implant. Microspectrofluorometric analysis indicated that these fibers were noradrenergic. These findings, which confirm the initial observations of Bjorklund and Stenevi (Br. Res. 31:1, 1971), indicate that noradrenergic axons are capable of sprouting and regeneration after injury. The results of the autoradiographic study, which will be presented in detail, suggest that some of these fibers originate in the locus coeruleus.

2323 LIMITED TARGET CELL INFLUENCES ON AXONAL GROWTH IN THE CNS OF THE LEECH. Sheryl A. Scott and Kenneth J. Muller. Dept. of Embryology Carnegie Institution of Washington, Baltimore, Md. 21210.

In each segmental ganglion of the medicinal leech there is one S-interneuron; it extends an axon both anteriorly and posteriorly halfway down the connectives that link ganglia to make an electrical synapse exclusively with the tip of the next S axon. When an S-cell axon is severed it regenerates along its surviving distal axonal stump to synapse selectively with its usual target. The target does not grow and remains electrically coupled to the severed axonal stump. We have investigated whether the target directs S-cell regeneration, and whether maintained contact with the stump prevents the target from sprouting. Single S-cells were selectively removed from the nervous system of an otherwise intact leech by injecting the somata with a protease; the axon of one or both adjacent S-cells was then severed. Axonal growth was followed by dissecting chains of ganglia at various times after the operation, and injecting the S-cells through recording electrodes filled with horseradish peroxidase, Lucifer Yellow or both for examination with light and electron microscopy.

When the target S-cell was destroyed, the adjacent injured axon regenerated as usual along its severed but persistent distal stump to the old synaptic region, and then stopped growing. Axons never regenerated beyond the region of normal synapse or the end of the distal stump. Physiological recordings and injections of Lucifer Yellow, which readily crosses normal and regenerated S-cell synapses, indicated that regenerated cells did not make aberrant synapses. Thus, axons in the CNS can regenerate in a normal fashion to the usual site of synapse in the absence of the target cell, and they do not choose alternative synaptic targets.

Axons of intact S-cells that had been connected to killed cells neither grew nor retracted and did not make other connections. Surprisingly, such intact axons could be stimulated to sprout and extend along the connective if another, distant axon of the same cell was injured. Together these results suggest that during normal regeneration the intact target cell still would not grow if the distal stump were removed. Moreover, injury apparently turns on a growth mechanism that can be expressed throughout the cell by those axons, injured or not, which have lost contact with their target.

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2324 THE TIME COURSE OF INCREASED GLUCOSE UTILIZATION IN HYPOGLOSSAL NUCLEUS NEURONS DURING REGENERATION. Philip A. Singer and Sharon Mahler*. Dept. Neurology, Kansas City Veterans Medical Center, Kansas City, Mo. 64128.

Motorneurons undergo major anabolic changes during regeneration. Watson (J. Physiol. 180:741, 1965) and others have shown increased uptake of RNA and protein precursors beginning 48 hours following axotomy. The energy metabolism during this reaction is controversial however, with some single neuron studies showing increases in oxidate metabolism in both axotomized and control nuclei (Hamberger and Sjostrand. Acta Physiol. Scand. 67:76, 1966) and others showing decreases. (Watson J. Neurochem., 13: 849, 1966) Numerous histochemical determinations of respiratory enzymes likewise show both increases and decreases. However, glycogen disappears from large anterior horn cells 72 hours after axotomy (Campa and Engel. Science 171:198, 1971) indicating increased glucose use. Other studies suggest that glycolysis (Watson. J. Physiol. 198:77, 1968) and the hexose monophosphate shunt (Nandy Arch Neurol. 18:423, 1968) are increased.

We used the 14C2 deoxyglucose technique to study glucose utilization. The left hypoglossal nerve was sectioned at the level of the carotid artery in 150 gm male rats. 14C2 deoxyglucose, 15uCi/100 gm, was injected in awake or nembutol anesthetized rats at 24 hours, 36 hours, 48 hours, 72 hours, and 14 days. The animals were sacrificed 45 minutes later and frozen sections of the brainstem exposed to x-ray film. The autoradiographs were enlarged and the optical densities of the sectioned and control hypoglossal nuclei determined. There was a marked increase in glucose utilization 48 hours after axon section which was maximal at 72 hours but unabated at 14 days. This data indicates that glucose utilization is increased very early and energy metabolizing enzymes may be the initial target of the signal for regeneration.

The studies listed above suggest that glycolysis and the hexose monophosphate shunt may be the only pathways for this increased metabolism. However, the magnitude of the increase suggests that oxidative systems may also be increased.

2325 ADULT CEREBELLAR PLASTICITY: REGENERATION OF PURKINJE CELL DENDRITES IN REHABILITATED UNDERNOURISHED MICE. Lucy Singer-Beck* and J. J. Pysh. Dept. Anat., Northwestern Univ. Med. & Dent. Schs.

It is well established that postnatal undernutrition reduces the final number of granule cells and the growth of Purkinje cell dendrites in the cerebellum. The purpose of this study was to investigate the capacity for regrowth of Purkinje cell dendrites previously retarded by postnatal undernutrition.

This study utilized a total of 22 B6D2F1 hybrid mice which were randomly cross fostered to enable littermate pairs, matched for sex and parentage, to be raised from birth to 60 days of age with different nutritional intake. Control litters were normally nourished while experimental litters were undernourished before weaning by the method of enlarging nursing litter size and after weaning by restricting access to mouse chow. At 60 days of age, undernourished animals weighed 70% less than controls. At this point rehabilitation was initiated by reinstating ad lib access to mouse chow. One group of experimental and control mice were sacrificed at 145 days of age and another at 250 days of age for a morphometric cerebellar analysis utilizing the Golgi-Kopsch technique.

At the end of 145 days of age, partial recovery of all parameters was noted including body weight (-15%), brain weight (-12%) vermis sectional area (-15%), granular layer area (-19%), molecular layer area (-11%), Purkinje cell field areas (-9.4%) and Purkinje total dendritic length/cell (-9.6%). By 250 days of age a significant difference was still noted in brain weight (-9.0%), vermis area (-8.5%) and granular layer (-11.8%) however, almost complete recovery occurred in the area of the molecular layer (-5.8%), Purkinje cell dendritic field area (-1.4%) and total dendritic length/Purkinje cell (-1.3%).

It is remarkable that the cerebellar molecular layer and Purkinje cell dendritic tree maintain their plasticity and can be induced to regenerate in the adult. Since it is unlikely that new granule cells could be formed during the rehabilitation period, it would appear that parallel fibers must be capable of lengthening and producing new presynaptic processes which induce further growth in the Purkinje cell dendritic tree.

These data offer evidence that the Purkinje cell dendritic tree retains a capacity for plasticity into the adult period.

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2326 INFLUENCE OF CULTURED NEURAL AND NON-NEURAL CELLS AND COLLAGEN ON RAT SCIATIC NERVE REGENERATION. Barry H. Smith, Vivian A. Betton*, Calvin S. Hawkins*and Arthur M. Banks*. SNB, NINCDS, NIH Bethesda, Maryland 20205.

Several factors influence the success or failure of peripheral nerve regeneration. In an effort to better understand the cellular factors involved we have utilized a vein graft to repair rat sciatic nerves. The vein graft serves as a chamber into which to introduce various agents such as collagen or cultured human or rat neural or non-neural cells to determine what effects they may have on nerve regeneration.

Both Osmond-Mandel and inbred Fisher rats have been utilized. The sciatic injury is produced by sharp transection in the thigh and the vein graft is taken from a segment of the inferior vena cava of a second rat of the same or different line, depending on the desired conditions. After a heparin flush, the vein so obtained is sewn to the proximal and distal ends of the transected nerve using four 8-0 Ethilon sutures. Microcrystalline collagen ("Avitene") is placed in the graft prior to suturing the graft. Cultured cells in phosphate-buffered saline or Hanks are injected into the graft after it is in place.

Cells for injection into the graft cavity have consisted of human glioma tumor cells (line L.M.), reactive post-injury rat peripheral nerve cells, and fetal rat cells derived from dorsal root ganglia, spinal cord, cerebellum, and cortex as well as fibroblastic elements. All cells were originally obtained as surgical specimens and then grown in Ham's F-10 medium with 10% fetal calf serum in a 5% CO_2 - 95% air humidified atmosphere at 36^{0}C and characterized in our laboratory by light as well as electron microscopic techniques. Controls have consisted of simple transection, suture reapproximation and vein-graft only groups. Animal are followed for periods of time beginning at 1 week and extending to six months. Quantitative light and electron microscopic techniques are being utilized to determine extent of regeneration.

Results to date include determination of an inhibitory effect of microcrystalline collagen on regeneration within the vein graft. This may be due in part to a mechanical problem but also involves a prominent cellular reaction in the graft. Use of an Osmond-Mandel vein graft in a Fisher rat nerve (or vice versa) is clearly deleterious to regeneration as is the presence of any infection. The presence of human glioma tumor cells appears to produce improved regeneration patterns from four to twelve weeks post-injury, although the tumor cells themselves are quiescent in the graft and disappear after several weeks. We are currently investigating the influence of the various rat fetal and adult lines on the regeneration process.

2327 SYNAPTIC RESTORATION IN THE RAT SUPERIOR CERVICAL SYMPATHETIC GANGLION FOLLOWING NEONATAL PREGANGLIONIC CHAIN SECTION. Arnold J. Smolen. Department of Anatomy, The Medical College of Pennsylvania, Philadelphia, PA 19129.

The superior cervical sympathetic ganglion (SCG) of rats was studied during the process of synaptic restoration which follows neonatal preganglionic chain (PGC) section. Litters of rat pups, aged 3 days, were divided into 3 groups. In one group, the left PGC was sectioned, a procedure which we have previously shown results in the eventual recovery of normal synapse numbers in the SCG. In the second group, the entire length of the left PGC in the neck was removed. This procedure was designed to prevent regeneration of the preganglionic axons. The third group served as unoperated controls. The litters of rats were killed on days 7, 15, 21 and 29, and the left SCGs were processed for electron microscopy. Quantitative ultrastructural methods were used to yield estimates of the numbers of synapses and postsynaptic membrane specializations (PMSs) unapposed by a presynaptic terminal (presumably vacated PMSs).

In both groups of operated animals, 90% of the synapses were lost by the 4th postoperative day. The remaining synapses presumably belong to intrinsic ganglionic neurons. In the rats in which the PGC was sectioned, restoration of synapse number began at 21 days. However, in the animals in which the PGC was removed, there was no restoration of synapse number. Thus, the intrinsic ganglionic neurons failed to sprout to produce additional synapses in response to the massive deafferentation caused by removal of the preganglionic input.

The number of vacated PMSs equalled only about 10% of the number of lost synapses in both groups of operated neonatal animals on the 4th postoperative day. This is in contrast to the findings in the deafferented SCG of the adult rat, where vacated PMSs equal about 50% of the number of lost synapses.

These findings demonstrate that the mechanism of synapse restoration in the neonate differs from that in the adult. In the adult, the regenerated presynaptic terminals reestablish contact with the vacated postsynaptic sites. In the neonate, however, the vacated postsynaptic sites are lost and synapse restoration appears to recapitulate normal synapse development.

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2328 INDUCED PLASTICITY IN VISUAL CORTEX OF ADULT CATS. D. N. Spinelli, Frances E. Jensen* and Leanna Standish*, Department of Computer and Information Science and Psychology, University of Massachusetts, Amherst, MA 01003.

During the critical period of development (6 to 10 weeks) the visual cortex of kittens exhibits remarkable plasticity. Kittens viewing vertical lines with one eye and horizontal lines with the other during this period lose binocular cells in visual cortex and exhibit instead neurons which are monocular and selective for lines of orientation similar to that viewed by the eye that activates them. These effects cannot be reproduced in adult cats, suggesting that plasticity and neuronal growth might be related. Colchicine has been shown to produce temporary chromatolysis, nucleolar enlargement, retraction of presynaptic terminals and loss of synaptic transmission when applied locally to neural tissue. These effects are reversible in 3 to 4 weeks. We wanted to investigate the possibility that during recovery plasticity might be present. To this end, a solution of colchicine (1 millimole) was applied to the visual cortex of one hemisphere in adult cats for 30 minutes. After recovery from surgery the cats were exposed to goggles, with vertical lines for one eye and horizontal lines for the other, 4 hours every day for a period of 2 months. The animals were kept in a dark room at all other times. At the end of two months, single cells were recorded from the visual cortex of the colchicine-treated hemisphere and from the untreated one, which served as control, and their receptive fields were mapped. The data shows that extensive reorganization of receptive field orientation, sensitivity and binocularity took place in the colchicine-treated hemisphere. Here, cells were mostly monocular and responded to bars of the same orientation viewed by the driving eye during recovery. A few cells were found that responded to vertical lines when tested through the eye that had viewed vertical lines, and also to horizontal lines when tested through the eye that had viewed horizontal lines. These cells are never found normally. Cells in visual cortex of the untreated hemisphere exhibited normal properties and receptive field types, i.e., they were mostly binocularly driven, and were distributed evenly in terms of orientation sensitivity. Histologycal analysis indicates that some neuronal rearrangement had taken place in the treated cortex. The results will be discussed in terms of the interaction between experience and the induced neuronal modifications.

2329 AUTORADIOGRAPHIC AND VISUAL ANALYSES OF TWO TYPES OF IPSILATERAL RETINOTECTAL PROJECTIONS IN GOLDFISH. Alan D. Springer. Dept. Physiol., Univ. Ill. Med. Ctr., Chicago, IL 60680

Following ablation of a hemitectum in goldfish, the severed optic nerve fibers regenerate and innervate the remaining ipsilateral hemitectum (IOT). Both the pattern of IOT innervation and the pathways taken by regenerating fibers to reach the IOT were examined with autoradiography. Two types of surgical preparations were examined: Type I - one eye and hemitectum ablated on the same side of the fish and Type II - both eyes intact and one hemitectum ablated.

Examination of tectal labeling in Type I fish revealed a normal laminar pattern of innervation in most, but not all fish. The majority of the regenerating nerve fibers appeared to reach the IOT via the ipsilateral optic tract (IOTr). In contrast, the IOT projection in Type II fish was discontinuous and few fibers appeared to reach the IOT via the IOTr. Discontinuity of the IOT projection in Type II fish may have resulted from the presence of the contralateral optic nerve fibers mechanically blocking the regenerating nerve fibers from reaching the IOT via the IOTr. Thus, the discontinuous IOT projection may in part be a consequence of a large number of fibers failing to reach the IOT, rather than of all fibers reaching the IOT and subsequently becoming restricted to discrete patches of the optic tectum.

A further experiment in Type II fish injected with labeled proline into the contralateral eye revealed that the existing projection, which had not been surgically altered, had become discontinuous as a consequence of superinnervation. Fish injected with labeled proline into both eyes indicated that the entire remaining hemitectum was innervated in a continuous fashion by both eyes.

Visual testing of Type I fish following regeneration indicated that most animals responded to an overhead moving shadow by reducing their respiration. Fish that had a sparse IOT projection, on the basis of subsequent autoradiographic analysis, did not respond to the shadow. Type II fish responded equally well with either eye in the shadow test. However, an optomotor response could only be elicited from the contralateral, but not the ipsilateral eye. These results suggest that the IOT projection in Type II fish is only partially functional.
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2330 REGENERATING DORSAL ROOT AXONS ARE BLOCKED BY SPINAL CORD ASTROCYTES. L.J. Stensaas, P.R. Brugess and K.W. Horch. Dept. Physiol., Univ. Utah Col. Med., Salt Lake City, UT 84108.

Axons regenerate rapidly in the dorsal root of the adult cat after an injury to the nerve several centimeters from the spinal cord. Regenerating axons are associated with Schwann cells in endoneurial portions of the nerve and eventually become remyelinated by them. However, the nerve fibers do not enter the spinal cord. They stop growing precisely at the root entry zone which represents the boundary between central and peripheral nervous tissue. Regenerating axons were not observed to penetrate the layer of astrocyte processes at the surface of the spinal cord in animals surviving up to two years. Myelinated and unmyelinated axons at the root entry zone end blindly. Many contain organelles characteristic of regenerating axons, some have terminal enlargements, but none give rise to the unmyelinated axons characteristic of a neuroma. The fact that astrocytes block the regenerating nerve fibers even though the initial injury was situated some distance away from the root entry zone and did not produce a glial scar indicates that they possess growth inhibiting properties. The results of this investigation suggests that the mature astrocyte may constitute the principal impediment to axon regeneration in the central nervous system of mammals.

2331 RETINAL GANGLION CELL RESPONSE TO NERVE GROWTH FACTOR AND ITS ANTISERUM IN THE INTACT AND REGENERATING VISUAL SYSTEM OF THE GOLDFISH. James E. Turner, Rebecca K. Delaney and James E. Johnson. Dept. Anat., Bowman Gray Sch. Med., Wake Forest Un., Winston-Salem, N.C. 27103

In recent publications we have reported pronounced effects elicited by NGF and its antiserum (anti-NGF) on the retinal ganglion cell body response to axotomy and optic nerve regeneration in the newt. In this abstract we wish to report that NGF and anti-NGF produced opposing and pronounced changes in the goldfish retinal ganglion cell body response to axotomy. Adult goldfish received one 4 µl intraocular injection of 2.5S NGF into the intact eye or at the time of optic nerve crush. Anti-NGF was delivered as a 3.0 mg intraocular injection administered at either the time of nerve crush, at 7 days post axotomy (i.e. 7 DPA) or as a multiple series of injections given at 3, 6, 9, and 12 DPA. NGF controls received 4 µl BSA (1 mg/ml) and anti-NGF controls were injected with preimmune rabbit serum (3 mg/eye). Retinas were prepared for electron microscopy at 7 DPA in the NGF group and at 14 DPA in the anti-NGF groups. Electron microscopic morphometric analyses were performed on randomly selected retinal ganglion cells. NGF treatment elicited a dramatic hypertrophy of the goldfish retinal ganglion cell at 7 DPA. The cytoplasmic area was increased by a factor of approximately 2.0 with accompanying significant increases in the nuclear area, the cytoplasmic/nuclear ratio and the nucleolar area. Mitochondrial, Golgi field, RER and nucleolar densities were the same for both NGF and control groups at 7 DPA. NGF elicited no detectable alterations in the retinal ganglion cells whose axons have not been crushed but remain intact. Anti-NGF treatment given as a multiple series of injections caused a significant reduction in the cytoplasmic area of neurons at 14 DPA. There was also an accompanying decrease in the nucleolar area and some evidence for retinal ganglion cell necrosis. These NGF and anti-NGF mediated changes, although similar to those seen in the regenerating newt visual system are more pronounced and dramatic. These results have led us to conclude that an NGF-like molecule may be ultimately important in the successfully regenerating vertebrate visual systems.
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2332 VIABILITY OF PNS TISSUE TRANSPLANTED INTO THE CNS. Ellen L. Weinberg* and Dr. Cedric S. Raine. Albert Einstein College of Medicine, Bronx, New York, NY 10461.

Previous studies from this laboratory (Brain Res.:138,423,1977) have shown that rat iris tissue transplanted into the autologous rat midbrain will be innervated by CNS axons, some of which have PNS myelin formed by graft Schwann cells. The present experiment follows a similar protocol. Segments of rat peripheral nerve were transplanted into rat midbrain. Animals were anesthetized and, under sterile conditions, tibial nerve grafts 3mm in length were placed through a burr hole in the cranium into the midbrain at the edge of the periaqueductal gray matter, in a rostral-caudal position. The overlying skin was sutured. Animals remained healthy and showed no clinical abnormalities. Animals were perfused at 3 weeks and 6 weeks post-implantation and the midbrain tissue was prepared for morphological study.

The implants became vascularized rapidly. The original axons of the graft had undergone Wallerian degeneration, and the myelin debris was degraded by graft Schwann cells and hematogenously-derived macrophages. At three weeks, there was clear evidence by EM of regenerating axons throughout the graft in association with Schwann cells. Elongated Schwann cell processes encircled individual axons or groups of axons.

At six weeks, the regenerated axons in the graft had PNS myelin around them, distinguishable from CNS myelin by its periodicity and the presence of a basal lamina. Several groups of remyelinated axons were fasciculated in the pattern of a regenerating peripheral nerve.

In the CNS parenchyma bordering the implant, myelination of the regenerating CNS axons was observed. Along most of the border of the implant was a clearly-defined rim of recently-proliferated oligodendroglial cells and thinly-myelinated CNS axons presumably en route to the transplanted tissue.

The findings of this study are consistent with the concept that transplanted myelinating cells may produce soluble factors which are attractive to regenerating axons.

2333 EFFECT OF TRIIODOTHYRONINE ON THE AMINO ACID UPTAKE OF THE BRAIN AND SPINAL CORD AFTER SPINAL HEMISECTION IN ADULT RATS. M. R. Wells, S. A. Lofton* and J. J. Bernstein. Dept. Neurosci., Coll. Med., Univ. of Fla., Gainesville, FL 32610

The thyroid hormones triiodothyronine (T_3) and L-thyroxine have been used to enhance regeneration in the peripheral nervous system (Cockett and Kiernan (1973), Exp. Neurol. 39:389) and in the central nervous system (Harvey and Srebnik (1967) J. Neuropath. Exp. Neurol. 26:661). The following experiments were designed to examine possible metabolic substrates for the action of T_3 on the central nervous system which may result in enhanced regeneration. Adult male rats (200-250g) were divided into experimental groups of unoperated animals, animals subjected to a vertebral laminectomy at T_2, or animals receiving a left spinal hemisection at T_2, to be examined at 1, 3, 7 and 14 days post-operation. Animals received a daily injection of vehicle or 1 µg/kg body weight of triiodothyronine in a bicarbonate buffer for the postoperation or equivalent time period. One hour prior to utilization, animals were given a subcutaneous injection 200 µci of [^{3}H]lysine. Tissue samples of brain taken bilaterally from somatomotor and occipital cortices. Spinal cord samples were taken bilaterally extending 14 mm rostral and caudal from the site of spinal hemisection. Samples were dissolved and the protein precipitated in 5% trichloroacetic acid (TCA). The radioactivity of TCA precipitable protein and TCA soluble fraction were determined by scintillation counting. In measurements adjusting the protein radioactivity to the soluble pools by ratio (relative radioactivity, RR), the protein uptake of [^{3}H]lysine in the brain and spinal cord of unoperated T_3 injected animals was highest ($p < 0.05$) at 3 days. In T_3 treated animals only, the RR in both brain and spinal cord of laminectomized and spinal hemisected animals showed significant differences over time post-operation. In spinal hemisected, T_3 treated animals, a hemispheric assymetry of [^{3}H]lysine uptake was present in brain at three days postoperation. The right hemisphere is somatomotor cortex had a higher ($p < 0.05$) protein radioactivity than the left. These data suggest that T_3 may affect some aspect of amino acid metabolism in the central nervous system of adult animals. Furthermore, there are indications that protein metabolism of CNS intrinsic neurons which have been axotomized may be stimulated by T_3 similar to peripherally projecting axotomized neurons (Rhodes et al. (1964) Exp. Neurol. 10:251).

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2334 RESPONSE OF LAMPREY DORSAL CELLS TO AXOTOMY. H.S. Yin*, K.K. Wellerstein* and M.E. Selzer. University of Pennsylvania, Philadelphia, PA 19104.

Both by antidromic activation and by retrograde labeling with horseradish peroxidase, it was determined that at least 85-90% of dorsal cells in the larval sea lamprey, P. marinus, project to the rostral spinal cord. Therefore axotomy of more caudally located dorsal cells was achieved by transection of the spinal cord at the level of the last gill. Following varying recovery times in fresh water at 22°C the dorsal cells were examined either histologically, in toluidine blue stained wholemounts, or physiologically, by intracellular recording. Light microscopic changes were maximal at about three weeks and were seen in progressively fewer cells at increasing distance from the transection site. These changes included: 1) loss of most cytoplasmic staining and concentration of chromophilic substance in a thin ring around the nucleus, 2) an increase in the proportion of cells with eccentric nuclei (defined as nucleus contacting the outer cell margin), 3) an increase in nuclear size and a decrease in cell diameter with a consequent striking increase in nucleus/cell diameter ratio from .34 to .43 in the first 5 mm caudal to the transection. By comparison, electrophysiological changes were less striking, and showed little correlation to the distance of the cell from the transection. Input resistance (Rn) increased by about 33% at between three and five weeks (control Rn = 8.67 MΩ), and thereafter returned to normal. It is possible that this change is in part related to the reduction in cell size. Resting membrane potentials decreased slightly from a control value of -56 mV ± .56 s.e.m. to -49 mV ± .59 at four to six weeks, and then increased to 61 mV ± 1.15 by eleven weeks. Both voltage and current threshold for spike activation dropped slightly, then greatly increased at between four and six weeks and thereafter returned toward normal. Axonal conduction velocities gradually increased from .51 m/sec ± .01 s.e.m. to .66 m/sec ± .02 at six weeks, and then returned toward normal. These and other electrophysiological changes could not be interpreted as representing any single change in basic membrane properties. The histological and electrophysiological changes noted above occurred during the period of behavioral recovery and axonal regeneration previously reported in the spinal transected larval lamprey. It is not yet known whether these changes are functionally related to the process of axonal regeneration.

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2335 SELECTIVE REGENERATION OF OPTIC NERVE FIBERS INTO THE OPTIC TECTUM AFTER TECTAL REIMPLANTATION OR TRANSPLANTATION IN ADULT GOLDFISH. Myong G. Yoon and F. A. Baker*. Dept. of Psychology, Dalhousie University, Halifax, N.S., Canada.

In normal goldfish, a majority of optic fibers invade the tectum via the stratum opticum, and terminate at the stratum fibrosum et griseum superficiale in a consistent topographic pattern. In the present study, the passages of regenerating optic fibers within the altered cytoarchitecture of the surgically operated tectum are examined after reimplantation of the tectal tissue, or reciprocal transplantations between a tectal piece and a cerebellar or forebrain tissue in adult goldfish. If a tectal tissue is reimplanted after 90° or 180° rotation around the dorsoventral axis, the tectal reimplant receives those regenerating optic fibers which previously innervated the reimplanted area. These ingrowing optic fibers are eventually redistributed within the rotated graft according to the original topographic polarity of the tectal tissue. These tectal reimplants, stained by Golgi methods, show that their neurons and radial glial cells remain in a more or less normal orientation with respect to the dorsoventral axis. Autoradiographic examination of the operated tectum after intraocular injection of H^3-proline reveals that only the stratum opticum and the stratum fibrosum et griseum superficiale are intensely labelled within the reimplanted tectal tissue. This suggests that regenerating optic fibers selectively invaded the tectal reimplant via their normal passages. On the other hand, if a piece of tectal tissue and a similar piece of cerebellar or forebrain tissue are reciprocally transplanted, regenerating optic fibers do not invade the transplanted cerebellar or forebrain tissue. Instead, they bypass the interposed foreign tissue by making dramatic detours on their way towards their appropriate target zones in the caudal tectum. In further experiments, a piece of tectal tissue is reimplanted after inversion of its entire laminar structure. Golgi stainings of these inverted tectal reimplants show no sign of reorganization in their cytoarchitectures. Neurones and radial glial cells within the tectal graft remain inverted upside-down (for as long as one and a half years). Autoradiographic examination of the operated tectum reveals that a group of incoming optic fibers abruptly dip towards the optic ventricle to find the stratum opticum within the inverted tectal tissue, and invade it via the proper passages inspite of its inversion. These results suggest that advancing tips of regenerating optic fibers are able to select not only the proper tectal tissue from other neural tissues but also the particular layer for their passages within the tectal tissue. (Supported by grants from NRC and MRC of Canada.)

SENSE ORGANS

2336 DISTRIBUTION AND HISTOCHEMISTRY OF "ONE BAG" SPINDLES IN DORSAL NECK MUSCLES OF THE CAT. G.J. Bakker* and F.J.R. Richmond, Department of Physiology, Queen's University, Kingston, Ontario. Canada. K7L 3N6.

The intrafusal fibre complement of most mammalian muscle spindles includes at least two nuclear bag fibres which may be differentiated by their histochemical and ultrastructural profiles. In most muscles however a small number of spindles contain only one nuclear bag fibre and these "one-bag" spindles account for a large proportion of spindles in dorsal neck muscles of the cat. Serial reconstruction of biventer cervicis has shown that 19% (71/369) of the spindles contained only one nuclear bag fibre. In complexus and splenius such spindles account for 34% (138/192) and 31% (146/466) of the population respectively.

The histochemical profiles of nuclear bag fibres have been examined in spindles of biventer cervicis and complexus using an ordered battery of staining techniques: myofibrillar ATPase (mATPase); mATPase subsequent to acid preincubation; mATPase subsequent to alkali preincubation. These techniques differentiate the nuclear bag fibres of two-bag spindles into 2 types in agreement with descriptions of spindles in cat hindlimb. One of the bag fibres (bag_1) reacts intensely for mATPase activity following acid preincubation but shows only light staining following alkali preincubation. The second fibre (bag_2) stains intensely following alkali preincubation and with moderate intensity following acid preincubation. By comparing the staining profiles in one-bag spindles with the profiles of two-bag spindles, the type of bag fibre present in one-bag spindles can be determined. In all one-bag spindles which have been so far reconstructed in complexus, the nuclear bag fibre which is present has a staining profile consistent with that of a conventional bag_2 fibre. Whether the loss of bag_1 fibres in these spindles has a major effect on the encoding properties of the primary ending remains to be determined.

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2337 A REGULATORY MECHANISM FOR OTOCONIAL GROWTH. Joanne Ballarino* and Howard C. Howland, Division of Biological Sciences, 136 Langmuir Laboratory, Cornell University, Ithaca, New York 14853.

Preliminary studies of otolithic organ growth in centrifuged chicks indicated that embryos of day 17 or older had significantly lighter otolithic organ weights than stationary controls (Howland & Ballarino, in press).

In this study we have repeated and refined this preliminary experiment by: a) performing all dissections in such a fashion that the dissector did not know the nature of the embryo being dissected; b) weighing the dissected otolithic organs twice, namely before and after dissolution of the otoconia in dilute HCl in order to calculate true otoconial weight; c) comparing the otoconial weights of rotated experimental animals raised at 2 g net acceleration with otoconial weights from both stationary embryos and embryos rotated at the center of the centrifuge grown at 1 g.

Under these conditions the mean otoconial weight (n=56) of the experimental animals was 11.6% lighter than that of the stationary controls (n=55, $p \leq 0.05$, 1 tailed T test) and 14.7% lighter than that of the rotated controls (n=47), $p \leq 0.0005$, 1 tailed T test).

Scanning electron micrographs were taken of the utricular otolithic organ of experimental and control embryos of 20 days of age. The otolithic organ of a 20 day old control embryo was found to have a few shrunken otoconia, similar to those found in adult guinea pigs by Lim (1973). Two experimental embryos of the same age had otoliths with cracked and shrunken otoconia. Further scanning electron micrographs of the otoliths of control and experimental embryos will be presented.

Howland, H. C. & J. Ballarino (in press). Is the growth of the otolith controlled by its weight? in The Satellite Symposium of the 1978 Neuroscience meeting: Vestibular Function and Morphology.

Lim, D.J. (1973). Formation and fate of the otoconia: Scanning and transmission microscopy.

2338 PERIPHERAL AND CENTRAL EFFECTS OF TONIC VIBRATORY STIMULI TO DORSAL NECK AND EXTRAOCULAR MUSCLES IN THE CAT. H. Barbas and B. Dubrovsky. Neurophysiol. Lab., Allan Memorial Institute, McGill Univ., Montreal, P. Q. Canada.

Extraocular and dorsal neck muscle afferents show converging excitatory and inhibitory effects on neurons of the frontal cortex of chloralose anesthetized cats at latencies of 5-50 ms (Exp. Neurol. 55: 680-693, 1977; Neurosc. Abstr. III: 468, 1977). We now report that neurons in these regions, which correspond with the frontal eye fields, also respond to vibratory stimuli (25-350 Hz, 20-200 μm) applied to extraocular and dorsal neck muscles. The observed evoked neuronal responses occurred 18-90 ms following the onset of vibration, and were characterized either by a phasic burst of cellular activity, or tonic responses which lasted for the duration of stimulation. Neurons could be further classified into those whose response depended on the frequency of vibration, and those that did not. In 12 cats 37 neurons showed consistent responses to vibration of either the biventer cervicis or the rectus capitis dorsalis major muscles of the dorsal neck. Of these nine showed an increase in firing activity as the frequency of vibration was increased (100-300 Hz) at amplitude displacements of 20-50 μm. Intravenous injection of the depolarizing neuromuscular blocking agent succinylcholine (30-40 μg/kg), known to activate preferentially the primary endings of muscle spindles, also activated these neurons. In nine cats 39 neurons responded to vibration of the superior rectus muscle of the eye at amplitudes of 50-140 μm, and 25-300 Hz frequencies, or 0.5-1.5 mm single stretches of the muscle. Consistent increases in firing activity with increases in vibration frequencies (100-300 Hz) were observed in seven of these neurons, including two which showed increased responses to faster single pulls of the muscle. The majority (69%) of the responsive neurons to extraocular muscle vibration showed tonic responses. The vibratory stimuli induced tension increases (40 mg - 2 g over the baseline) and electromyographic activity in the rectus capitis dorsalis major muscle of the dorsal neck, and the superior rectus muscle of the eye, which was abolished with administration of the paralytic agent gallamine triethiodide. This result demonstrated that a tonic stretch reflex can be elicited in both dorsal neck and extraocular muscles with vibratory stimuli, suggesting the presence of a proprioceptive feedback control system in both of these muscle structures. The neuronal responses to low threshold afferents originating in muscle receptors further suggests that small head and eye movements can activate these frontal brain regions, which may participate in mechanisms underlying coordinated eye and head movement.

H. B. is now at Harvard Neurological Unit, Beth Israel Hospital, Boston, MA.

2339 MOTION SICKNESS IN SQUIRREL MONKEYS: EFFECTS OF ROTATIONAL RATE, PHENOTYPE, SEX, DIURNAL CYCLES, AND ABLATION OF AREA POSTREMA ON INCIDENCE OF EMESIS. T. Beavers, K. R. Brizzee, W. R. Mehler, J. M. Ordy and P. M. Klara*. Delta Regional Primate Research Center, Covington, LA 70433, NASA-Ames Research Center, Moffett Field, CA 94035, and Dept. Anat. Tulane University, New Orleans, LA 70112.

To determine the susceptibility of the squirrel monkey to motion sickness, combined horizontal rotation and vertical motion tests were carried out to determine the specific effect of 10, 25 and 50 r.p.m. rotational rates with a vertical excursion of 6" every 2 seconds, Colombian and Bolivian phenotype, sex, morning and afternoon diurnal cycles, repeated exposure to motion tests, and ablation of area postrema (AP) on the incidence of emesis. The highest incidence of 80% emesis was observed in the Bolivian phenotype at 25 r.p.m., compared to 30% at 50, and 20% at 10 r.p.m. ($p<.05$). The incidence of emesis was also significantly higher in Bolivian males than in females ($p<.01$). No significant effects on incidence of emesis were observed in early morning compared to late afternoon tests, or with repeated test exposure involving a one day interval between tests. Following complete bilateral ablation of the AP, three monkeys from a group of 12 that exhibited an emetic response to 50 r.p.m. did not respond one month after postoperative recovery. Of six monkeys with partial ablation of AP, three monkeys did not respond to 50 r.p.m., whereas three others did respond. Three sham-operated controls that exhibited an emetic response to 50 r.p.m. before the operation, also continued to respond one month after postoperative recovery. From the results of this study, it can be concluded that the Bolivian squirrel monkey can be used succesfully as a model for determining the effects of lesions and drugs on emetic mechanisms related to motion sickness in a higher diurnal primate that is of the same taxonomic order as man. Supported by NASA-Ames Grant No. NSG-2139.

2340 FREQUENCY RESPONSE OF DEITERS' NEURONS TO NATURAL STIMULATION OF NECK AND MACULAR LABYRINTH RECEPTORS. R.Boyle* and O.Pompeiano. Ist. Fisiol. Umana, Catt.II, Univ. di Pisa, 56100 Pisa, Italy.

Frequency response of Deiters' (LVN) neurons to stimulation of neck and labyrinth receptors has been evaluated in precollicular decerebrate cats during sinusoidal rotation (10° peak amplitude at 0.026 Hz): a) of the cervical axis while maintaining the head horizontally (neck input), b) of the whole animal in both directions of the median plane (labyrinth input), and c) of both the head and neck while maintaining the body horizontally (neck and labyrinth input). 70 out of 120 units (58.3%) tested during neck stimulation and 77 out of 102 units (75.5%) tested during labyrinth stimulation showed a periodic modulation of the firing rate in response to the sinusoidal inputs, the phase angle being mainly related to the extreme neck or head position. The average sensitivity of the responses, expressed in per cent increase of the mean firing rate per degree of displacement, was higher for the neck (3.26 ± 3.77, S.D.) than for the macular input (2.54 ± 2.71, S.D.). Among the units responsive to the neck input, 34/46 (i.e., 73.9%) were located in the rostroventral LVN (cLVN) (sensitivity: 3.84 ± 4.54, S.D.), while 36/74 (i.e., 48.6%) were located in the dorsocaudal LVN (lLVN) (sensitivity: 2.73 ± 2.84, S.D.). Among the units responsive to the labyrinth input, 31/34 (i.e., 91.2%) were located in cLVN (sensitivity: 3.62 ± 3.4, S.D.), while 46/68 (i.e., 67.6%) were located in lLVN (sensitivity: 1.81 ± 1.83, S.D.). It appears therefore that the sensitivity to the neck and labyrinth inputs is greater in the forelimb (cLVN) than in the hindlimb (lLVN) region of Deiters' nucleus.

Among 100 units tested to neck and labyrinth stimulation, 52 responded to both inputs and were mainly positional sensitive, most of these units (73.5%) were excited during side-down tilt of the animal, while fewer units (46%) were excited during side-down rotation of the neck. Rotation of the head alone, leading to combined stimulation of neck and macular receptors, yielded a response whose sensitivity and phase angle were significantly correlated with the expected vectorial values derived from the analysis of the individual responses. Due to the significant convergence and predictable interaction of both neck and macular inputs, the LVN represents an important premotor structure for the integration of the two inputs during the cervical and labyrinthine control of posture.

2341 VISUAL-VESTIBULAR INTERACTION IN VERTICALLY SENSITIVE OTOLITH-DEPENDENT UNITS. N.G. Daunton, D.D. Thomsen*, and C.A. Christensen. NASA-Ames Research Center, Moffett Field, CA 94035 and Vassar College, Poughkeepsie, NY 12601.

The effects of combined visual and vestibular stimulation in the horizontal plane on otolith-dependent units in cat vestibular nuclei have been discussed in an earlier paper (Daunton, Thomsen and Christensen, Neurosci. Abs. 4: 610, 1978). These studies have shown that in units responding to horizontal translational motions, the addition of congruent visual input results in an increase in the gain of the response and a shift in response phase toward maximum velocity of motion, as compared with gain and phase obtained without visual input.

In the present study responses of units sensitive to both visual and vestibular sinusoidal stimulation along the vertical axis were recorded under the following conditions: 1) vestibular stimulation; 2) visual stimulation; 3) congruent visual-vestibular stimulation, produced by moving the animal in the lighted but stationary visual enclosure. In addition, responses to vestibular, visual and congruent visual-vestibular stimulation along right-left and fore-aft horizontal axes were recorded, if a unit was sensitive to horizontal motion. These recordings were obtained from chronically prepared cats relaxed with Flaxedil and artificially ventilated.

Results show that the addition of the congruent visual input increased the gain of responses to vertical linear acceleration in a majority of units. However, the actual amount of the gain increase was less than that found with horizontal stimulation. Analyses of phase relationships with vertical stimulation show that unlike the horizontal stimulus condition no consistent shift in phase toward maximum stimulus velocity was found. These analyses also show that the magnitude of phase shift was smaller for vertical than horizontal stimulation.

The data suggest that vision plays a less important role in sensing vertical motion than it does in sensing horizontal motion. Several hypotheses may explain these findings. They may be due to differences between utricular and saccular function or to differences in the nature of the vertical vs. the horizontal vestibular stimulation (pure linear acceleration vector vs. rotating linear acceleration vector). A three-dimensional vectorial display of unit responses will be used to aid the evaluation of potential hypotheses.

2342 FAST-ADAPTING STRETCH RECEPTOR ORGANS: PERIODIC STIMULATION WITH AND WITHOUT PERTURBATIONS. Oscar Diez-Martinez* (SPON: J. P. Segundo). Department of Anatomy and Brain Research Institute, University of California, Los Angeles, CA 90024.

Isolated fast-adapting stretch receptor organs (FAO) of crayfish were submitted to sinusoidal length modulations with depths of .09 to .75 mm and frequencies of .2, 1.0, 3.0 and 10.0 cps. Sinusoids were imposed either by themselves ("clean") or perturbed by fast, erratic length flunctuations or "jitter." Different jitter amplitudes were explored from x.05 to x.66 of the modulation. Afferent discharge rate was measured over "bins" of about 1/10 of the period, and plotted as a function of time. Average Lissajous displays of rate vs. length and rate vs. velocity were examined.

Experiments without jitter: At .2 and 1.0 cps, shallow modulations caused no discharge. With deeper modulations, the rate vs. length display commonly had a clockwise loop with a flat extension to the left. Occasional loops were counterclockwise. At 3.0 cps, "phase-locking" occurred very frequently, and at 10.0 cps almost invariably. The peak rate increased with the modulation depth except when extremes were achieved, in which case it decreased. The bin-rate variability was low for bins with high rates. The rate vs. velocity plots were counterclockwise loops with a base upon the abscissa. Sensitivity at different frequencies (evaluated by comparing the rate-swings using identical bin-widths and depths) was contingent upon bin-width: with small bins it was highest for 10.0 cps, but for large ones it was highest for .2 cps.

Experiments with jitter: Jitter changed the response at all frequencies. At low frequencies, when clean modulation did not produce discharges, jitter elicited firing; in every case it reduced or eliminated contacts with the abscissa and converted the displays into folium-like loops; bin-rates and peak-rates were increased. At high frequencies, jitter eliminated "locking", and frequently reduced peak-rates, rate-swings and the variability in the bins with high variability without jitter. The effect upon rate vs. length plots was to displace the loops from the abscissa and to flatten them.

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2343 HORSERADISH PEROXIDASE IDENTIFICATION OF FOUR SEPARATE GROUPS OF VESTIBULAR EFFERENT NEURONS IN THE ADULT PIGEON. Avrim R. Eden and Manning J. Correia. Depts. Otolaryng., Physiol. and Biophys. Univ. Tex. Med. Br., Galveston, TX 77550.

Horseradish peroxidase (HRP) was injected and confined (16-21 hrs.) to the endolymphatic space of one labyrinth in five adult pigeons. Amounts of 0.5µl of 50% HRP were delivered through a micropipette glued into the transsected anterior duct every 10-15 mins. (total 7-10µl). HRP-stained endolymph was seen exiting both ends of the transsected posterior duct confirming circulation. The brain and labyrinth were fixed by bilateral transcardiac intracarotid catheterization. HRP was reacted by the tetramethylbenzidine (TMB) blue reaction process. Schwartz et al (Brain Res. 155:103-107, 1978) injected HRP into the lateral crista of one pigeon and reported a bilateral cluster of DAB-labelled vestibular efferent neurons in the nucleus reticularis pontis caudalis. Using the techniques described above, we have extended these findings by identifying four distinct ipsilateral and two distinct contralateral groups of labelled vestibular efferent neurons.

Within the reticular formation the labelled, large multipolar efferent neurons were distributed equally bilaterally in all 5 pigeons (mean ± SD no. of cells each side: 49±25). The majority of these cells were clustered close together in the nucleus reticularis pontis caudalis immediately ventro-latero-caudal to the abducens nucleus. A separate, smaller cluster of labelled, similar sized, multipolar neurons was also noted within the same nucleus, adjacent and medial to the nucleus paragigantocellularis lateralis and ventro-latero-caudal to the first group of labelled cells.

Within the ipsilateral vestibular nuclear complex, labelled efferent neurons (mean ± SD no. of cells: 77±25) were noted to cluster in distinct groups in 3 of 5 pigeons. A group of medium sized bipolar labelled neurons were located within the tangential nucleus, adjacent to and intermingled with, larger multipolar labelled neurons in the latero-caudal part of the inferior (descending) vestibular nucleus. A second group of large multipolar labelled neurons (Deiter's cells) interspersed with labeled medium-sized round and triangular cells were noted to cluster in the medial pole of the lateral vestibular nucleus adjacent to the nucleus laminaris.

A further group of small, labeled neurons were also noted in the ipsilateral nucleus laminaris and may represent cochlear efferent neurons. (Supported in part by the Deafness Research Foundation and NASA Contract NAS 9-14641.)

2344 STIFFNESS COEFFICIENT OF THE CUPULA IN THE SEMICIRCULAR CANAL OF THE SKATE. Lawrence S. Frishkopf and Richard D. Kunin*. Department of Electrical Engineering and Computer Science and Research Laboratory of Electronics, Massachusetts Institute of Technology, Cambridge, MA 02139.

Cupula displacements have been measured in the semicircular canal of the skate, Raja erinacea, under known loads. The cupula stiffness coefficient -- the ratio of applied torque to angular displacement -- has thereby been directly determined. All procedures were carried out in artificial skate perilymph. The excised labyrinth was dissected to expose the ampulla which was cut from the canal with fine iris scissors. The ampulla was trimmed to enlarge the openings at both ends; contact with the cupula was carefully avoided. Attachment of the cupula at both the crista and at the vault of the ampulla and the integrity of the hair cell cilia were assessed using Nomarski interference contrast optics. Preparations which by these criteria appeared undamaged were studied further under the Nomarski microscope.

Small pieces of aluminum foil were placed upon the cupula and resulting cupula displacements at one or more points were measured. Displacements were in the range from 10 to 160 micrometers, large compared to estimates of the physiological upper limit (3-5 micrometers) in the same species. Displacements at different points were consistent with the notion that the cupula moves as a unit about an attachment region at the crista. The location of the foil allowed estimation of the torque about the crista and thereby determination of the stiffness coefficient of the cupula. In some preparations several pieces of foil were placed upon the cupula and thereby successive increments of displacement were measured; the stiffness coefficient increased as additional load was placed on the cupula. Measured stiffness coefficients were between 4×10^{-4} and 4×10^{-3} dyne-cm/rad in different preparations. These values are consistent with those that have been inferred in other species by less direct means. (Supported by NIH Grant NS11080).

2345 ANALYSIS OF THE HORIZONTAL VESTIBULO-OCULAR REFLEX OF THE ALERT RHESUS MONKEY AS A FUNCTION OF ROTATIONAL ACCELERATION MAGNITUDE. Joseph M. Furman, Dennis P. O'Leary. Dept. Otolaryngology, Div. of Vestibular Disorders, Univ. of Pittsburgh, Sch. of Med., Pittsburgh, PA 15213 and James W. Wolfe. USAF School of Aerospace Medicine, Brooks AFB, San Antonio, Texas 78235.

The horizontal vestibulo-ocular reflex (VOR) of three adolescent male rhesus monkeys was evaluated with rotational acceleration in the dark. Vestibular stimulation included single frequency sinusoidal as well as pseudorandom binary sequence (PRBS) rotational acceleration of various amplitudes. Each animal performed a behavioral task during the experiment. Eye position was measured from chronically implanted bitemporal ball electrodes with a D/C coupled amplifier. A PDP 11/40 computer controlled the vestibular stimulus and simultaneously stored eye position using a 12 bit A/D converter sampling at a rate of 100 Hz.

A computer analysis related slow phase eye velocity to the rotational stimulus velocity in order to yield gain and phase estimates at discrete frequencies in the range 0.008 to 1.28 Hz. The magnitude of the vestibular stimulation was varied from 8 degrees/second/second to 256 degrees/second/second in order to elucidate the amplitude dependencies of the VOR. Gain and phase estimates revealed that over the range of stimulus amplitudes employed, no consistent amplitude dependencies were evident; This was found for both sinusoidal and PRBS testing.

These results suggest that the slow phase movements of the VOR can be effectively studied with linear systems techniques over a wide range of stimulus intensities.

2346 VISUAL INFLUENCES ON VESTIBULAR COMPENSATION. David W. Jensen, T. Kubo* and M. Igarashi. Baylor College of Medicine, Houston, Texas 77030.

After a unilateral vestibular neurectomy in guinea pigs, the intensities of spontaneous eye nystagmus and lateral head deviation abate in consistent and quantifiable manners. This is part of a process called Vestibular or Equilibrium Compensation. We have begun to examine the influence of certain visual deprivations upon the compensatory rates of eye nystagmus frequency and lateral head deviation over the first 24 post-operative hours, when approximately 90% of the "tonic compensation" takes place. All animals of this study had chronic Ag-AgCl ball electrodes which were used to record eye nystagmus. Three of these individuals also had skull screw electrodes over the left and right visual cortices, where visual evoked potentials were readily obtained. These three animals subsequently had their corneas opacified to eliminate pattern vision. Three groups of animals were analyzed: (i) 8 normal-sighted individuals that compensated in light, (ii) 3 corneal opacification individuals that compensated in light, and (iii) 3 normal-sighted individuals that compensated in darkness. Eye and head asymmetries of Group (i) individuals immediately decreased from maximum values recorded 1 hour post-operatively and by 24 hours had compensated 89 and 86 percent, respectively. In contrast eye and head asymmetries of Group (ii) individuals remained near their maximum values for several hours post-operatively and by 24 hours had compensated 52 and 36 percent, respectively. Eye and head asymmetries of Group (iii) individuals also remained near their maximum values for the first few post-operative hours and by 24 hours had compensated 74 and 62 percent, respectively. Also, Group (i) and (iii) individuals were capable of visually suppressing spontaneous nystagmus (tested 24 hrs. post-op) while Group (ii) individuals were not.

Thus, vestibular compensations of eye and head asymmetries in guinea pigs are affected by conditions that influence the visual system. Two interpretations of the data are possible: The faculty of pattern vision is necessary for vestibular compensation to proceed normally, and/or visual deprivations cause a decreased alertness which slows compensation.

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2347 TRANSFER CHARACTERISTICS OF PRIMARY-AFFERENT SEMICIRCULAR-CANAL UNITS IN THE PIGEON. Jack P. Landolt and Manning J. Correia D.C.I.E.M., Downsview, Ontario, Canada M3M 3B9 and UTMB, Galveston, TX, 77550.

Analyses of single unit activity from semicircular canal units in the pigeon to sinusoidal accelerations indicate that the transfer function, G(s), relating output (neural activity) to input (angular acceleration) of each unit is of the form $G(s) = Cs^k/(T_L s+1)$, for frequencies f = 0.01 to 10 Hz. (s^k is a fractional order differential operator with $0<k<1$, C is a gain constant with units in impulses $.s^{-1}$/degrees $.s^{-2}$, and T_L is the so-called long time constant of the classical torsion pendulum model (tpm).) This transfer function differs in two ways from that of the classical tpm that is used to describe semicircular canal function: i) T_L is not single-valued as it is in the tpm; rather it is unit dependent and takes on a range of values from T_L = 4.45 s to T_L = 18.61 s (mean ± SEM = 10.24 ± 1.20 s; a value comparable to T_L derived from biophysical properties). This indicates that individual or small groups of contiguous receptor cells (units) likely have different mechanical properties from those of other groups. This suggests that information is coded and passed to the central nervous system differently in different regions of the vestibular epithelium and may be important in orientation. ii) The classical tpm does not include the term s^k, which can be expanded as a transfer function containing product terms in s in the numerator (zeros) and denominator (poles). The first term in this expansion is of the form, $H(s) = KT_1 s/(T_1 s + 1)$, where T_1 is an adaptation time constant and K is a gain constant. This expression had previously been defined from nystagmoid studies (Malcolm, '68) as the input-output transfer function, H(s), for the phenomenon of "adaptation" -- an unusual response degradation probably resulting from within the receptor cell (which is not predicted by the tpm). A mean (±SEM) T_1 = 77.18 (±13.06)s was determined. The data indicate that adaptation is not only unit dependent but also that it is much more complex at the neural level than is envisaged from the form of H(s). It appears likely that s^k represents a relaxation phenomenon comprising of a time-varying intracellular Na^+/K^- process; components of which are summed with the generator potential in the receptor hair cell. Further analyses indicated that the degree of regularity of the spontaneous discharge, as determined by the coefficient of variation, CV, was significantly correlated with k in G(s). The larger the CV, the larger is the corresponding k and, consequently, the amount of adaptation.

2348 NON-VISUAL CONTROL OF VESTIBULO-OCULAR REFLEX SUPPRESSION IN MAN. Ruth A. Maulucci* and Richard M. Herman. Dept. Rehab. Med., Temple Univ. Health Sciences Center, Philadelphia, PA 19140.

In man, when retinal information is coupled to head motion, suppression of the vestibulo-ocular reflex (VOR) is marked at low frequencies of oscillation (e.g., gain of 0.1 at 0.1 - 0.5 Hz). In the absence of retinal information, image of the coupled visual signal yields a nystagmus pattern with VOR gains enhanced four to sevenfold. In the dark, when head movement is associated with knowledge of the motion of the eyes in the orbit (induced by sensory feedback training) rather than with an image of the visual signal, VOR gain and fast phase saccades are suppressed to a level equivalent to the response observed with retinal information. This magnitude of suppression persists without further training for a considerable period of time (at least 12 months). Attempts to distract the subject by cognitive tasks do not lead to marked alteration in VOR suppression. Phase advance of eye displacement relative to head displacement, observed with retinal information, is modified under all test conditions in the dark to an in-phase response. The results suggest that under these test conditions: 1) non-visual control of VOR suppression can be achieved by matching a "sense of eye movement or fixation" to head motion, and 2) gain and phase control of the VOR may function independently.

2349 CORRELATIONS OF SPONTANEOUS ACTIVITY CHARACTERISTICS WITH INNERVATION PATTERNS IN THE ISOLATED GUITARFISH HORIZONTAL SEMICIRCULAR CANAL. Dennis P. O'Leary and Robert F. Dunn. Dept. Otolaryngology, Div. of Vestibular Disorders, Univ. of Pittsburgh, Sch. of Med., Pittsburgh, PA 15213.

Spontaneous activity of different VIIIth nerve semicircular canal fibers shows a wide range of mean (μ), standard deviation (σ) and coefficient of variation (CV = σ/μ) of interspike intervals. We investigated whether these parameters were correlated with spatial position of fibers in the guitarfish horizontal ampullary nerve (HAN), shown previously to be comprised of 5 to 9 separated bundles (O'Leary, Dunn and Honrubia, Nature 251: 225-227, 1974) Epochs of 40 seconds of spontaneous activity from 280 afferent neurons were digitized on a PDP 11 computer system, with an interspike interval resolution of 100 microseconds, and edited to remove artifacts. Interspike interval histograms and parameters μ, σ and CV were computed for each afferent. Each parameter was tested separately, using the Kruskal-Wallis test, to determine whether it was distributed uniformly among 7 different bundle areas of the HAN.

The results showed that both σ and CV were distributed differently in the nerve ($p < 0.0002$), with greater values ("irregular" cells) occurring in central HAN nerve bundles, and lesser values ("regular" cells) occurring in extreme rostral and caudal HAN bundles. Differences in μ distributions were not significant at an acceptable level. The systematic differences in σ and CV can be correlated also with the projection patterns of the guitarfish HAN to the crista described by Dunn (J. Comp. Neurol., 183: 779, 1979), and with differences in HAN dynamic response characteristics described by O'Leary, Dunn and Honrubia (J. Neurophysiol., 39: 631, 1976). In general, fibers from central HAN regions projecting toward the crista crest, have faster response dynamics and exhibit "irregular" spontaneous activity. Fibers from peripheral HAN regions projecting toward the crista slopes, have slower response dynamics and exhibit "regular" spontaneous activity.

2350 REGIONAL INNERVATION DIFFERENCES IN THE UTRICLE OF THE GOLDFISH INNER EAR. Janet F. Ott* and Christopher Platt. Dept. Biological Sci., Univ. Southern California, Los Angeles, Calif. 90007.

The utricle of the vertebrate inner ear is an otolith organ that generally is considered a gravistatic receptor. Fishes have only the cylindrical Type II hair cells in their vestibular sensory epithelia, but within each otolith organ macula there are some structural distinctions between different regions. The utricular macula usually has a band, the striola, in which the hair cells are larger, with larger ciliary bundles, than elsewhere in the macula. We now have investigated whether goldfish have a pattern of utricular innervation showing distinctions between striolar and non-striolar regions.

Utricles and their attached branch of the eighth nerve from goldfish 40-50 mm long were fixed and imbedded by techniques conventional for electron microscopy. Blocks were sectioned either transversely or along the axis of the nerve trunk. Light microscopy was used for 1 μm sections stained with toluidine, and electron microscopy was used for thin sections stained with uranyl acetate and lead citrate.

Nerve fiber diameters range from less than 1 μm up to 15 μm. In the utricular trunk leading to the macula, fibers of up to 5 μm diameter tend to be clustered in bundles in the core of the nerve, while the largest fibers occur around the periphery. The fibers in the core of the nerve appear to interdigitate with the large fibers, as these small fibers course up to and through the basement membrane in non-striolar regions. Large fibers penetrate the basement membrane under the striolar region of the macula. There are roughly 200 fibers of more than 5 μm diameter in the utricular branch of fish of this size; these represent less than 20% of the total fiber population in the utricular branch.

We conclude that the goldfish utricle has a distribution of innervating fibers suggesting from their diameters that the fastest conduction times and shortest latencies might arise from striolar regions. The sparse numbers of large fibers to the abundant hair cells of the striola also suggests great convergence, which could provide high sensitivity by averaging a signal from a field of inputs.

2351 BEHAVIOR OF SEMICIRCULAR CANAL PRIMARY NEURONS SUGGESTS NEW MODEL B.N. Segal* & J.S. Outerbridge* (SPON: G. Mandl). Dept. Otolaryngol. & Biomed. Eng. Unit, McGill Univ., Montreal, Canada H3G 1Y6.

The activity of 51 single neurons innervating the horizontal semicircular canal of the bullfrog was examined during prolonged, horizontal-plane, angular velocity stimuli, both sinusoidal and triangular, having different amplitudes (10-320°/s) and frequencies (0.01-0.3 Hz). During the prolonged stimulation of most cells, the peak neural response in successive cycles gradually declined over a 1-4 min. interval and thereafter remained essentially constant. The late, non-declining response was considered to be the steady state neural response, and attempts were made to fit it with a model composed of a linear element, corresponding to the Goldberg & Fernandez equations (J. Neurophysiol.34:661, 1971), followed by a biased rectifier element. This model was inadequate since the slope and x-intercept of the fitted biased rectifier were strongly dependent on stimulus magnitude, which would imply that neural sensitivity and threshold were input dependent. Moreover, it can be shown that such neural behavior cannot be explained by any model composed of an arbitrary linear system followed by an arbitrary static nonlinearity (eg. power law, etc.).

Simulation studies based on this data suggest a new form of model for the rotational afferent pathway, which is consistent with findings of others. It is well known that an essentially static nonlinearity exists at the level of hair cell transduction. Furthermore, recent studies suggest that adaptation and high frequency lead are introduced proximal to this nonlinearity. Therefore, it appears that the rotational afferent pathway can be modelled, to a first approximation, by a linear Steinhausen element, representing the semicircular canals; a static nonlinear element representing hair cell transduction; a linear, adaptation-like, high frequency lead element, possibly representing the hair cell primary afferent synapse, and finally, a biased rectifier representing primary afferent action potential generation. This model could be approximately fitted to the steady state responses of 8 out of 12 suitably selected cells, thus explaining the apparent dependence of neural sensitivity and threshold on input amplitude. Furthermore, the presence of the static nonlinearity preceding the adaptation element in the new model accounts for the decline in neural response observed during prolonged stimulation, and appears to explain a number of previously reported nonlinear phenomena in several animals. Finally, the model supports the notion that nonlinear behavior is more commonly reported in cold-blooded than in warm-blooded animals because hair cells of warm-blooded animals are biased to normally operate in the more linear portions of their transduction characteristic.
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SLEEP

2352 EFFECTS OF ELECTRICAL STIMULATION OF AREA POSTREMA/NUCLEUS OF THE SOLITARY TRACT ON RAPHE UNIT ACTIVITY AND CORTICAL EEG IN THE ANESTHETIZED RAT. J. D. Bronzino*, P. J. Morgane, A. Johnson*, and W. C. Stern. (Spon: W. L. McFarland) Trinity College, Hartford, CN 01606, Worcester Fndn. Exptl. Biol., Shrewsbury, MA 01545 and D. Dix Hosp., Raleigh, NC 27610.

It is well established that electrical or chemical stimulation of the area postrema/nuc. tractus solitarius (AP/NTS) of the medulla produces EEG cortical synchronization and/or sleep. Recently, an anatomical projection from the NTS to the nucleus raphe dorsalis was identified in the rat using the horseradish peroxidase method (Brain Res. 1977, 122: 229-242). Because of the involvement of the anterior raphe and AP/NTS in sleep mechanisms and the indication of an anatomical projection to the raphe from the AP/NTS we investigated whether stimulation of the region of the AP/NTS would influence the activity of single units of the anterior raphe nuclei.

Fourteen adult male rats were anesthetized with urethane, 1000 mg/kg, and had the dorsal medulla exposed for positioning of stimulating electrodes. Results from 44 raphe units showed that in these acute preparations stimulation of the AP/NTS at 0.1-0.3 mA, 1 Hz or 10 Hz (occasionally 1.0 mA was used) was without marked effects. Approximately 50-60% or the units showed no change in firing rates, 30-40% exhibited temporal suppression of firing, and 10-20% were excited during the stimulation. For all units the average duration of suppression was 57 and 16 msec at 1 Hz and 10 Hz, respectively. The magnitude of the suppression was only about 1/3 to 1/2 that previously observed after stimulation of the lateral habenula or substantia nigra (Stern et al., 1979, Exptl. Neurol. and Brain Res. Bull. in press). Also, in several instances there was a complete dissociation between changes in raphe unit activity and cortical synchronization or desynchronization. Cortical synchronization was often observed to occur without a change in raphe unit activity, and in some cases raphe unit activity was altered without a change in cortical EEG waves.

In summary, the present data suggest that in the rat there is not a strong influence of AP/NTS region on anterior raphe units, and that the cortical EEG synchronization produced by electrical stimulation or the AP/NTS is not mediated by changes in unit activity in the anterior raphe. (Supported by NSF grant ENG 77-04271.)

2353 MACROMOLECULAR REGULATION OF REM SLEEP AND ITS INTERACTION WITH CHOLINERGIC MECHANISMS. Georges Dreyfus C.*, Rosa María Espejel*, Donají Gutierrez*, Laura Chavez-Noriega*, René Drucker-Colín. Depto. de Neurociencias Centro de Investigaciones en Fisiología Celular, UNAM, México, D.F. México.

Recently we have gathered evidence showing that protein synthesis inhibitors(PSI), produce a specific decrease of REM sleep, and that such decrease comes about through a reduction of the frequency of REM periods without affecting their duration. In addition we have shown that PSI reduce some of the phasic elements of REM sleep such as eye movements(EM), and multiple unit activity(MUA) bursts(Drucker-Colín et.al. Exp. Neurol., 63, 458 1979). The purpose of these experiments is to gather further evidence for the role of macromolecules in REM sleep and to determine their interaction with cholinergic systems. In the first series of experiments 14 cats were administered 100mg/Kg Chloramphenicol for 12consecutive days and their sleep-wake cycle was determined in days 1,5,9,10,11,12, and compared to baseline recording; the results showed that there was a specific decrease in REM sleep of 42,25,30,17,31 and 27% respectively on each of the above mentioned days, no rebound effect was seen, and slow wave sleep (SWS) was unaffected. Since chronic administation of PSI may have a peripheral effect, 6 cats implanted with cannulae in midbrain reticular formation were topically administered 3mg, 1.5mg, and 300ug of anisomycin, the results of these experiments showed a dose dependent decrease of REM sleep, while again SWS was unaffected. In a third series of experiments 24 cats, were sleep deprived(SD) of REM sleep for 72 hours, following they were administered either 2mg of atropine, 100 mg/Kg of Chloramphenicol, both drugs or saline; the results showed that for the chloramphenicol group, REM sleep rebound seen after SD was blocked, and both EM and MUA bursts decreased. REM rebound under atropine was unaffected but PGO waves were reduced by 57%. Combination of drugs produced a further decrease of REM and phasic events. Results suggest that macromolecular systems interact with cholinergic ones to trigger REM sleep.

2354 **ASSAYS OF MONOAMINE METABOLITES IN LUMBAR CSF SAMPLES FROM NORMAL HUMAN CONTROLS AND HYPERSOMNIAC PATIENTS.** **Kym F. Faull,* Christian Guilleminault, Patricia J. Anderson* and Jack D. Barchas. Dept. Psychiatry, Sch. Med., Stanford Univ., Stanford, CA 94305.**

At the Stanford Sleep Disorders Clinic 35% of the patients who complained of Excessive Daytime Sleepiness (EDS) did not fulfill the criteria for narcolepsy or obstructive sleep apnea syndrome. In this group of patients, the EDS was the sole symptom and this was objectively confirmed by multiple sleep latency test scores. In an attempt to find a CNS neurochemical defect characteristic of this disabling yet poorly-defined hypersomniac condition, we collected lumbar CSF samples from 8 patients and 15 normal control subjects. At an interval of 24 hours, two lumbar punctures (LP) were performed on each individual; the second LP was performed after an oral probenecid dose. The samples were assayed by gas chromatography-mass fragmentography to determine the concentrations of the neutral and acidic monoamine metabolites HVA, DOPAC, MHPG, and 5-HIAA. Results indicated that after the probenecid dose there was a significantly elevated concentration of HVA and 5-HIAA in the CSF samples taken from the patient population, suggesting increased turnover of dopamine and serotonin.

2355 THE EFFECT OF AN EXPANDING EPIDURAL MASS ON THE INTRACRANIAL PRESSURE PROFILE IN THE SLEEPING MONKEY. Gündüz Gücer and Chandrasekharan Nair*. Division of Neurological Surgery, Baltimore City Hospitals, Baltimore, Maryland 21224.

Normal intracranial pressure waves called plateau waves have been demonstrated to occur almost exclusively during desynchronized sleep epochs (Gucer and Viernstein). The average amplitude of these waves are 170.6 + 6.04 mm H_2O, the average duration is 6.8 + 1.38 minutes. It is felt that they reflect the increase cerebral blood flow which occurs during desynchronized sleep. During slow wave sleep infrequent one per minute waves whose amplitudes are 50 - 100 mm H_2O occur normally (1).

We have studied in five Macaque monkeys trained to fall asleep in primate chairs the intracranial pressure profile during sleep as a mass is slowly expanded in the epidural space. All monkeys were fitted with telemetric intracranial pressure monitor, EEG, EOG, EMG, and epidural balloons under general anesthesia. Each animal had two days of baseline recording to obtain the normal profile before expansion of the balloon.

Consistent finding were that during slow-wave sleep ICP waves at one per minute appeared whose amplitude ranged from 100 - 500 mm H_2O and appeared to be identical to Lundberg B-waves. During desynchronized sleep waves appeared whose shape and characteristics appeard to be like plateau waves. However, amplitudes of these waves were 500 - 1000 mm H_2O. Their duration increased to 5 to 20 minutes. After deflation of the balloon a period of persistent abnormal wave phenomenon was followed by a return to normal intracranial pressure profile.

1. Gücer, G. and Viernstein, L.J. Intracranial Pressure in the Normal Monkey While Awake and Asleep, Journal of Neurosurgery, 1979.

2356 A NEW APROACH TO THE STUDY OF THE BIOCHEMICAL REGULATION OF REM SLEEP: IMMUNOLOGICAL STUDIES. María del Carmen Gutiérrez, Marietta Tuena*, Georges Dreyfus C.*, René Drucker-Colín. Depto. de Neurociencias, Centro de Investigaciones en Fisiología Celular, UNAM, México D.F., México.

Several lines of evidence from our laboratory have suggested that macromolecules may participate in the regulation of REM sleep (Drucker-Colín et al. Exp. Neurol. 63, 458, 1979). The experiments to be described below provide a novel approach to the study of sleep, and one that provides a more direct corroboration of the role of macromolecules. Sixty cats were used in this study half of those cats were perfused with a push-pull cannula in the midbrain reticular formation (MFR). The perfusates were dialyzed, liophylized and resuspended in Ringer. This material was then inyected into rabbits and antibodies obtained by standard procedures. These antibodies were purified in a DEAE-celulose column, and were then concentrated in a millipore filter (45 um). These antibodies were then inyected into the MFR at 10,50,100,500, and 1000 ug/50 ul doses. The effects of these antibodies on the sleep-wake cycle was compared to Ringer pre-immune serum, antibodies to cat serum, antibodies neutralized with whole antigen, or partial antigen from serum. All concentrations were identical. The results of these experiments showed that the 100,500 and 1000 ug dose of antibodies to MRF proteins decreased REM sleep by 70% without affecting slow wave sleep (SWS). However the two highest doses affected SWS transiently through an increase in its latency. This decrease in REM sleep came about through a decrease in the frequency but not in duration of REM periods. In addition it was observed that this antibodies decrease the phasic events of REM sleep, i.e eye movements and multiple unit activity much in the same way as protein synthesis inhibitors do. Since antibodies neutralized with whole antigen did not affect REM sleep whereas partial neutralization with cat serum antigen did, it can be suggested that a special class of brain proteins seem to be involved in the triggering mechanisms of REM sleep.

2357 DEVELOPMENT OF COUPLING BETWEEN AUTONOMIC, CNS AND SOMATIC VARIABLES IN INFANTS AT RISK FOR THE SUDDEN INFANT DEATH SYNDROME. R. M. Harper, B. Leake*, T. Hoppenbrouwers*, M.B. Sterman and J. Hodgman* (SPON: H. Lesse). Dept. Anat. & Brain Research Inst., UCLA, and Newborn Division, LAC/USC Med. Ctr., Dept. Pediatr., Los Angeles, CA 90024.

Disturbance in the normal organization of physiological parameters that define sleep state, such as the absence of muscle tonus during epochs of quiet sleep (QS) or the absence of respiratory variation during active sleep has been observed in acutely ill infants (Monod et al., Biol. Neonate 11:216-247, 1967). Victims of the Sudden Infant Death Syndrome (SIDS) frequently succumb during sleep at a developmental period (3 months) associated with rapid changes in sleep-state organization. Since SIDS victims may have experienced some degree of disorganization in physiological parameters used to characterize sleep, establishment of trends in coalescence of physiological parameters could aid in identifying factors that differentiate infants at risk for SIDS. Therefore, we examined the degree of coupling between physiological parameters at ultradian periodicities during development. Twelve-hr polygraphic recordings were obtained in 21 normal infants and 21 siblings of SIDS victims. Recordings were obtained at 1 week and at 1,2,3,4 and 6 months of age. Heart and respiratory rate and their variability, somatic activity, including EOG and EMG, filtered and integrated EEG at 13, 4 and 1.5 Hz were digitized and compressed for each min of every 720 min of recording. The resulting 720-point time series were subjected to spectral analysis, and coherence values between pairs of selected variables were calculated at ultradian periodicities from 0.1 to 5 cycles/hr. ANOVA procedures were used to assess statistical trends over age and between risk groups. Coupling between physiological variables, as measured by coherence, was very high at low frequencies over all ages; coherences at higher frequencies tended to be lower. At higher frequencies, several peaks appeared with development. A principal peak was noted at 1 cycle/hr. Although coupling at this frequency was present as early as the first week of life for some pairs of variables, it did not become prominent in other pairs of variables until 3 months of life. Differences in coupling between pairs of variables were observed between the normal group and SIDS-risk group. These included decreased coupling between pairs of physiological variables at frequencies higher than 1 cycle/hr in the SIDS group, reflecting a difference in organization of sleep states between normal and risk infants.

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2358 EFFECT OF ACUTE HEROIN WITHDRAWAL ON SLEEP AND OTHER BEHAVIORAL PARAMETERS IN HUMANS.[1] Richard C. Howe, Jerry L. Phillips* and Frederick W. Hegge*†. Dept. of Physiology, Eastern Virginia Medical School, Norfolk, VA 23501 and †Dept. of Military Medical Psychophysiology, Walter Reed Army Institute of Research, Washington, DC 20012.

The purpose of this study was to evaluate the effects of acute heroin abstinence on sleep and other overt behaviors in humans. Electrophysiological data were recorded on a 24-hour per day basis for 5-7 continuous days. Behavioral observations were also noted throughout the study. An observation was noted only when it differed from the preceding one. Recording environment was an isolated hospital ward. Data were collected from young heroin dependent individuals who were generally in good health, had short heroin use histories (1-3 months), used few other drugs concurrently, and were addicted to 95-98% pure heroin. Matched drug-free control subjects were simultaneously studied. Informed consent was obtained from all subjects. All EEG records were scored according to standard techniques in one-minute epochs and were transferred to a PDP8E computer for subsequent analyses.

The heroin dependent patients during withdrawal showed a severely disrupted sleep-waking pattern which peaked on Day 2 of withdrawal. The disrupted sleep in these patients was also associated with frequent attempts at sleeping throughout the 24-hour day. Total sleep per 24-hour day was 304 minutes in the drug dependent patients during withdrawal compared to 456 minutes in the controls. Sleep Stages II, III and IV averaged 218 minutes per 24-hour day during withdrawal compared to 295 minutes in the controls. The heroin dependent patients during withdrawal showed 29 minutes of REM sleep per day whereas the controls averaged 94 minutes. Analysis of the behavioral observation data also confirmed that the heroin dependent patients during withdrawal were awake more of the time and considerably more active. The following behavioral observation categories were significantly higher in the heroin dependent patients during withdrawal than in the controls: drinking, excretion, in bed, out of bed, prone, restless, and rolling.

These results suggest that the central nervous system mechanisms responsible for the normal sleep-waking patterns have been markedly disrupted during heroin withdrawal. This disruption is further reflected by the increase in overt behavioral activities observed in the heroin dependent patients during withdrawal.

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2359 POSITIVE RESULTS IN TWO CASES OF NARCOLEPSY TREATED WITH THYROTROPIN-RELEASING HORMONE (TRH). J.L. Jurado*, J.M. Rhodes, A. Fernández-Guardiola and C. Valverde-R*. Inst. Nal. de Neurol. & Neurocirug., Unidad de Investigaciones Cerebrales, Mexico 22, D. F.

The action of thyrotropin-releasing hormone (TRH) on serial sleep recordings in two Narcoleptic patients was evaluated. In one case, 20 mg of TRH, v.o., was given for seven days and in the other case for eleven days. Both cases reported alleviation of Excessive Day-Time Sleepiness and associated symptoms. In contrast to normals, both subjects showed increase in Delta sleep. In one case with early onset REM, the latency was shifted to a normal or longer period with a return to early onset at the cessation of TRH. The other case showed one early onset REM after beginning TRH. It is proposed that TRH is acting as Neuroregulator in a balancing fashion. Further, that TRH may be useful in both the diagnosis and treatment of Narcolepsy.

2360 SLEEP CIRCADIAN RHYTHMICITY FOLLOWING ENRICHED AND IMPOVERISHED REARING: RELATIONSHIPS BETWEEN PARADOXICAL SLEEP AND MEMORY STORAGE PROCESSES. David R. Kleinman, Baruch M. Gutwein* and William Fishbein. Psychobiology Lab., Dept. Psych., The City College of New York, New York, N.Y. 10031

Sleep cycle circadian rhythmicity following either enriched or impoverished environmental rearing is examined in this study. Mice are reared in either super-enriched (SEE), regular enriched (REE), social control(SC), or isolate environment (IE) for 30 days. SEE and REE groups show a general increase in total slow-wave sleep (SWS) in the 24 hr. cycle but the number of SWS episodes, mean duration of SWS episodes, and per cent SWS of total sleep time(TST) is not significantly different from the SC group for the day cycle (0700-1900). Enriched rearing produces a significant and selective increase in the number of PS (Paradoxical Sleep or REM sleep) episodes, mean duration of PS episodes, total amount of PS time, and percent PS/TST throughout the 24 hr. cycle. IE reared mice also show a general increase in SWS primarily during the day with significant reductions in all measures of PS occurring exclusively in the day cycle. It is suggested that alterations of chronobiological sleep rhythms after differential rearing may be mediated by the interaction of the suprachiasmatic nucleus (SCN) with the Gigantocellular Tegmental Field (FTG) and locus coeruleus (LC). Our results provide additional support for the hypothesis that PS occuring over a protracted time period is a requisite neurobiological mechanism for the processing, maintenance, and storage of long-term memory.

2361 CONSCIOUSNESS AND LUCID DREAMS. Fred K. Lenherr. Berkeley Brain Center, Box 2249, Berkeley, CA 94702.

The phenomenon of lucid dreams offers a number of tantalizing clues concerning the brain mechanisms underlying human consciousness. A so-called "lucid" dream is one in which the dreamer is clearly aware, while the dream is still going on, that he or she is dreaming. Lucidity generally arises out of an on-going normal dream. Three classes of factors may precipate lucidity. First, extreme terror or other strong emotion may cause one to realize that "this is only a nightmare." Subjects commonly report that they are then able to escape by waking themselves up. Secondly, extreme illogicality or bizarreness in the events of the dream can sometimes cause the dreamer to become lucid. Finally, unusually active manipulation of the dream environment by the dreamer sometimes results in lucidity. In all cases, the dreamer, once lucid, is able to control and explore the dream world with considerable freedom from the physical constraints of the real world, and usually reports a general increase in the vividness and verisimilitude of the dreamscape.

These findings suggest that significant interactions between frontal, pre-frontal, limbic, and reticular areas may be present during some dreams, and that the dream state and the waking state may be more closely related than hitherto believed. A further finding is that experienced subjects can sometimes retain ego consciousness, while still asleep, even after the lucid dream ends. Whether this occurs during NREM periods or only during tonic REM periods (REM periods lacking phasic REMs) is unclear at present. In any case, it appears possible that higher cortical functions like planning (manipulation) and language (logic) are able to function during normal dreams, and that when these functions are triggered by suitable events in the dream, they are able to alter the pattern of ascending reticular activation to produce an experience similar to normal waking consciousness in the almost total absence of external sensory inflow.

2362 RESPIRATORY NEURONS OF THE PNEUMOTAXIC CENTER DURING SLEEP AND WAKEFULNESS

Ralph Lydic and John Orem, Department of Physiology, Texas Tech University School of Medicine, Lubbock, Texas 79430.

Extracellular recordings, using tungsten microelectrodes, were made from the pneumotaxic center (PNC) of intact, unanesthetized cats during sleep and wakefulness. Contrary to previous work using decerebrate cats (Feldman et al. Brain Res. 104: 341, 1976) the awake, intact cat demonstrated respiratory neuron activity which was stongly respiratory modulated. During wakefulness, phasic neuronal bursts with average frequencies up to 50 Hz were observed superimposed on a tonic background. There was a decline in the activity of these cells during sleep. This decreased activity began in NREM sleep and was more fully expressed in REM sleep where the discharge rate was only 30-40% of the wakefulness value. These results suggest a loss of respiratory drive to the PNC during sleep.

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2363 PARADOXICAL SLEEP WITHOUT ATONIA IN CATS IS ACCOMPANIED BY EXCESSIVE EXPLORATORY BEHAVIOR IN WAKEFULNESS. Adrian R. Morrison, Graziella Mann*, Joan Hendricks*, and Catherine Starkweather*. Laboratories of Anatomy, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104 USA

Paradoxical sleep without atonia is a dramatic phenomenon created by small bilateral lesions in the pons (centered at P2.1, L2.0, H-3.5, Berman[1]) in cats and is characterized as follows: After slow wave sleep, when paradoxical sleep with muscle atonia would normally appear, cats raise their heads, make body righting movements, exhibit alternating movements of the limbs, and even attempt to stand. Throughout an episode, which shows all other aspects of paradoxical sleep, including unresponsiveness to visual stimuli, cats act as if they are being startled, searching and sometimes attacking an object. In wakefulness they show minor cerebellar signs. We have postulated that the lesions disrupt pontine excitation of the medullary inhibitory area and inhibition of a brainstem locomotor center during paradoxical sleep.[2]

In six cats we tested whether these sleep effects were transferred in some way to wakefulness. All exhibited in wakefulness an increase of 23-127% in exploratory locomotor activity, which consisted of moving about a room marked into squares and investigating various parts of the room rather than aimless pacing. This behavior was measured 1 hour/day for 5 days pre- and postoperatively.

The existence of a parallel effect in wakefulness and paradoxical sleep produced by pontine lesions supports our hypothesis that events of normal paradoxical sleep, which is a hyperalert brain state, are the result of exaggeration of a brainstem mechanism designed to dampen responses to sudden, novel stimuli in wakefulness lest the animal over react and run blindly into danger prior to stimulus analysis. The results also suggest that paradoxical sleep without atonia is more than a simple abolition of the brainstem inhibition of spinal motor neurons which occurs in normal sleep and that the animals are not acting out normal "dreams".

1. Berman, A. L. The Brainstem of the Cat, University of Wisconsin; Madison, 1968
2. Morrison, A. R. Brainstem regulation of behavior during sleep and wakefulness. In J. M. Sprague and A. N. Epstein (eds.), Progress in Psychobiology and Physiological Psychology, 8. Academic Press, New York, p. 91-131, 1979

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2364 MEDULLARY RESPIRATORY NEURON ACTIVITY: RELATIONSHIP TO TONIC AND PHASIC REM SLEEP

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This study analyzed the relationship of brain stem respiratory neuron activity to the tonic and phasic events of rapid eye movement (REM) sleep. Dorsal and ventral medullary respiratory neurons were recorded in sleeping cats. Discharges of inspiratory and expiratory cells increased in number and average frequency with increases in ponto-geniculo-occipital (PGO) spiking (phasic REM activity). The correlations between PGO wave frequency and respiratory neuron activity were positively related to the discharge levels of the neurons: the more active the cell, the greater the relationship to PGO activity. Tonic REM influences on respiratory neurons were calculated by extrapolating from the regression line relating PGO frequency and neuron activity to the hypothetical state of no PGO activity. These calculated levels, when compared to non-REM sleep levels, showed that tonic REM mechanisms decruited some neurons and activated others. Decruited cells tended to be found in the ventral medullary respiratory group; activated cells were generally in the dorsal group. These results demonstrate an association of brain stem respiratory activity to non-respiratory REM sleep variables.

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2365 NUCHAL MUSCLE TONUS DURING SLEEP, WAKEFULNESS AND TONIC IMMOBILITY IN THE RABBIT. R.T. Pivik, P. Sussman* and C. Braun*. Dept. of Psychiatry University of Ottawa, School of Medicine, Faculty of Health Sciences, School of Psychology, University of Ottawa.

A cardinal characteristic of paradoxical sleep (PS) in mammals is inhibition of nuchal or facial muscle tonus. Tonic immobility (TI), a reversible state of immobility induced by rapid dorsoflexion and characterized by loss of righting reflexes and relative unresponsiveness, is reported to exhibit tonic inhibition of nuchal musculature and spinal reflexes similar to that occurring during PS. Previous reports examining muscle tonus in the rabbit have been descriptive and have not provided quantitative inter-or-intrastate comparisons. In the present study nuchal EMG activity was quantified by means of resetting integrators and comparisons made between states of sleep, wakefulness and tonic immobility.

Seven (7) New Zealand White adult male rabbits were chronically implanted for recording EEG, nuchal EMG (teflon coated stranded stainless steel wire) and EOG activity. Recordings began one week after surgery. Spontaneous sleep-waking cycles were recorded in unrestrained animals in a light, sound and temperature controlled environment. TI was induced by rapidly inverting the animals in a "V" shaped trough. TI data were gathered in the late afternoon or early evening after sleep recordings. EMG activity was quantified (resets/second) by a resetting integrator pulses from which are proportional to EMG voltage. Quantified muscle activity was categorized according to the following conditions: active wakefulness (AW), quiet wakefulness (QW), transition to sleep (drowsy, D), slow wave sleep (SW), PS and TI. The transition from SW to PS was also examined. Statistical comparison among states was conducted using analyses of variance with post-hoc t tests when appropriate.

Muscle activity progressively decreased from AW through states QW, D, SW and PS. EMG activity during PS was least of all conditions, but differred significantly ($p < .001$) only from AW. Muscle activity decreased during the transition (1 minute) from SW to PS, but the reduction during PS relative to the immediately preceeding SW epoch was not statistically significant. Tonic muscle activity during TI was at a level more similar to SW than PS.

Tonic levels of nuchal EMG activity during PS and TI were quite variable. In our experience, neither state is consistently characterized by tonic atonia.

2366 EFFECT OF SLEEP ON BENZODIAZEPINE BINDING IN BRAIN. M. K. Poddar, D. A. Urquhart and A. K. Sinha. Department of Physiology & Biophysics, College of Medicine and Dentistry of New Jersey-Rutgers Medical School, Piscataway, N.J. 08854, USA.

In vitro specific ^{3}H-diazepam binding was determined in the cerebral cortex (C), upper brain stem (UBS), cerebellum (Cr) and pons-medulla (P-M) of adult, male, hamsters (body wt. 100-120 g) after sleep and wakefulness. Animals were decapitated following 20 or 50 min. of non-rapid-eye-movement sleep or a comparable period of wakefulness. Immediately after sleep the specific binding of diazepam increased in all four regions of brain in contrast to liver, kidney and lung were no changes in specific binding of ^{3}H-diazepam were detected between sleep and wakefulness. This increased diazepam binding was found to be dependent on length of the sleeping time.

Sleeping time	% increase of ^{3}H-diazepam binding in brain regions			
(min)	C	UBS	Cr	P-M
20	36	21	64	57
50	54	34	107	87

The increased diazepam binding in brain regions after sleep may be due to (1) a change in the number of binding sites (Bmax) and/or (2) a change in the binding affinity ($1/K_D$). Schatchard plot analysis of diazepam binding in cerebral cortex, upper brain stem and pons-medulla showed a significant increase in the affinity of the receptor for the ligand after sleep whereas no significant change was found in the number of binding sites between sleep and wakefulness.

Brain regions	% increase (with respect to awake) after 50 min sleep	
	K_D	Bmax
C	37.30 ($p<0.01$)	NS
UBS	20.30 ($p<0.05$)	NS
P-M	30.47 ($p<0.05$)	NS

This increased diazepam binding affinity in brain regions after sleep suggested that there may be a possibility of an increase in concentration of gamma-aminobutyric acid and/or a decrease in concentration of diazepam binding inhibitors, including any possible endogenous ligand for the benzodiazepine receptor. Studies on cross mixing experiments of diazepam binding with synaptic membrane in presence of 30,000 g supernatant of sleeping and awake brain regions indicate the presence of a diazepam binding inhibitor in awake brain. Further studies are now in progress to characterize the inhibitor present in the 30,000 g supernatant of the awake animal's brain. [Supported by grants from NSF (BNS-76-18615) and from NIH (NS 13118)]

2367 EFFECT OF ALCOHOL ON SLEEP AND NIGHT-TIME PLASMA GROWTH HORMONE AND CORTISOL LEVELS. T. A. Roehrs*, P. N. Prinz, E. D. Weitzman and M. Linnoila* (SPON: W. Dong). Univ. of Wash., Seattle, WA 98195, Montefiore Hospital, Bronx, NY 10467 and Duke Univ., Durham, NC 27710

The acute and chronic effects of alcohol and alcohol withdrawal on sleep patterns and on growth hormone and cortisol fluctuations occurring during sleep were studied. Before going to bed five healthy men, aged 21 - 26, received a placebo drink for three baseline nights, alcohol (1g/kg) for eight nights, and no drink on a final withdrawal night. After two adaptation nights in the laboratory, standard all night polygraphy sleep recordings and blood samples, obtained every 20 minutes through indwelling venous catheters, were collected for one night each in the baseline, acute (alcohol night 1), chronic (alcohol night 8), and alcohol withdrawal conditions.

On both drug nights mean blood alcohol levels peaked within an hour of bedtime at 80 mg percent and the effect of alcohol on sleep stage percentages was confined to the first half of the night. Acute and chronic alcohol significantly reduced REM sleep. Slow wave sleep (stage 3 and 4) was increased significantly after acute alcohol, but returned to baseline levels on the chronic alcohol night. On the withdrawal night there was a slight, but non significant, increase in REM sleep and no change in slow wave sleep relative to baseline levels. The other sleep stage measures were not altered significantly by alcohol administration and withdrawal.

Alcohol, on both the acute and chronic nights, significantly suppressed (by 70%) plasma growth hormone levels across the whole night. All measures of the growth hormone response, total for the night, hourly rate, and peak level, were affected similarly by alcohol. There was no rebound in growth hormone levels on the alcohol withdrawal night. Night-time plasma cortisol levels were not affected by acute and chronic administration and withdrawal of alcohol.

The theoretical and clinical implications of the alcohol effects on sleep patterns and on growth hormone release will be discussed.

2368 VESICULATION OF ASTROGLIAL CELLS IN HAMSTERS FOLLOWING 24-HR SLEEP DEPRIVATION. Arabinda K. Sinha, Ching-Shin Lee*, and Bijan K. Ghosh. Dept. Physiol. & Biophysics, CMDNJ-Rutgers Med. Sch., Piscataway, NJ 08854

Five male hamsters (120 g) were sleep deprived for 24 hr by manual or mechanical manipulation. Their brains were fixed in situ by perfusing a mixture of glutaraldehyde and formaldehyde through the heart. Control animals were prepared identically. Pieces of frontal cortex, occipital cortex, and liver were treated with osmium tetroxide, dehydrated and embedded. This sections were examined by electron microscope. Stereological analysis was performed by taking low power (5,600 x) pictures of randomly encountered neurons and astroglial cells in five different areas of the frontal cortex of both the control and experimental animals. The partial volumes, relative surface areas and relative number of subcellular components were determined in the micrographs projected on a standard morphometric test-grid.

The cell-bodies of neurons from the IVth and Vth layers of frontal and occipal cortex were scanned for structural alterations. Nuclear membrane, chromatin distribution pattern, mitochondria, Golgi apparatus, and cell membrane show no changes. The partial volumes of endoplasmic reticular cisternae and the surface area of the endoplasmic reticulum remained constant in the neurons of sleep deprived animals. Also, the number of neuronal lysosome decreased by 46% in frontal cortex. No changes in the liver cells were detected. The astroglial cells of the sleep deprived animals were found to contain large clear vacuoles. In 80% of the sleep deprived astroglial cells the endoplasmic reticulum vesiculated with partial desolution of its membrane as compared to only 14% in the control animals. There is no change in the surface area of endoplasmic reticulum. The volumes of astroglial endoplasmic reticular cisternae increased by 162% in frontal cortex and 122% in occipital cortex of the sleep deprived animals. The fact that the surface area of the endoplasmic reticulum remains the same but the volume increases gives support to the idea that the membrane bound spaces seen in the astroglial cytoplasm are vesiculation of the endoplasmic reticulum rather than its new formation. Incapacitating behavioral decrement associated with sleep loss may be a consequence and symptomatic expression of the type of subcellular disorganization observed in this study. (Supported by grant #78-3532A from Air Force Office of Scientific Research).

2369 ON GENERATION OF PONTO-GENICULO-OCCIPITAL(PGO) WAVES, RELATIONSHIP BETWEEN THE GENICULATE AND OCCIPITAL PGO AND THE EFFECTS OF NON-VISUAL SENSORY STIMULI. Clara Torda. N.Y.C.PA.TR., N.Y., N.Y., 10028.

PGO waves coincide with rapid eye movement sleep(REM), PGO-type waves with the saccades of alert animals. Therefore, PGO waves may be manifestations of a mechanism designed for image holding and related processes. The here presented work addressed some of the characteristics of PGO waves, e.g. their composition and sensitivity to non-visual sensory stimuli. The effects of various interventions on the occipital PGO waves were studied in the cat following a combined method of Brooks and Gershon (Neuropharmacol., 11:499, 1972) and Calvet et al. (J.Neurophysiol., 28:893, 1975). Intracellular recordings were obtained from the pontine tegmentum by the method of Segundo et al. (J.Neurophysiol., 30:1194, 1967) and from the lateral geniculate nucleus (LGN) and marginal gyrus following the method of Singer (Brain Res., 35:55, 1973). The occipital PGO (recorded from the marginal gyrus) was compared to both: (1) the PGO recorded from the pons and the LGN, and (2) the "directly" transmitted PGO waves recorded from the marginal gyrus of cats either after surgical removal of the LGN, or transsection of the geniculo-cortical pathways. Two methods were used to study the effects of non-visual stimuli on the geniculate and the two types of occipital PGO waves: (1) electrical stimulation of the medial part of the ventral LGN, and (2) non-visual sensory stimulation (clicks, skin of foot, etc.) administered in presence and absence of reserpine. The results suggest that a potential mechanism for encoding PGO waves consists of vestibular activation of specific neurons of the pontine paramedian tegmentum under the influence of a specific set of pontine pacemaker cells. The occipital PGO waves of intact animals result from combination of the geniculate and the "direct" occipital PGO waves (a cortical activity under the direct influence of the pontine PGO that ascended by bypassing the LGN). The differences between the "direct" occipital PGO and those of intact cats are statistically significant and consist of the presence of a first positive going spike and greater differences in shape and amplitude of the occipital waves found in the intact cat. Mathematical treatment of the data suggested that the occipital PGO is a vectorial sum of the geniculate and "direct" occipital PGO. The first positive going spike seems to be related to the activity of the intrageniculate terminal of the optic tract as changed in amplitude and shape by the activity of LGN. The PGO waves were affected by endogenous and exogenous non-visual sensory processes, more in the LGN than in the occipital cells alone.

2370 INTERSPECIES COMMONALITIES IN MOTILITY PATTERNS DURING SLEEP AND WAKEFULNESS. Lewis P. Zeidner* and Evelyn B. Thoman. Dept. of Biobehavioral Sciences, University of Conn., Storrs, Ct.06268.

Motility recordings of infant and adult rats and rabbits, and of human infants from birth to six months of age were used to demonstrate commonality in motor expression of sleep and waking states. The analog output from a motility sensor was produced on a single channel, with respiration signals apparent during quiescence and motor movements superimposed on respiration during activity. The resulting composite patterns of motility were judged for quiet sleep, transitional sleep, active sleep, sleep-wake transition, and quiet or active wakefulness. Judgements of state in each species were made from motility recordings only, and judges were unfamiliar with state observations in that species. Comparisons of judgements with records obtained by direct state observation during the motility recording were used to assess the validity of the judgements. Although the behavioral expression of states across ages and in different species varies, the quality of motility patterns reflect a basic organismic expression of state that shows continuity phylogenetically and ontogenetically.

SOMATOSENSORY SYSTEMS

2371 DEGENERATION OF TRIGEMINAL PRIMARY SENSORY NEURONS AFTER SKIN REMOVAL. Jan Arvidsson* and Gunnar Grant. Department of Anatomy, Karolinska Institutet, S-10401, Stockholm, Sweden.

In previous studies it was shown that transection of peripheral branches of trigeminal primary sensory neurons in adult rats and cats resulted in degeneration of their centrally projecting branches. Ultrastructural studies of this type of axonal degeneration, termed transganglionic degeneration, revealed alterations similar to those occurring during Wallerian degeneration. Studies of the trigeminal ganglion following peripheral nerve transection showed a substantial cell loss at the light microscopical level, as well as ultrastructural signs of degenerating and dying nerve cell bodies. Since the transection in those studies was made far out peripherally, the question was raised whether simply disconnecting the nerve cell body from its receptors might itself induce transganglionic degeneration. As a first approach small areas of skin were removed supraorbitally (2 rats), infraorbitally (2 rats) and in the mental region (2 rats). The edges of the incisions were apposed and sutured. After postoperative survivals of 14 or 24 days the rats were perfused and sections from the brain stem processed according to the Fink-Heimer technique. In all cases there were small amounts of degeneration in the rostral part of the ipsilateral trigeminal nucleus caudalis. There was no degeneration in other trigeminal nuclei. The location of the degeneration was strictly within the same dorso-ventral and medio-lateral areas as after supraorbital, infraorbital or mental nerve transection shown in previous studies. The results support the view that disconnection of the nerve cell body from the receptor area may be an important factor for provoking transganglionic degeneration. Degeneration in the central nervous system of adult animals following skin removal does not seem to have been shown before. It clearly demonstrates the possibility of using this approach for mapping the body surface on the primary sensory relay nuclei. Hopefully further studies will give an answer to the question whether this approach will be useful also for mapping the central representation of different types of receptors.

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2372 SYMPATHETIC EFFERENT MODULATION OF FACIAL MECHANORECEPTORS IN THE CAT. David J. Barker and Paul D. Shepard*. Dept. of Physiology, North Texas State University Health Sciences Center/Texas College of Osteopathic Medicine, Fort Worth, TX 76107.

Chronic hyperactivity of the sympathetic innervation of a tissue is often a prevalent finding in many clinical conditions, including post-traumatic pain syndromes. In previous work (Bereiter & Barker, Soc. Neurosci., 1976, 2, #946) we found that the estrogen induced enlargement of cutaneous receptive fields could be duplicated by chemical or surgical lesions of the sympathetic innervation. The present study was undertaken to examine the quantitative effects of sympathetic efferent stimulation on rate and pattern of firing in cat first-order facial mechanoreceptors.

Single units were recorded from the trigeminal ganglion of anesthetized cats. Cutaneous mechanoreceptors were stimulated with controlled mechanical stimuli (trapezoid, pulse, sine) before and after electrical stimulation of the cervical sympathetic trunk, which was dissected free of the vagus and cut centrally. Both mechanical and electrical stimulus parameters were systematically varied.

Among 25 neurons studied intensively (2 to 10 hours) we observed the following results: (1) Intervals between repeated mechanical stimuli of less than 20 sec. resulted in significant decreases in firing rate, possibly due to fatigue. No changes were observed for interstimulus intervals of 1 min. or more. (2) Adaptation rate could be varied with stimulus amplitude. (3) Sympathetic stimulation lasting longer than 1 sec. can produce significant artifacts due to mechanical movement of the tissue. (4) Many rapidly adapting units showed a decrease in rate of firing and increased latency to firing following sympathetic stimulation. (5) Many slowly adapting units show an increase in firing rate following sympathetic stimulation. (6) "Stroke" units (responds to movement across the field) show variable or no effects of sympathetic stimulation. (7) Sympathetic stimulation can result in changes in interspike interval pattern. The effects of sympathetic stimulation can be both transient and maintained, and can be blocked with phentolamine. Our results are consistent with previous findings in the frog (Roberts and Carlof, Soc. Neurosci., 1977, #1572) that some units are facilitated while others are inhibited by sympathetic stimulation. Our results also suggest that great care must be taken in choosing both mechanical and electrical stimulus parameters in order to minimize the possibility of artifacts. The present findings show that in addition to cat vibrissae (Nilsson, Acta physiol. scand., 1972, 85, 390-397), other types of cutaneous receptive fields (glabrous rhinarial skin, fur patch) can be influenced by sympathetic efferent stimulation. Supported by AOA Grant 78-11-169.

2373 CONTROL OF SENSORY TRANSMISSION BY ELECTRICAL STIMULATION WITHIN THE CAUDAL RAPHE NUCLEI OF THE CAT. P.S. Blum. Department of Physiology, Thomas Jefferson University, Philadelphia, PA 19107.

Sensory-evoked activity of single spinothalamic tract neurons can be inhibited by electrical stimulation within the nucleus raphe magnus, nucleus raphe pallidus, and nucleus raphe obscurus (caudal raphe nuclei, CRN), Willis et al., J. Neurophysiol. 40: 968, 1977. The present experiments were designed to investigate the location within the CRN where electrical stimulation can inhibit the activity in ascending pathways evoked by sensory stimulation and to determine whether or not the inhibition is directed preferentially to sensory-evoked activity in the spinothalamic tract. Adult cats were anesthetized with chloralose (60 mg/kg) and prepared for acute electrophysiological experiments. Single shock electrical stimuli were delivered to the superficial radial (SR) nerve (50 to 500 microamp) via a hook electrode and to nine sites in the CRN (25 to 100 microamp) with an array of three bipolar concentric electrodes. The compound action potential was monitored with a hook electrode on the SR nerve and averaged slow-wave recordings were taken contralateral to the SR nerve from the medial lemniscus (ML) at the midbrain and spinothalamic tract (ST) at C1. The amount of sensory-evoked activity in the ML and ST was determined by measuring the height of the initial monopolar portion of the averaged ST and ML response following SR stimulation. The height of the evoked potentials following SR stimulation was compared to the height of the potentials when the SR stimulus was preceeded by stimulation at each of the 9 CRN sites. Reduction in the size of the SR-evoked potential by CRN stimulation was defined as inhibition. In a total of five experiments, 37 brain stem sites were stimulated within the CRN. Stimulation at 19 of these sites produced inhibition of the sensory-evoked ST potential, while stimulation at three sites produced inhibition of the sensory-evoked ML potential. No sites were found where stimulation caused an inhibition of both sensory-evoked potentials. Within each experiment, the total percent inhibition of the ST was calculated for each CRN stimulus site. The inhibition-producing sites were ranked and a value of 100% of maximum inhibition was assigned to the site producing the greatest total inhibition in each experiment. Sites that showed 90-100% of maximum ST inhibition were located within the ventral part of nucleus raphe magnus and the rostral part of nucleus raphe pallidus. These data suggest that activity originating from neurons in the ventral part of nucleus raphe magnus and the rostral part of nucleus raphe pallidus function to reduce the amount of sensory-evoked activity in the spinothalamic tract.

2374 EFFECTS OF TEMPERATURE ON NEUROPHYSIOLOGICAL AND PSYCHOPHYSICAL FREQUENCY CHARACTERISTICS IN THE SOMATOSENSORY SYSTEM. S. J. Bolanowski, Jr.* and R. T. Verrillo. Institute for Sensory Research, Syracuse University, Syracuse, NY 13210.

The effects of temperature on both the neurophysiologically measured frequency characteristics of isolated mechanoreceptors (Pacinian corpuscle) and the vibrotactile frequency response obtained psychophysically on humans are compared. The neural frequency characteristics established for a criterion of approximately 1 spike/stimulus cycle are U-shaped functions that shift toward higher frequencies with increasing temperature. The best frequencies for these functions are directly proportional to temperature. The low-frequency slopes do not change significantly with temperature but remain at about 12 dB/octave. The U-shaped functions do not shift significantly along the intensity axis as a function of temperature. Similar results were obtained for lower firing rate criteria. The psychophysical frequency characteristic shows a flat, low-frequency (10-50 Hz) portion of low sensitivity and a U-shaped, high-frequency (50-700 Hz) portion of high sensitivity at normal skin-surface temperatures (30°C). A breakpoint in the curve occurs where the two portions meet (∿50Hz). Considerable evidence indicates that the high-frequency portion of the characteristic is mediated by the Pacinian corpuscle population. For skin temperatures below normal physiological levels, the best frequency (high-frequency portion) of the vibrotactile frequency characteristic is shifted to lower frequencies. Skin temperatures above normal produce shifts toward higher frequencies. These results are in fundamental agreement with the neural data. Because the shape of the psychophysical curve reflects interactions of at least one other receptor population, graded temperature effects produce additional changes not seen in the physiological data from individual Pacinian corpuscles. Below normal surface temperatures, the low-frequency (flat) portion is elevated and both the bandwidth and sensitivity of the high-frequency portion are decreased. This produces a shift to lower frequencies in the breakpoint between the flat and U-shaped portions of the frequency characteristic. For temperatures above normal, the bandwidth of the high-frequency portion is increased without much effect on the overall sensitivity. These additional effects may be explained in terms of different cutaneous receptor populations, all of which are effected by temperature.

2375 INTRACORTICAL AND THALAMIC CONNECTIONS OF THE SUPPLEMENTARY SENSORY AND SUPPLEMENTARY MOTOR AREAS IN THE MONKEY. R.M. Bowker*, E.A. Murray and J.D. Coulter. Marine Biomedical Institute, Departments of Psychiatry & Behavioral Sciences and Physiology & Biophysics, University of Texas Medical Branch, Galveston, Texas 77550.

Recent studies from this laboratory have described the organization of spinal projections from the medial posterior parietal cortex of area 5, termed the supplementary sensory area, and from the medial cortex of area 6 comprizing the supplementary motor area in primates. Here we report the intracortical and thalamic connections of these two cortical areas determined by the retrograde horseradish peroxidase (HRP) and anterograde autoradiographic tracing methods.

Cortical HRP injections were made by multiple penetrations with 0.3-0.6 µl of 25-50% HRP solution. The tissue sections were then reacted with the tetramethylbenzidine technique. Anterograde studies were performed by injections of 0.03-0.07 µl (100 µCi/µl) of a mixture of tritiated proline and leucine into either cortical region followed by routine processing of the tissues for autoradiography.

The supplementary sensory area receives somatotopically organized projections from the posterior parts of SI (mainly areas 1 and 2), from the lateral part of area 5, from the lateral area 6 on the convexity of the hemisphere and from the medial part of area 6 comprizing the supplementary motor area. The supplementary motor area similarly receives somatotopically organized projections from the SI cortex (mainly areas 1, 2 and 3a), from the lateral part of area 5, from the primary (area 4) motor cortex (MI) and from the supplementary sensory area (medial area 5).

Thalamic afferents to the supplementary sensory area arise primarily from the dorsolateral part of the lateral posterior nucleus (LP) with a small number of neurons being seen scattered as far anterior as the caudal ventrolateral nucleus (VLo) and as far posterior as the caudal ventroposterolateral nucleus (VPLo). Thalamic afferents to the supplementary motor area arise from the lateral and dorsal parts of the ventrolateral nucleus (both VLc and VLo), extending forward into the ventral anterior nucleus (VA) and caudally into the lateral-most part of the ventroposterolateral nucleus (VPLo).

Both the thalamic, as well as intracortical pathways (with the exception of part of the SI projection) for the supplementary sensory area and supplementary motor area are reciprocal.

In view of the connections of these two cortical areas, the supplementary sensory area and supplementary motor area would appear to be capable of analyzing and programing complex sensory and motor behavior. (Supported by NS12481).

2376 AN AUTORADIOGRAPHIC STUDY OF THE ASCENDING PROJECTIONS OF THE PRINCIPAL SENSORY TRIGEMINAL NUCLEUS IN THE RAT. William C. Broderick*, Leo C. Massopust, and Paul A. Young. Francis and Doris Murphy Neuroanat. Res. Lab., Dept. Anat., Sch. Med., St. Louis Univ., St. Louis, MO 63104.

The purpose of this study was to investigate the ascending projections of the principal sensory trigeminal nucleus in the rat. Tritiated leucine was ejected iontophoretically from a glass micropipette into the nucleus using a surgical stereotaxic approach. The tissue was processed according to the method described by Cowan et al. (1972). Three sets of projections were seen coursing from the ejection site. The first projection was a small group of diffuse fibers which coursed dorsomedially through the pons immediately ventral to the abducens nucleus and locus coeruleus. Some fibers terminated in the ipsilateral pontine reticular formation while others decussated and synapsed in the contralateral pontine reticular formation. The second group decussated, ascended rostrally and dorsally, and terminated in a diffuse pattern in the region of the inferior medial geniculate nucleus or the posterior thalamic region of Lund and Webster, (1967), the adjacent reticular formation and the pretectal nuclear complex. The third projection was a large ventral tract which decussated between the level of the principal sensory trigeminal nucleus caudally and the level of the interpeduncular nucleus rostrally. This tract took a position dorsomedial to the medial lemniscus. The trigeminal tract continued rostrally through the midbrain located between the red nulceus and the substantia nigra. Projections from this tract terminated densely in the ventral part of the zona incerta and to a lesser degree in the posterior thalamic area. The remainder of the trigeminal tract terminated in the medial portion of the ventral posterior nucleus of the thalamus. Even though it has been suggested that there is an ipsilateral projection to the thalamus from the principal sensory trigeminal nucleus in the rat, there was no evidence in this investigation to support that claim.

2377 ABSENCE OF ALTERATIONS OF DORSAL HORN SOMATOTOPY AFTER UNILATERAL L_7 SECTION IN CAT. Paul B. Brown, H. Richard Koerber , and Robert P. Yezierski . Dept. of Physiology and Biophysics, West Virginia University Medical Center, Morgantown, WV 26506.

Single units were recorded in laminae I-VI of the left dorsal horn of adult cats at various survival times after ipsilateral section of L_7 dorsal root, using T_{12}-spinalized, urethane-anesthetized animals. Light touch receptive fields were mapped on standard cat leg drawings, spontaneous activity was characterized, and recording sites were marked by the Prussian Blue method. Statistical analysis revealed no changes in the somatotopic map (distance from tips of toes as a function of mediolateral position of cell for each segment), spontaneous activity, or receptive field geometries (area and length/width ratio as functions of distance from tips of toes), at survival times ranging from 0 to over 200 days, compared with normal data from earlier studies. However, at early survival times it was difficult to find cells with receptive fields within the focus of the L_7 dermatome (the toes), especially in the L_7 segment, where the toes are most heavily represented.

It is concluded that, with regard to light touch receptive fields, the somatotopy of the dorsal horn is not significantly altered after cutting one root (L_7), even though dendritic changes have been demonstrated in these cells after L_7 section (Brown, Busch and Whittington, 1977, 1979). This is presumably due to the overlap of the L_7 dermatome by other dermatomes. Future experiments will examine the physiological effects of cutting two, three, and four dorsal roots. Ongoing experiments are examining sprouting of afferents and propriospinal connections in the same preparations.

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2378 PHASE DEPENDENT GATING OF SOMATIC SENSORY INPUT TO SMI CORTICAL CELLS DURING LOCOMOTION By John K. Chapin and Donald J. Woodward, U. Tx. Health Sci. Ctr. at Dallas, Dallas, Texas 75235

This study was conducted to test the postulate that statically defined receptive field properties in SmI cortical neurons of awake, freely moving rats may be subject to internal gating according to specific parameters of movement.

Cells with purely tactile receptive fields on the ventral surface of the forepaw were isolated and post-stimulus time histograms (PSTH's) were generated by repetitively touching the skin with a manually held probe. The responsiveness of these cells to "active touch" of their receptive fields was tested by averaging unit activity around foot contact during locomotion. About 56% of these cells were found to respond strongly at footfall (Strong FF cells), but 43% discharged only weakly at footfall (Weak FF cells) even though they responded strongly to equivalent parameters of passive touch stimulation. In subsequent studies, the somatic responsiveness of such units was also tested by electrical stimulation of receptive field areas through subcutaneously implanted bipolar electrodes. The latency and form of the PSTH's produced by this method were very similar to those derived from natural touch with a probe. For this reason such electrical stimulation was considered to be a reliable means of quantitatively testing the transmission of somatic sensory input during movement. The quantitative level of the excitatory responses of these cells to electrical stimulation delivered at 1-3 Hz during various phases of the forelimb step cycle were plotted by computer. In this method the baseline level of unit activity for each step cycle phase was calculated and automatically subtracted from the averaged post-stimulus excitatory responses. The "peri-footfall stimulus effectiveness plots" generated by this technique showed that the sensory responsiveness of most cells was modulated over the step cycle. In particular, the responsiveness of the "Weak FF" cells were found to be phasically deppressed 10 to 50 msec before footfall. These units responded vigorously to tactile or electrical stimuli delivered during the early swing phase. Conversely, the "Strong FF" cells were found to be phasically facilitated just before footfall. Such neurons could not be driven by any form of stimulation during the early swing phase.

These findings suggest that this sample of cells may be partitioned into separate perceptual systems. One group may be actively configured to respond only to the anticipated floor contact of the foot occurring during locomotion, whereas the other may respond only to unanticipated stimuli delivered during other phases.

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2379 PROPERTIES OF DIRECTION-SENSITIVE NEURONS IN PRIMARY SOMATOSENSORY CORTEX OF ALERT MONKEYS. Richard M. Costanzo and Esther P. Gardner, Dept. of Physiology & Biophysics, NYU School of Medicine, New York, NY 10016.

In SI cortex of alert monkeys, we recorded from 60 direction-sensitive neurons (DSNs) which were effectively driven by moving stimuli traversing the receptive field in particular directions, but responded poorly to punctate cutaneous stimuli. Fine brushes or calibrated bars (2-30 mm in length) were used to define the direction of movement eliciting the strongest response (on-direction) and that evoking the weakest activity (off-direction). On- and off directions were usually diametrically opposed, but in a few DSNs were separated by only 90 deg. Individual DSNs responded to trajectories within a range of angles from 90-360 deg. Responses were graded with angular displacement from the on-direction, becoming progressively weaker as the off-direction was approached. DSNs with hand receptive fields tended to be more narrowly tuned than those on the forearm. All directions of movement were represented in our sample. On the forearm, 2/3 of the direction preferences were oriented along the distal-proximal axis; hand direction preferences were uniformly distributed.

Three classes of DSNs were found: 1) Multidirectional neurons (37%) responded to movements in all directions, but were preferentially excited by those in the on-direction. 2) Unidirectional cells (57%) gave strong responses to on-direction stimuli, and only 0-4 spikes to off-direction stimuli. 3) Opponent direction cells (6%) were spontaneously active, increasing their firing rate to on-direction trajectories, and decreasing firing below spontaneous levels to off-direction stimuli. All three cell types fired continuously and uniformly throughout the stimulus trajectory. Instantaneous frequency histograms showed no peak in responsiveness as the stimulus crossed the field center, or any other individual point in the field. Thus DSNs seem to ignore information about the exact position of the stimulus at any moment in the trajectory.

In addition to coding direction of movement, many DSNs were able to differentiate parallel trajectories in the on- and off-directions. Only some DSNs with on-directions oriented transversely across the forearm or hand showed position insensitivity. 2/3 of DSNs tested also discriminated the size of moving bars, some preferring narrow, others wide bars.

The broad tuning properties, plus the multiplicity of information encoded in the firing patterns of individual DSNs (direction, specific trajectory, size of stimulus), suggest that activity distributed across the population of DSNs must be evaluated to determine the precise characteristics of the moving stimulus.
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2380 SPINOTHALAMIC TERMINATIONS IN THE VENTROPOSTEROLATERAL NUCLEUS OF THE CAT. A.D. Craig, Jr. and H. Burton. Depts. of Anatomy-Neurobiology and Physiology-Biophysics, Wash. Univ. Sch. Med., St. Louis, MO 63110

Recent evidence of a projection from n. caudalis to the ventroposteromedial n. in the cat thalamus (Burton et al., JCN '79) prompted a reconsideration of the reported absence (Boivie, EBR '71; Jones & Burton, JCN '74) of spinothalamic projections to the ventroposterolateral n. in this species. Following single or multiple injections of horseradish peroxidase (HRP) into individual or conjoint spinal levels and/or laminae, the distribution of labeled terminal projection zones was examined after the application of the highly sensitive tetramethylbenzidine chromogen reaction. These procedures enabled visualization of anterogradely transported label and often permitted observation of terminal, pericellular arborizations and "boutons" with Golgi-like clarity. These experiments confirmed the most recent previous studies in that terminals occur in the spinal part of the ventrolateral n., medial part of the posterior n., magnocellular part of the medial geniculate n., and the centrolateral and parafascicular nuclei. Several additional termination zones have been identified. One of these is VPL, in which labeled terminals are distributed in two patterns. First, isolated small patches of terminals are seen sparsely scattered within the central portion of the topographically appropriate part of VPL. A second narrow zone of terminals can be observed within the extreme ventral aspect of VPL where they surround the cells (many of which are small) found in this location; these terminations are also distributed somewhat topographically. Recordings obtained from single neurons in this ventral zone indicate that some of these cells respond to a wide dynamic range of cutaneous stimulation.

These results, along with the recent results on projections from n. caudalis (Burton et al., '79; Ganchrow, JCN '78), suggest that spinothalamic terminations in the ventroposterior nuclei of the cat and monkey are qualitatively homologous in both their spinal and trigeminal components.
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2381 PROJECTIONS OF HINDLIMB DORSAL ROOTS TO SPINAL CORD OF CAT. James L. Culberson and Paul B. Brown. Depts. of Anatomy and Physiology/Biophysics, West Virginia University Medical Center, Morgantown, WV 26506.

The Fink-Heimer degeneration stain was used to study projections of dorsal roots L_3-S_2 to segments L_2-S_3 of the cat spinal cord. Unilateral dorsal root section was effected transdurally between the ganglion and entry of the root into the intrathecal space. In each animal, one dorsal root was sectioned, on the left side; roots L_3-S_2 were sectioned, each of them in at least two animals. The animals were perfused transcardially with 0.9% saline and 10% formalin 5-7 days after rhizotomy, and the cords were stored in 10% formalin for at least two weeks. Cords were cut into blocks at intersegmental boundaries, and serial 40 μm thick frozen sections were cut in the transverse plane. Alternate serial sections were stained by the Fink-Heimer method, and with cresyl violet acetate.

Degeneration was seen in all laminae, although it was uncommon in ventral lamina II - dorsal lamina III, and lamina VIII. Excluding projections to Clarke's column, the rostrocaudal extent of degeneration observed for each root was: L_3 projects to segments L_2-L_6 (examined L_2-S_2); L_4 root to segments L_2-L_5 (L_2-S_1 examined); L_5 to segments L_2-S_2 (examined L_2-S_2); L_6 root to L_3-S_2 (examined L_3-S_2); L_7 root to L_4-S_2 (examined L_7-S_2); S_1 root to L_4-Ca_1 (examined L_3-Ca_1); and S_2 root to L_6-S_3 (L_5-S_3 examined). Ventral horn distributions were usually limited to the deafferented segment ± segment.

Since it is now known from HRP injections of cutaneous nerve fibers (A. G. Brown et al.) and peripheral loading of cutaneous nerves with HRP (Brown and Koerber) that large cutaneous fibers end in laminae III-IV and that deep afferents do not (A. G. Brown et al.), projections to laminae III-IV were plotted on dorsal view maps of left dorsal horn to examine their somatotopy. No simple rule can be devised to describe the projections of all dorsal roots, either in terms of anteroposterior or mediolateral extent. Thus, for example, there is no consistent medial or lateral shift of projections rostral or caudal to entry segment of a given root. However, cutaneous afferents in a single root project consistently to the same dorsal horn region in different cats. The projection target is predictable on the basis of somatotopy: a dorsal root projects to those regions of dorsal horn where the root's dermatome is represented in physiological mapping experiments.

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2382 TEXTURED SURFACES EXPLORED WITH THE MOVING FINGERTIPS: THEIR REPRESENTATION IN THE DISCHARGE OF DIGITAL NERVE FIBERS. Ian Darian-Smith, Linda Oke*, Ian Davidson* and Kenneth Johnson. Sensory Processes Laboratory, Department of Physiology, University of Melbourne, Parkville 3052, Australia.

Textured surfaces actively explored with the fingers, or moved across the stationary finger, are among the commonest stimuli that we identify and characterise tactually. Experiments were designed to examine the representation of the spatial and temporal features of two geometrically patterned surfaces moving across the skin in the discharge patterns of single mechanoreceptive fibers, and in assemblies of these fibers. The stimulator used in these experiments applied the moving surface to the skin for a specified time with a particular contact force and at a particular velocity. The surfaces used were fine gratings defined by their spatial frequency, and geometric arrays of elevated 'dots' defined by the dot diameter and the spacing of these dots.

Single mechanoreceptive fibers innervating the fingertip pad were isolated by dissection of the median nerve in the anesthetised monkey (Macaca nemestrina). Each fiber was then identified as a slowly adapting (SA), quickly adapting (QA), or Pacinian afferent.

Fibers responded to gratings moving across the fingertip with a very regular discharge which, however, reflected the temporal frequency (spatial frequency x velocity) rather than the spatial features of the surface. The spatial characteristics of the surface could be represented only in the discharge pattern of a spatial assembly of these fibers. Coarse gratings moving slowly across the skin (temporal frequency of 20 - 40 Hz) were best represented in the responding SA fiber population; fine gratings moving at a high velocity across the skin (temporal frequency > 100 Hz) were best represented in the discharge of the Pacinian fiber population, and stimulus combinations generating a temporal frequency in the range 30 - 100 Hz were precisely represented by discharge in the QA fiber population.

Specification of the mechanoreceptive fiber responses to the two-dimensioned dot patterns involved a more complex data collection. With each successive stimulus presentation the moving surface was translated 125 microns laterally relative to the direction of movement of this surface. The response array generated by this procedure defined the representation of the stimulus surface both along an axis at right angles to its line of movement, and in the line of movement.

2383 OCULAR COUNTERROLLING DURING CONSTANT VELOCITY ROLL IN NORMAL HUMANS AND IN PATIENTS WITH UNILATERAL VIII NERVE SECTIONS. Shirley G. Diamond and Charles H. Markham. Dept. Neurology, UCLA School of Medicine, Los Angeles, CA 90024.

Nine subjects were rolled about their naso-occipital axes at a constant velocity of 3°/sec after having been securely strapped into a rotating chair. The head was positioned and stabilized by a bite bar. Trials began with a roll to 90° right ear down, reversed and rolled to 90° left ear down, again rolled to 90° right ear down, then back to 90° left ear down, and finally back to the upright baseline. At each 90° position, subjects were held steady for 30 sec before being rolled to the opposite direction. A camera mounted on the rotating chair photographed both eyes at each 10° of roll, and at each 10 sec while subjects were held motionless at 90° tilts.

Counterrolling was measured by a dual projector system described in an earlier study (Diamond, Markham, Simpson & Curthoys, Acta Otolaryngologica, in press, 1979). Mechanical accuracy of the measuring device is 1 minute of arc; practical accuracy is 15 minutes.

Results in six normal subjects showed that contrary to general belief, the counterrolled position of the eyes is not held constant as long as the tilt is maintained. During the 30 sec intervals when subjects were held motionless at 90°, 28 observations were recorded and 22 of these showed the subjects' eyes continued to make occasional rotational movements. This torsion during the stationary interval was as much as 5°, with 2° being common. Direction of change during the static period was about equally divided between more and less counterrolling. When subjects began rotation back toward the upright baseline, frequent torsional eye movements in the "wrong" direction were observed. These were corrected fairly rapidly, and no normal subject showed "wrong" direction torsion in the area bounded by 30° to the right or left of the upright position. The amplitude of counterrolling was approximately equal on both sides.

Three patients with right vestibular nerve sections performed 2 to 5 years earlier showed distinctly abnormal patterns when they were rolled contralateral to the lesion, i.e. left ear down. These abnormal patterns consisted of reduced amplitude and/or more instability in the form of irregular "wrong" direction torsion, persisting longer than in the normal subjects and appearing closer to the upright position. Counterrolling was not conspicuously different than normals when patients were rolled to the side ipsilateral to the lesion, i.e. right ear down. Further studies are underway to delineate the differences between normals and persons with unilateral lesions to aid in the diagnosis of utricular dysfunction. (Partly supported by NASA NGR 05-007-418.)

2384 ORGANIZATION OF THE SOMATOSENSORY CORTEX IN CEBUS MONKEYS. D.J. Felleman*, R.J. Nelson, M. Sur* and J.H. Kaas, Depts. of Psychology and Anatomy, Vanderbilt Univ., Nashville TN 37240.

The somatotopic organization of the post-central somatosensory cortex was revealed by determining receptive field locations from approximately 2700 recording sites in four (4) Cebus monkeys, (Cebus albifrons). As in previous studies in macaque, squirrel and owl monkeys, two separate representations of the cutaneous body surface were found. In position, these representations appear to correspond to architectonic Areas 3b and 1. As in other monkeys, the two representations are essentially mirror-images of each other, with receptive field positions matched for recording sites along the common border. Both representations show a similar medio-lateral organization to other primates studied, with tail and hindlimb represented medially and forelimb and face represented laterally. Glabrous digit tips point rostrally in the anterior representation and caudally in the posterior representation. As in macaques (Sur et al., Neuro. Abst. 4:559,'78), the occiput is represented medial to the arm in the rostral representation. The tail representation is unusually large, occupying at least 12 sq. mm spanning both representations. Much of the tail representation is occupied by the tip of the tail. The ventrum of trunk is represented anteriorly and caudally with the dorsal midline represented at the junction of the two representations. The inner arm and thigh are represented rostrally and caudally in the two representations respectively; the lateral surfaces of the limbs are represented at the common border. All regions of the face are represented in each of the cutaneous representations.

Comparisons between previously studied species indicate that there are two basic patterns of organization of the representations of the hairy surfaces in monkeys, while the orientation of the glabrous digits appears to be consistent. Thus, the distal digits are represented at the extremes of the representations and the proximal surfaces are represented at the border of the two representations in all monkeys. However, in cebus and squirrel monkeys, the ventral trunk and the medial limb surfaces are represented at the rostral and caudal extremes. The remaining surfaces of the limbs and trunk are systematically represented, either from ventral to dorsal, by way of the lateral surface, in the case of the trunk, or from medial to lateral, by way of the anterior surface, in the case of the limbs. The opposite is true of the organization of the representation of the hairy surfaces in macaques. Therefore, while there appears to be a common pattern of organization for the glabrous surfaces, the hairy surfaces may vary, in toto in the cortical representations across species.

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2385 FUNCTIONAL MODIFICATION OF CAT SOMATIC SENSORY-MOTOR CORTEX (SmI) BY SELECTIVE DEAFFERENTATION. Joel Ira Franck*[1] and Jacqueline Metzler (SPON: Carole C. LaMotte). Dept. Neurosurgery, Yale Univ. Sch. Med., New Haven, CT 06510.

In order to further analyze the somatotopic projection to SmI, single units were recorded from 6 awake, paralyzed, adult cats, using N_2O analgesia and mechanical stimulation. Chronic recording chambers were implanted over the left SmI. Cells were classified as to whether they responded to superficial stimulation (S cells), such as hair movement or light touch, or to deep stimulation (D cells), such as pressure or joint manipulation.

The 144 units studied during 9 control recording sessions revealed that SmI is organized in a highly specific manner, with 93% of the cells belonging to a single submodality and having a discrete receptive field (RF). The body surface is represented in SmI as a series of overlapping strips, oriented at about 45° relative to the cruciate sulcus, with rostral body segments lying caudo-laterally and caudal segments rostro-medially. D cells were concentrated rostro-laterally and S cells caudo-medially. Within each segmental strip, cells with dorsal RF's tended to be located caudo-medially while cells with ventral RF's were found rostro-laterally.

All dorsal roots caudal to L3, with the exception of L7, were then intradurally sectioned on the right side in 3 cats, 2 of which had been studied as controls. These animals were examined several times over the next 8 to 55 days, during which time 169 units were isolated in 9 sessions. Nearly 67% of the post-rhizotomy cells had the highly specific patterns of activity observed in the control recordings. However, 33% of the units studied after deafferentation, as compared to 7% of those examined in the control preparations, exhibited unusual properties which emerged within 8 days of dorsal-root section. These cells responded in a nonspecific manner to stimulation, often to multiple subclasses, of uncharacteristically shaped, large, or stocking-like, contralateral, bilateral or multiple RF's. The somatotopic map of SmI changed progressively over time. Rostral body segment representations in the lateral regions of SmI "migrated" into the partially deafferented medial regions of SmI which had represented the hind limb in the control state. Previous reports indicate that the widespread connections unmasked in this chronic study may be present throughout the somatosensory system of intact animals but are usually latent or ineffective (Wall, 1977, Philos. Trans. R. Soc. Lond. B 278: 361-372).

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2386 QUANTITATIVE EXAMINATION OF PRESYNAPTIC PROFILES CONTAINING FLATTENED VESICLES IN NUCLEUS GRACILIS OF GALAGO. D. J. Friedenbach. Dept. Anat., Sch. Med., UND, Grand Forks, ND 58202.

The rostral portion (RG) of nucleus gracilis of Galago has been examined by quantitative methods. Six bushbabies were routinely prepared for electron microscopic examination. A series of thick and thin sections were taken from RG for orientation and examination purposes. Sections were randomly selected and photographed to produce 100 micrographs at a final magnification of 40,000X. The point reticule method of Weible was used to determine the percent area of identifiable neuropil for three classes of synapses: axodendritic (FD) or axosomatic (FS) with flattened vesicles, and axoaxonic (AA). For each identified synapse several parameters were directly measured. ANOVA was used to examine for significant differences ($P<0.05$) between synaptic classifications or parameters within animals, among animals or significance ($P<0.05$) between synaptic types. AA synapses (5%) compose the smallest percentage of presynaptic elements defined as having flattened vesicles (FD-19%, FS-14%). No other differences were detected when comparing the size of the presynaptic profiles (1.9-2.6μm^2) or the number of synaptic sites found on each presynaptic profile (1.1-1.3). AA synaptic lengths (28nm) were shorter than either FD (410 nm) or FS (680nm) synapses. A significantly higher percentage of AA synapses (99%) were characterized as Gray type II synapses (FD-62%, FS-50%). By shape criteria FD synapses (1.2) were significantly more round than FS (1.8) or AA (1.7). The cross-sectional area of vesicles in FS terminals ($1.9 \times 10^3 nm^2$) was larger than in FD or AA synapses (1.5 and $1.4 \times 10^3 nm^2$). There were no differences in the variance of these parameters: the arc of the synapse (1.2-1.3), the percent of the area of the presynaptic profile occupied by vesicles (42-54%), or the number of vesicles per presynaptic profile (12-19).

Inhibitory afferents have been demonstrated to the rostral nucleus gracilis of other animals. If the flattened vesicle-containing profiles are inhibitory then it is plausible that they represent inputs from three different sources. Work is currently underway to investigate the nature of the terminals differentially terminating in RG.

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2387 PATTERNS OF PROJECTIONS OF THALAMIC VENTROBASAL NEURONS TO THE FIRST SOMATIC SENSORY AREA OF MACAQUES. D.P. Friedman and E.G. Jones. Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

Cortical projections from physiologically identified neuronal groups within the thalamic ventrobasal complex (VPLc and VPM) were studied using the autoradiographic method. In each experiment the areas of the thalamus receiving somatic sensory input were mapped using standard microelectrode recording techniques. A 1 µl Hamilton syringe was guided into VB according to these maps. The final position of the injection was selected by recording from a microelectrode which had been cemented to the barrel of the syringe so that the tip of the electrode was less than 500 µ from the bevel of the syringe barrel. This pair was placed into the brain so that the electrode was immediately posterior to the opening in the bevel. 10-50 nl of tritiated leucine and proline (50 µci/µl, New England Nuclear) was expelled after determining the response properties of the nearby neurons. Such injections commonly produced a heavily labelled central core of less than 1 mm^3. Virtually all of this zone was posterior and lateral to the syringe barrel, thus enveloping the recording site.

Injected clusters responding to stimulation of deeper tissues were always most dorsally placed within VB, though sometimes at its anterior border with VPLo. Injections made into clusters of neurons responding to light tactile stimuli were more ventrally placed within VB.

The pattern of terminal labelling on the cortex was studied using series of 20 µ thionin counterstained autoradiographic sections cut in either sagittal or horizontal planes. Following injections into clusters responding to light tactile stimuli, labelling was confined to area 3b. Such labelling was seen in vertical columns or bands within layers IIIb and IV. An individual column 250-400µ wide was separated from an adjoining column by an equivalent sized gap. Within 3b columns located on adjacent sections were often arranged in register so as to form a pattern of repeating strips or bands of variable orientation with respect to the axes of the postcentral gyrus. The more dorsal injection sites,placed where units responded to stimulation of deeper tissues, produced labelling mainly in areas 1 and 2. Within those areas labelled terminals appeared in elongated bands completely within layer III. Because the labelling in these areas was extremely light a finer structure could not be clearly resolved.

2388 SOMATOSENSORY FUNCTION FOLLOWING LESIONS IN MIDBRAIN LEMNISCAL PATHWAYS.
Gabriel P. Frommer. Dept. Psychology, Indiana University, Bloomington, IN 47405

Although the consequences of lesions in somatosensory pathways of the spinal cord and in their projections in the forebrain have been investigated extensively, few data are available on the effects of lesions in the somatosensory afferent pathways in medial lemniscus and related midbrain structures. The present experiments investigated the effects of such lesions placed at sites from which brief, short-latency, contralaterally originating somatosensory evoked potentials could be recorded. The primary behavioral measure was performance on a series of roughness discriminations. Cats were required to press the rougher (or smoother) of two pedals to obtain food reinforcement. Rats had to choose the arm of a T-maze that had the rougher (or smoother) aluminum floor. Terminally, evoked potentials elicited by foreleg stimulation were recorded under barbiturate anesthesia from somatosensory cortical projections. Additional electrophysiological data were obtained from other cats prepared with electrodes chronically implanted at various sites in the somatosensory afferent pathways before and after midbrain lesions were made.

All five cats were capable of making the tactile roughness discriminations after anatomically verified major damage to lemniscal pathways in the midbrain. All four that survived long enough reached or exceeded preoperative levels of discrimination performance after extended testing. However, the terminal cortical evoked potentials were similar in amplitude, latency, and configuration to ones recorded from unoperated control animals. Evoked potential data from the chronically prepared cats showed that more extensive midbrain damage extending beyond the anatomically defined boundaries of lemniscal pathways were required to attenuate markedly the cortical somatosensory evoked potentials. Only one of six experiments comparing rats with midbrain lemniscal lesions to control rats demonstrated statistically reliable disruption of performance on roughness discriminations. Rats with lemniscal lesions relearned preoperatively mastered discriminations more slowly than did control animals. Rats also showed little if any effect of midbrain lesions on cortical somatosensory evoked responses. Lesions in the rat's medial lemnicus also produced a transient syndrome of hyperreactivity to tactile and auditory stimuli, postural and gait abnormalities, and aphagia which was apparently secondary to the motor deficits and hyperreactivity. Supported by NIH grants MH 10852 and 29204.

2389 PHYSIOLOGICAL SPECIALIZATION OF TRIGEMINAL NUCLEI? A COMPARISON OF STIMULUS-RESPONSE RELATIONSHIPS IN VIBRISSA-ACTIVATED NEURONS OF NUCLEI INTERPOLARIS AND ORALIS OF RAT. John M.Gibson, James D. Woodburn* and Wally I. Welker. Department of Neurophysiology, University of Wisconsin, Madison, Wisconsin 53706, USA.

The nuclei of the trigeminal complex exhibit cytoarchitectonic and projectional differences which suggest the possibility of differential processing of mechanosensory information. Physiological studies have, however, produced highly contradictory results regarding the issue of trigeminal nuclear specialization. We are exploring this issue through a broad-based methodology using an extensive battery of precisely-controlled stimuli in conjunction with a variety of quantitative analytic procedures.

Single-unit action potentials are recorded extracellularly with tungsten microelectrodes in the trigeminal complex of the barbiturate-anesthetized albino rat. Stimuli consist of quantitatively controlled deflections of single mystacial vibrissae. Data are collected and analyzed by means of digital computers.

The responses of units of nuclei interpolaris and oralis are qualitatively similar. (1) About half the receptive fields include more than one vibrissa. (2) Angular displacement and velocity thresholds range over three orders of magnitude. (3) Adaptation rates vary widely and are highly dependent on stimulus amplitude.

Quantitative methods, however, reveal several differences. (1) Receptive fields of interpolaris units tend to be smaller than those of oralis. The upper quartile receptive field size of interpolaris neurons is 4 vibrissae, compared with 6 for oralis. About 1/3 as many interpolaris neurons as oralis neurons have receptive fields which include more than 10 vibrissae or a patch of common fur. (2) Angular displacement and velocity threshold distributions of interpolaris neurons lie above those of oralis, although below those of first-order neurons. (3) Interpolaris neurons tend to adapt more rapidly than those of oralis. The adaptation index (defined for a step deflection of about 7^{o} as the ratio of firing rate during the third 100-msec. period of stimulation to that during the first 100-msec. period) was approximately zero (i.e., "rapidly adapting") for almost 2/3 of interpolaris neurons, compared with about half those of oralis. The upper quartile value of the adaptation index of interpolaris neurons was about 2/3 that of oralis neurons.

Although stimulus-response relationships in nuclei interpolaris and oralis are qualitatively similar, quantitative differences suggest physiological specialization for differential processing of mechanosensory information by these nuclei.

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2390 INTERACTION OF ACTIVITY IN FROG SKIN AFFERENT UNITS.
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Single axonal discharges in response to mechanical stimulation of two innervated spots of a dorsomedial skin 'touch' receptive field are recorded with a suction electrode from cutaneous nerve in decapitate spinally pithed frogs. The preparation is immersed in Ringer solution (pH=7.1, T=~21°C); the in situ orientation of skin to underlying tissues is maintained. Each mechanical stimulator consists of a 0.3 mm OD rounded tip glass rod glued to the cone of speaker driven over a range of voltages which yields proportional mechanical output. Unit identification criteria include: 1) no resting discharge; 2) rapid adaptation to a long (>50 msec) constant displacement to either spot; and 3) the single impulse evoked by a just threshold amplitude, 1 msec duration mechanical stimulus to one spot is abolished when preceded at >50 msec by a single impulse similarly evoked from the other spot, but can reobtain at the same interval with a suprathreshold amplitude stimulation. Interspot distances are >1500 µm.

The recovery cycle- tested 1/2-3 sec at one spot by conditioning with 1 impulse elicited from the other spot- declines continuously to the unconditioned threshold level in ~200 msec. However in other units, early (5-20 msec) and later (80-150 msec) discontinuities occur. Significantly, the same mechanical conditioning applied to an uninnervated region between the spots did not alter the threshold for mechanical impulse initiation at the test spot.

In other experiments, trains of 1 msec mechanical pulses are delivered 2-4/sec to one spot recurrently and to the other for ~3 sec periods randomly with respect to the recurrent train; each spot tested separately drives the parent axon 1:1. During simultaneous dual spot stimulation, the resultant impulse train resembles the combined sequential stimulus train, except for shorter interstimulus intervals corresponding to earlier periods of the recovery cycle.

These results illustrate two modes of activity interaction within the neural substrate of the receptive field: (1) RESET of threshold for impulse initiation from one spot by an antidromically elicited recovery cycle, and (2) MIXING of activity evoked from different peripheral sites.

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2391 THE RELATIONSHIP BETWEEN THE PATTERN OF COMMISSURAL TERMINATIONS AND THE MAPS OF SOMATOSENSORY CORTEX (Sm I and Sm II) IN THE GREY SQUIRREL. H. J. Gould, III. Dept. Anat., Univ. Cincinnati College of Medicine, Cincinnati, Ohio 45267.

Interhemispheric somesthetic connections were studied in the grey squirrel using the Fink-Heimer technique for degeneration. The total pattern of connections was correlated with the electrophysiological maps of Sm I and Sm II. In six squirrels the corpus callosum was sectioned through the medial wall of one hemisphere six days prior to mapping the contralateral somatosensory cortex. At the end of each recording session small reference lesions were made at known points within each map. The animals were then perfused and the tissue was flattened and sectioned parallel to the surface for analysis according to the technique of Welker and Woolsey ('74, J. Comp. Neur., 158: 437).

The densest degeneration is observed along the margins and in special zones within Sm I. This degeneration is related to the midline representations of the body including representations of the upper and lower lips, neck, trunk, genitalia and tail, while the representations of distal body parts including the vibrissae, corner of the mouth, forepaw and foot demonstrate little if any degeneration. Moderate degeneration is often seen related to representations of the proximal limbs. These data corroborate the observations of others that only midline and proximal limb structures are connected through the corpus callosum. In addition, dense degeneration is observed within the special unresponsive zone (UZ) of Sm I. This supports the prediction of Sur et al., ('78, J. Comp. Neur., 179: 425) that UZ is a major commissural recipient zone. In Sm II moderate degeneration is found to be related to both midline and distal body parts within the erect representation of the body (Nelson et al., '79, J. Comp. Neur., 184: 473). The central portion of Sm II is relatively free of degeneration. These results support recent physiological studies in the cat that suggest the existence of interhemispheric connections between representations of distal limbs in Sm II (Innocenti et al., '74, Exp. Brain Res., 19: 447; Robinson, '73, Exp. Brain Res., 18: 131).

It is therefore possible that callosal connections serve different functions in Sm I and Sm II since the dense interhemispheric connections in Sm I are related specifically to midline structures and the connections in Sm II are related to all body parts. It may be that the connections in primary sensory cortex, Sm I, are essential for midline fusion of the two halves of the body within the cortex while the connections in secondary sensory areas, Sm II, are more important in the processing of complex sensory discrimination. (Supported by National Science Foundation Grant BNS-81824).

2392 THE PYTHON AND THE RATTLESNAKE: A COMPARISON OF INFRARED TRIGEMINO-TECTAL PATHWAYS USING HORSERADISH PEROXIDASE AND CHOLINESTERASE TECHNIQUES. Edward R. Gruberg*, Eric A. Newman*, and Peter H. Hartline. (SPON: Daniel Kurtz). Eye Research Institute of Retina Foundation, Boston Ma. 02114 and Massachusetts Institute of Technology, Cambridge Ma. 02139.

Infrared receptors of pit viper and boid snakes project to a specialized primary trigeminal nucleus, the nucleus of the lateral descending trigeminal tract (LTTD). Previously, using horseradish peroxidase (HRP) techniques, we showed that the LTTD of the rattlesnake projects to the contralateral optic tectum via an intermediate nucleus in the ipsilateral ventro-lateral medulla, the nucleus reticularis caloris (RC). Now, using HRP, we have traced the LTTD projection to the tectum in the python (P. reticulatus). Following HRP injection into the intermediate layers of the tectum, the Mesulam benzidine blue method revealed large (35-70 μm in diameter) multipolar cells scattered throughout the main body of the contralateral LTTD. The smaller, more numerous LTTD cells were not stained. This demonstrates that, in contrast to the case in rattlesnakes, a direct LTTD to tectum connection exists in the python and shows that only the large LTTD cells project to the tectum. We stained python and rattlesnake (Crotalus viridis) brains for acetylcholinesterase by a two step cupric sulfate-ferricyanide method. In python, the large LTTD cells stained heavily for cholinesterase. The rattlesnake LTTD lacks cells over 25 μm in diameter. However, the cells of the rattlesnake RC showed heavy cholinesterase activity; these cells (25-45 μm in diameter) have a similar multipolar appearance as the large cells of the python LTTD. In both species, the large cells project to the contralateral tectum. We suggest that these large cells serve a similar function in the infrared systems of the two groups of snakes and may have a common origin. They differ in that they form a distinct nucleus in the rattlesnake but not in the python. Supported by NIH grants R01-EY01539, T32-EY07028, T01-EY00090, and by the Bell Telephone Laboratories.

2393 COLLATERAL BRANCHING OF ASCENDING SPINAL NEURONS IN MACAQUES. Nancy L. Hayes* and Aldo Rustioni, Depts. of Anatomy and Physiology and The Neurobiology Program, UNC, Chapel Hill, NC, 27514.

A double-labeling strategy has been employed in primates a) to identify simultaneously cells of origin of ascending spinal projections to the dorsal column nuclei (DCN) and to the thalamus, and b) to answer the question of whether at least some of these spinal neurons have axons which, by way of collateral branching, reach both targets. In adult monkeys (M. fascicularis) 10 μl of 30% HRP were injected unilaterally in the ventrobasal complex, and 0.5-1.0 μl (10-30 μCi) of tritiated, enzymatically inactive HRP (^{3}H-apo-HRP, New England Nuclear) were injected in the contralateral dorsal medulla. After 2 or 3 days animals were perfused with mixed aldehydes. 1-2 mm thick slabs through selected spinal segments were reacted in Hanker-Yates substrate for histochemical visualization of HRP, embedded in paraffin, sectioned at 15 μm, and processed for autoradiographic demonstration of ^{3}H-apo-HRP (Hayes and Rustioni, 1978).

Spino-thalamic neurons, labeled by HRP-positive granules, are found mainly in lamina I and in the lateral portions of laminae IV-VI throughout the side of the cord contralateral to the thalamic injection. Spino-DCN neurons are most numerous ipsilateral to the medullary injection, in medial portions of laminae IV-VI throughout cervical levels, but laterally, in the same laminae, in lumbar segments. At lumbar levels, spino-thalamic and spino-DCN neurons are also found along the lateral border of the ventral horn. Neurons in this location, labeled by either the thalamic or the DCN injection, are large (40-65 μm) and are indistinguishable from the spinal border cells described by Cooper and Sherrington (1940) as the source of the ventral spino-cerebellar tract in cats and monkeys. At lumbar levels, but not at cervical levels, some large dorsal horn neurons are labeled by both cytoplasmic HRP-positive granules and by reduced silver grains in the overlying emulsion. These double-labeled neurons are interpreted as having an axon which gives origin, presumably at spinal cord levels (Cajal, 1909), to collaterals ascending to both the ipsilateral DCN and the contralateral thalamus. Elucidation of whether and to what extent other ascending pathways to various supraspinal targets originate from common spinal neurons may provide new insights into the functional mechanisms of ascending spinal systems.

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2394 TOPOGRAPHIC ORGANIZATION OF THALAMIC NEURONS RESPONSIVE TO TEMPERATURE STIMULATION OF ORAL AND PERIORAL REGIONS OF THE CAT. H. Hirata, E.L. Auen, D.A. Poulos, and J.T. Molt, Dept. of Anatomy and Div. of Neurosurgery, Albany Med. Coll., Albany, N.Y. 12208.

The organization of thalamic neurons receiving trigeminal input arising from cutaneous mechanoreceptors has been the subject of many investigations. There have been fewer attempts to describe the organization of thalamic neurons responsive to cutaneous temperature stimulation. This report describes the spatiotopic organization within a zone of the cat thalamic nucleus ventroposterior medialis (VPM) that receives thermosensitive input from oral and perioral regions. The anatomic data reported here were acquired as part of an electrophysiological study of the response characteristics of single thalamic thermoreceptive neurons (see Poulos et al., this volume). Microelectrodes were stereotaxically placed into the thalamus of urethane-anesthetized cats and the responses of electrophysiologically isolated neurons considered to be thermosensitive were studied in detail. The size and shape of thermosensitive peripheral receptive fields were determined and electrolytic lesions were made to verify electrode placement. Cats were perfused with saline followed by 10% formol-saline, the brains were removed, blocked, frozen-sectioned at 25μ, and stained with either cresyl violet or thionin. Histological data are based on experiments completed in 14 adult cats. Reconstruction of histological sections combined with maps of thermoreceptive neuron depth within an electrode puncture, lesion sites, and neuronal peripheral receptive field size and location revealed a spatiotopic organization of thermoreceptive neurons within the dorsomedial zone of VPM. Neurons in the more rostral portions of this dorsomedial zone of VPM had small thermosensitive receptive fields (1-2mm^2) located on the ipsilateral lips, tip of the tongue, and nose. As the electrode was moved caudally within this zone the peripheral thermoreceptive fields appeared progressively larger (10mm^2), and were located on the more caudal portions of ipsilateral tongue. The medial to lateral spatiotopic organization within this zone was not as clearly defined as the rostral to caudal organization. The more lateral regions had ipsilateral receptive fields on the lateral edge of the tongue and lips and ventral surface of the tongue. The medial regions had ipsilateral receptive fields near the midline of the tongue and lips. A small area in the rostral medial border within the dorsomedial zone had small thermoreceptive fields located on the contralateral side of the tongue. A definitive spatiotopic organization was not found in the dorsal to ventral plane within the zone. While the trigeminal thermoreceptor organization in cat thalamus is not yet as well defined as that of mechanoreceptor input, it is clear that a spatiotopic organization does exist. Supported by NIH grant NS11384.

2395 ANATOMICAL DEMONSTRATION OF TRIGEMINAL AND SPINAL PROJECTIONS TO THE CAUDAL MEDULLA. Susan Hockfield and Stephen Gobel. Neurobiology & Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20205.

The grey matter of the caudal medulla (CM) between the obex and the spinal cord is made up of dorsal and ventral horns which are laminated much like the grey matter of the spinal cord. Our earlier work has shown that layers I, V and VI of the CM contain trigeminothalamic projection neurons that are likely to be involved in trigeminal pain pathways. Responses of layer I, V and VI neurons to noxious stimuli can be modulated by electrical stimulation of several CNS sites. In the present study, neurons that project to the CM from the cervical spinal cord and from the trigeminal nuclei are demonstrated by use of the retrograde transport of horseradish peroxidase (HRP). Unilateral injections of HRP were made in either the dorsal or dorsal and ventral horns of the CM of adult cats. Camera lucida drawings were made to determine the morphology and precise laminar or nuclear location of labeled neurons.

In the spinal cord, segments C6-C8 contain neurons that project to the CM ipsilaterally from layers I, IV and V and contralaterally from layers I and V. In C1-C3, neurons project ipsilaterally from all layers except layer II and contralaterally predominantly from layers I and V-VIII. Few labeled neurons are found in C4 and C5. Throughout the cervical spinal cord, layer II (the substantia gelatinosa layer) contains almost no propriospinal neurons.

In the CM, many labeled neurons are found contralaterally in layers I, IV, V, VII and VIII. Many labeled neurons in layers III and IV have apical dendrites that ascend into layers I and II. In layer VII, labeled neurons are found medially within the intermediate nucleus and laterally within the nucleus retroambiguous.

Most of the ipsilateral descending intratrigeminal projection neurons are found in trigeminal nucleus oralis. The other trigeminal nuclei contain far fewer labeled neurons and these lie in the medial part of the main sensory nucleus and in the dorsal part of trigeminal nucleus interpolaris.

This study shows that some ascending propriospinal neurons to the CM arise from layers which previously have been shown to contain long distance projection neurons, i.e., layers I, V and VI, suggesting that a single neuron may function as both a short intersegmental and as a long distance projection neuron. Trigeminal nucleus oralis is the major source of descending intratrigeminal neurons to the CM which is an important criterion for distinguishing it from the main sensory nucleus and nucleus interpolaris. The propriospinal and intratrigeminal projections demonstrated in this study must constitute an important source of axonal endings in the CM which may modulate the activity of trigeminothalamic projection neurons.

2396 SOMATOTOPIC ORGANIZATION OF DORSAL HORN PROJECTIONS OF CUTANEOUS NERVE TWIGS. H. Richard Koerber , and Paul B. Brown, Dept. of Physiology and Biophysics, West Virginia University Medical Center, Morgantown, WV 26506.

Brown and Fuchs (1975) proposed that the presynaptic cutaneous neuropil of the dorsal horn is somatotopically organized. We have examined projections of small cutaneous nerve twigs to cat dorsal horn. Peripheral cutaneous nerve twigs were cut and their proximal stumps were soaked in distilled water for 10 minutes. Then they were immobilized in plastic cuffs which had been sealed at one end and half-filled with 10% HRP, and the nerves were sealed in place by filling the rest of the cuffs with agar-saline. The cuffs were sutured to underlying muscle and the wounds were closed. After survival times of 2-5 days, the cats were perfused and the cords were processed according to the tetramethyl benzidine method of Mesulam (1978). The nerve twigs tested were: (1) a branch of the posterior femoral cutaneous nerve ($PFCN_3$) and (2) the lateral branch of the sural nerve (LSN). Cutaneous fields of these nerves were mapped before cutting and loading with HRP.

Central projections of these cutaneous nerves were consistent with the somatotopic organization of the dorsal horn. The $PFCN_3$ projects to L_6, L_7 and S_1, to a lateral position in the dorsal horn corresponding to the somatotopic representation of its innervation field (posterior aspect of distal thigh and calf), skirting more medial areas representing more distal skin. The LSN projects to L_7, nesting in a zone just medial to the $PFCN_3$, corresponding to the somatotopic representation of its innervation field (lateral foot, just distal to the $PFCN_3$ innervation). No other projections from these nerves were seen. All projections were to lamina I - dorsal lamina V.

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2397 HINDLIMB DERMATOMES OF CAT USING AVERAGING METHODS. David H. Kukulinsky* and Paul B. Brown. Department of Physiology and Biophysics, West Virginia University Medical Center, Morgantown, WV 26506.

Previous reports of cat hindlimb dermatomes are so contradictory that they cannot be used with any precision. For proper mapping of dermatomes, the following criteria must be met: (1) Stimuli must consistently activate cutaneous nerves but not nerves innervating deep receptors; (2) The dorsal root recording method must be sensitive enough to detect activity in small numbers of fibers, but insensitive to activity in other dorsal roots; (3) A means of quantifying the response must be used; (4) Mapping of response magnitudes must use a consistent means of representation. Previous studies have generally failed to meet these criteria.

Seventy standard loci on the hindlimb were tested, in five adult cats, using mechanical pulses (3 msec rise and fall times) applied to the mat of clipped fur. An amplitude of 20 μm was used, since this was demonstrated in single unit studies to activate hair and SAI afferents, but not deep receptors. Stimuli over 25 μm can activate deep receptors. Averages were obtained from dorsal roots L_4-S_1, sampling at 10,000/sec (each root) and averaging over 100 trials (each root) at each stimulus point. Baseline dc offset, $\bar{e}_{dc}$, was computed from a pre-response period of 25 msec.

$$\bar{e}_{dc} = \frac{1}{250} \sum_{i=1}^{250} \underline{s}_i ,$$

where $\underline{s}_i$ is the i^{th} sample point. The absolute-value noise level, $\bar{e}_N$, was computed from the same sample points:

$$\left| \bar{e}_N \right| = \frac{1}{250} \sum_{i=1}^{250} \left| \underline{s}_i - \bar{e}_{dc} \right| .$$

The response magnitude, $\bar{e}_R$, was computed from the next 250 sample points, which included the neural response:

$$\left| \bar{e}_R \right| = \frac{1}{250} \left[\left(\sum_{i=251}^{500} \left| \underline{s}_i - \bar{e}_{dc} \right| \right) - \left| \bar{e}_N \right| \right] .$$

Dermatomes were plotted as contour maps of response magnitudes on the hindleg, using three standard views for each dermatome. General locations agree with earlier reports, although individual reported dermatomes differ markedly from our results. There was little inter-animal variation.

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2398 ATTENUATION OF POST-CENTRAL RESPONSES TO PERIPHERAL INPUTS DURING ACTIVE MOVEMENT IN MONKEY. Y. Lamour*, H. Solis*, V. A. Jennings* and E. V. Evarts. Lab. Neurophysiol., NIMH, Bethesda, MD 20205.

Precentral motor cortex responses to peripheral stimuli are attenuated when stimuli are delivered during large active movements (Evarts, E.V. and Fromm, C., Neurosci. Lett. 5: 267-272, 1977). One of the structures which might be involved in this attenuation is the post-central sensory cortex, a region reciprocally connected to motor cortex by cortico-cortical fibers. This hypothesis was examined by recording post-central neuron responses to stimuli delivered at various phases of active movement.

Monkeys (Macaca mulatta) pronated or supinated a handle in order to maintain accurate positioning of the handle in a target zone defined by a visual display. Movements were similar in magnitude (20°) and in velocity (150°/sec) to the "ballistic" movements studied by Evarts and Fromm, but were different in that they were accurately terminated by the monkey rather than by an external "stop." Stimuli superimposed on these large active movements were changes in handle position (position pulses, 50 msec, 8-10°) delivered at different phases of the task: 100 msec after the visual cue triggering the active movements (but before any active displacement), during active movement, and during the holding period between active movements. In addition, we compared neuronal activity during unperturbed active movements with neuronal activity occurring during comparable passive movements induced by ramp displacements of the handle (150 msec, 20°).

Of 150 post-central neurons found to be sensitive to passive arm displacements, 30% showed a decreased response to position pulses delivered during large active movements (as compared to the responses to position pulses delivered during the holding period). For some neurons, responses to position pulses delivered immediately prior to onset of active movements were also decreased. Discharge during unperturbed active movements commonly differed from discharge during passive movements of similar extent and velocity. Usually the responses differed only quantitatively in amplitude and/or time course, but in some cases opposite sorts of responses were observed (e.g., excitation during active movement and no response or inhibition for the same direction of passive movement).

These results provide evidence for modulation of the responsiveness of post-central cortex neurons during active movement, and while not excluding modulation of peripheral input at subcortical levels, raise the possibility that post-central cortex is responsible, in part at least, for the modulation of peripheral inputs to motor cortex during active movement.

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2399 CHANGES IN THE SENSITIVITY OF CUTANEOUS MECHANORECEPTION DURING DEVELOPMENT AND AGEING. J. Leon* and A. J. McComas. Dept. of Neurosciences, McMaster Univ. Med. Ctr., Hamilton, Ontario, Canada. L8S 4J9

The sensitivity of type I slowly adapting cutaneous mechanoreceptors, 'touch domes', changes over the course of development. This change is mediated by two mechanisms. The first is a reduction in the density of touch domes per unit skin area during growth. The number of marked touch domes in the skin of groups of rats of different ages and in the skin of individual rats during their development was counted. Touch dome density is significantly lower in rats after weaning and continues to decrease with increasing age. From repeated observations on the number of touch domes within a given nerve field during development it appears that the observed changes in touch dome density can be accounted for entirely by growth of the skin receptive field of the nerve with the maintenance of a constant number of touch domes.

In addition to their density in the skin touch domes appear to alter their sensitivity to pressure as they age. The second mechanism involves an increase in threshold to mechanical stimulation as the animal reaches old age. The threshold of the touch domes was tested using a mechanical prodder which was monitored on line with a photoelectric cell that was previously calibrated to detect prodder movement. Responses were recorded from the third lumbar dorsal cutaneous nerve. The touch domes of juvenile rats (30-45 days old) are most sensitive to mechanical stimulation. The threshold of the touch domes increases with increasing age. The threshold of touch domes in aged rats (over 6 months old) is significantly higher than the threshold of touch domes in adult rats (45 days to six months old). With decreasing density and increasing threshold, the rat progressively loses tactile sensitivity through these receptors over the course of its life.

2400 COINCIDENCE OF FUNCTIONALLY, ARCHITECTURALLY, AND CONNECTIONALLY DEFINED BOUNDARIES IN S-II/r OF MACAQUES. P. R. Loe, B. L. Whitsel, and A. Rustioni, Depts. Physiol. and Anat., U. of N. C., Chapel Hill, N. Car. 27514.

Mountcastle and Powell's demonstration that a systematic relationship exists between structure and function in somatic sensory cerebral cortical organization has been confirmed repeatedly by other workers investigating the organization of the postcentral cortex in primates. Prominent cytoarchitectonic boundaries also exist within the parietal cortex occupying the superior bank of the Sylvian sulcus in macaque monkeys (Macaca mulatta and Macaca fascicularis), but their functional significance is uncertain. The objectives of the present study were (i) to establish criteria for identification of certain prominent transitions in cytoarchitecture within the rostral part of the second somatic sensory cortical projection field (S-II/r), and (ii) to obtain neurophysiological data that would provide information about the functional meaning of these transitions. In neurophysiological mapping experiments, we determined the receptive field locations and response properties of single neurons isolated during microelectrode penetrations that crossed the boundaries of interest. The information obtained was plotted along the electrode tracks identified in serial sections from each experimental brain. The boundaries between the hand representation and the neighboring representations of proximal body regions (arm and face) in S-II/r coincide with clearly visible cyto- and myeloarchitectonic boundaries. (In most oblique or coronal sections through S-II/r, there are at least two such boundaries, corresponding to the superior and inferior boundaries of the hand representation.) To obtain additional support for the existence of transitions in cortical architecture at the boundaries of the hand representation in S-II/r, the cortico-cortical connections of the regions in question were studied. Contralateral connections were identified by injections of HRP into functionally characterized (by single-neuron recordings) regions of the contralateral S-I and S-II/r; ipsilateral connections were identified by HRP injections placed in functionally identified regions of the ipsilateral S-I. The tissues were processed using the Hanker-Yates procedure. Analyses of the distribution of labelled cells revealed that (i) while neurons within the hand area of S-II/r do not send axons to the opposite S-I or S-II/r, many neurons in this area send axons to the ipsilateral S-I hand representation; and (ii) the boundaries of the acallosal S-II/r area determined by retrograde labelling correspond precisely with the boundaries of the hand area as determined neurophysiologically. (Supported by NS10865, DE02668, RR05333, and NS11737.)

2401 LOCALIZATION OF LARYNGEAL AFFERENTS IN THE CAT NODOSE AND CERVICAL SYMPATHETIC GANGLIA AS DETERMINED BY HRP AND FLUORESCENT DYE TRACER TECHNIQUES. G.E. Lucier and J.O. Dostrovsky. Faculties of Dentistry and Medicine, University of Toronto, Toronto, Canada M5G 1G6.

Previous electrophysiological studies (Mei, N. Exp. Brain Res. 11: 465, 1970; Sessle, B.J. Brain Res. 53: 333, 1973) have suggested that the superior laryngeal nerve (SLN) cell bodies are mainly concentrated in the region of the entrance of the SLN to the nodose ganglion. In order to determine anatomically whether there is a specific localization within the ganglion of SLN cell bodies axonal transport techniques employing fluorescent dyes and horseradish peroxidase (HRP) were used. In adult cats anesthetized with ketamine approximately 1 µl of 2.5% 4', 6-Diamidino-2-phenyl indol·2HCl (DAPI) - 10% Primuline in saline was injected into the right SLN, the nerve crushed at the injection site, and the animals allowed to recover. Four to five days later the animals were perfused with 10% buffered formalin and the nodose and superior cervical ganglia (SCG) removed. Frozen sections of the ganglia were cut at 30 µ and mounted on untreated slides. Sections were observed under a fluorescent microscope using a 360 nm excitation wavelength (van der Kooy, D. et al. Brain Res. 158: 189, 1978). Cell bodies containing DAPI-Primuline could be easily distinguished from the dark background by their blue fluorescence. Labelled cells were maximally concentrated at the rostral end of the nodose ganglion and extended caudally along the border of the SLN entrance region. In the extreme rostral end single and small groups of labelled cells were seen surrounded by axons of the vagus nerve. Fluorescent cells were also present in the SCG and although more sparse and scattered tended to be localized unilaterally along the periphery of the ganglion. Injection of HRP into the laryngeal wall resulted in a similar distribution of HRP-labelled cell bodies in the nodose ganglion. These studies indicate that the cell bodies of SLN are localized within specific regions of the nodose and cervical sympathetic ganglia.
(Supported by NIH and Canadian MRC).

2402 SOMATOTOPIC ORGANIZATION OF TRIGEMINAL SENSORY NUCLEI. Carl F. Marfurt* (SPON: Louis Misantone). Department of Anatomy, Temple University, Philadelphia, Pennsylvania 19140.

Central connections of sensory trigeminal nerve branches were identified in the cat trigeminal brainstem complex (main sensory and spinal trigeminal nuclei). In separate animals, the frontal, infraorbital, or mental nerve was cleanly transected and anchored in HRP-filled microtubing (50%, Boehringer-Mannheim). Forty-eight to ninety-six hours postoperatively, the animals were perfused according to the methods of Rosene and Mesulam (JHC, 1978). The tissue was reacted with tetramethylbenzidine (TMB), mounted on slides, and viewed, unstained, under darkfield microscopy for the presence of HRP reaction product.

Retrograde transport of HRP in transected fibers of the frontal, infraorbital, and mental nerves, respectively, labeled parent cell bodies in the anteromedial, intermediate, and posterolateral regions of the ipsilateral trigeminal ganglion. Transperikaryonal transport of HRP was detected within the central processes of these primary trigeminal neurons and was used to trace their terminations in the trigeminal brainstem complex. These central processes terminated somatotopically in the main sensory and spinal nuclei, with the mental nerve fibers terminating most dorsally, frontal fibers most ventrally, and infraorbital fibers primarily in an area between the latter two. The infraorbital nerve fibers were present in greater numbers and projected in modest amounts to the dorsal and ventral areas of the main sensory and spinal nuclei.

Labeled fibers from all three nerves terminated throughout the rostrocaudal extent of the trigeminal brainstem complex. However, fibers terminated in greater numbers in the main sensory nucleus and the rostral part of pars caudalis. Labeled processes or terminals could be found in the marginal, substantia gelatinosa, and magnocellular layers of pars caudalis but were most numerous in the magnocellular layer. Few labeled fibers could be identified below the first cervical segment.

Individual fibers entering the trigeminal sensory nuclei were occasionally observed coursing within intranuclear fiber bundles. However, the majority of entering fibers were seen in relation to, and presumably terminating in, areas containing nerve cell bodies. No labeled fibers were seen passing to the contralateral trigeminal complex.

Central connections of other branches of the trigeminal nerve are currently under investigation in this laboratory in an effort to refine our knowledge of somatotopic organization in the trigeminal brainstem complex. (Supported by Biomedical Research Grant RR054)

2403 FUNCTIONAL PLASTICITY IN THE CAT SOMATIC SENSORY-MOTOR CORTEX (SmI) DURING REVERSIBLE EPIDURAL ANESTHETIC BLOCKS. Jacqueline Metzler, Philip S. Marks* and Joel Ira Franck*[1]. Dept. Neurosurgery, Yale Univ. Sch. Med., New Haven, CT 06510.

The extracellular responses of single units in SmI of 4 awake, paralyzed, adult cats under N_2O analgesia were examined before, during and after short-term, reversible epidural anesthetic (bupivacaine) blocks of the L4-L5 dorsal roots. Chronic recording chambers were implanted over the left SmI and units were isolated in the rostral-medial region, the region receiving afferent projections from the contralateral upper hind limb. The somatotopic representation and response properties of the 213 units sampled in the control preparations were similar for all 4 cats and in general agreement with the results of other studies.

The 106 units studied during the subsequent blocking experiments all had receptive fields (RF's) on the upper right hind limb. Of these, 59 (56%) failed to respond to any mechanical peripheral stimulation while the epidural anesthetic was in effect. Most of these latter units were activated by cutaneous stimulation (hair movement or light touch) before and after the blocks. The remaining 47 units (44%) continued to respond during the anesthetic blocks, but with new RF's and, sometimes, to different subclasses of stimulation. These new RF's usually appeared on the abdomen or foot within 15 to 20 min after the injection of the anesthetic. Three cells had double RF's with separate excitatory fields on the foot and abdomen. The pre-block characteristics of each unit returned within 2 to 4 hrs as the epidural anesthetic wore off. Mechanical stimulation of the area of the new RF did not influence the cell's activity before and after the blocks were in effect. The response properties of the cells driven by stimulation of new body regions during the blocks differed from the pre- and post-block characteristics in several ways: (i) the responses frequently habituated to repetitive (>1/sec) stimulation; (ii) modality specificity was not always preserved; i.e., units initially classified as "cutaneous" sometimes became "deep", requiring strong mechanical stimulation to drive them; and (iii) RF sizes and shapes were not as stable and uniform.

The immediacy and reversibility of the observed effects rule out sprouting or other growth processes as an explanation. They do suggest that afferents from adjacent body areas exist in close proximity to a particular cell but that only some of these afferents are functionally effective in the unblocked state. An analogous explanation has been offered for similar effects observed in the spinal cord and dorsal column nuclei (Wall, 1977).

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2404 FUNCTIONAL CHARACTERISTICS OF NEURONS IN THE TRIGEMINAL NUCLEUS CAUDALIS-CERVICAL DORSAL HORN TRANSITION ZONE. S.G. Nord and D.E. Rolince*. Dept. of Neurology, Upstate Med. Ctr./SUNY, Syracuse, N.Y. 13210.

We demonstrated previously in the cat that afferents from the three trigeminal divisions and from the C_2 and C_3 dermatomes project to a single population of neurons in the dorsolateral gray matter of the medullary-spinal cord transition zone (Nord, S.G. et al , Neurosci. Abstr., 1978, 4: 556). The present experiments were undertaken to extend these findings. A region of the zone extending from 5.1-8.5 mm caudal to the obex was explored in the monkey for neurons which responded to mechanical stimulation of the face and body. In general, microelectrode penetrations were made at, or just rostral to, levels at which the C_2 dorsal roots could be seen entering the spinal cord. The locations of selected cells were marked electrolytically. The great majority of the neurons were localized histologically in laminae IV-VI. Although some responded only to noxious stimulation and others to light touch, more (63%) were of the "wide dynamic range" type, responding differentially to variations in stimulus intensity. The majority of the neurons (62%) had receptive fields which lay entirely within the NV distribution although the proportion of such units decreased with distance from the obex. These fields were innervated predominantly (89%) by afferents from the ophthalmicand maxillary divisions and all but one were restricted to the intermediate and peripheral zones of the classical onion-skin facial pattern. An additional 29% of the neurons had fields which consisted of contiguous trigeminal and cervical areas. These usually consisted of either ophthalmic division-dorsal C_2 or mandibular division-ventral C_2/C_3 combinations. A relatively small proportion of cells (9%), which increased with distance from the obex, had exclusively segmental fields. These typically were comprised of portions of the C_2 and/or C_3 dermatomes. No sharply defined modality or somatotopic organization was observed within the region explored. Our results reveal that a substantial, loosely organized pool of neurons in the monkey nucleus caudalis-cervical dorsal horn transition zone receives projections from both upper cervical and (predominantly) posterior trigeminal regions. They also confirm our previous findings in the cat and they support our proposal that this neuronal pool mediates an overlap of trigeminal and cervical sensation which is seen clinically in man and which has been demonstrated experimentally in the monkey.

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2405 THALAMO-CORTICAL INTERCONNECTIONS OF THE SOMATIC FIELD OF THE PROSIMIAN, GALAGO SENEGALENSIS. G.R.Penny* and R.S.Nowakowski* (SPON: I.T. Diamond). Dept. Psychol., Duke Univ., Durham, NC 27706.

The neocortex of Galago can be divided into three sensory fields, visual, auditory and somatic (Diamond, 1979), each of which can be subdivided into several cytoarchitectonic areas. The somatic field consists of Brodmann's area 4, a premotor area, a koniocortical area, and a belt between area 4 and the koniocortex. We have studied the thalamocortical and corticothalamic projections of single subdivisions of the somatic field by making small iontophoretic injections of horseradish peroxidase (HRP) or a mixture of tritiated leucine and proline.

The principal finding is that the labeled cells or terminals in the thalamus are distributed in bands which cross nuclear borders. The shape and position of the bands are a function of the cortical area injected. Each pattern involves at least one of the traditional relay nuclei (ventral lateral or ventral posterior) and extends into one or more of the intralaminar nuclei (central medial, central intralaminar, central lateral, centre median, paracentral or parafascicular). In many cases the band extends rostrally into ventral anterior and/or caudally into the posterior group. For example, injection of HRP into the face area of koniocortex leads to a band of labeled cells beginning rostrally in VPi and VPM and continuing dorsocaudally through VPM and VPs into CIN and Po. One branch extends from the main band medially through VM into Pc and Ce. A similarly placed amino acid injection shows that the thalamocortical and corticothalamic projections are reciprocal. Injection of HRP into area 4 labels a band of cells beginning rostrally in VL and extending caudally into rostral VPL and the intralaminar nuclei. One branch extends medially along the ventral surface of the ventral group into the zona incerta. The banding pattern produced by injection of tritiated amino acids into area 4 is remarkably similar, except that the band contains areas of heavy label in VL and CIN which are connected by thinner regions of lighter label. HRP injections confined to either the superficial or deep layers of somatic cortex and injections of HRP or tritiated amino acids into the thalamus show that different nuclei in the somatic thalamus receive from and project to different layers of the cortex. For example, VP receives fibers from layer VI and projects to layer IV, whereas CM receives fibers from layer V and projects to the layers superficial to IV.

These results confirm the generalization, which has emerged from similar studies of the auditory and visual fields, that each cortical area receives fibers from and projects to several thalamic nuclei and that the layer of origin of the corticothalamic projection and the layer of termination of the thalamocortical projection are distinct for different thalamic nuclei.

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2406 RESPONSE OF THALAMIC NEURONS TO THERMAL STIMULATION OF ORAL-FACIAL REGIONS IN THE CAT. D.A. Poulos, E.L. Auen, H. Hirata and J.T. Molt, Dept. of Anatomy and Div. of Neurosurgery, Albany Medical College, Albany, N.Y. 12208.

Recordings of extracellular discharges in response to cooling of trigeminal peripheral receptive fields (PRFs) were obtained from neurons located within the thalamic nucleus ventroposterior medialis (VPM) in urethane-anesthetized cats. Two types of thermally responsive neurons were encountered. One type (T units) had thermal response characteristics identical to those of "typical" specific cold units identified in peripheral nerve and in our earlier studies of the marginal zone in the cat spinal trigeminal nucleus. T units, (1) displayed ongoing temperature dependent activity over a wide range of temperatures held constant (45-9°C), (2) responded to each of a series of rapid cooling steps below 35°C with a dynamic increase in rate of firing, (3) responded to step-like changes in the warming direction with a transient decrease in activity and (4) were insensitive to mechanical deformation of their PRFs. The second neuronal type (T+M units) had thermal response characteristics that were indistinguishable from those of specific cold units but displayed in addition, a responsiveness to mechanical deformation of their PRFs. The mechanical sensitivity of T+M units varied from those requiring only light brushing of their PRFs (which were contiguous with their thermal fields) to those requiring firm pressure or stretch. While over 100 thermally sensitive cells were identified, 39 units (25 T, and 14 T+M) were held for sufficient time to allow detailed study. 80% of the cells within the zone of VPM explored had ipsilateral PRFs. Most PRFs were small (1-2mm^2) with the exception of those located on the proximal surface of the tongue which measured up to 3 x 10mm. Response latencies to percutaneously applied electrical stimuli averaged 16.6 msec consistent with an A delta fiber input. Two results obtained in the present study differ from our observations made within the marginal zone of spinal V. First, the thalamic T units all had "typical" response profiles whereas 3 types of specific T units were seen in medulla e.g., typical units, primarily static, and primarily phasic units. It is possible that the primarily static and primarily phasic medullary unit types project either to a region of thalamus not explored and/or to other CNS structures. Secondly, the T+M units described here were not observed in medulla. They may be located within a medullary region not explored or alternatively, thalamic T+M units may represent a convergence of specific thermal and specific mechanical inputs at the thalamic level. The thalamic neurons described in the present study appear capable of providing precise and specific information about the extent and location of facial skin cooling. Supported by NIH grant NS11384.

2407 EFFECTS OF MECHANICAL STIMULUS FORCE ON DISCHARGE OF RACCOON GLABROUS SKIN SLOWLY ADAPTING CUTANEOUS MECHANORECEPTORS. Benjamin H. Pubols Jr., Christine H. Maliniak*, and Ann F. Corson*. Department of Anatomy, College of Medicine, Pennsylvania State University, Hershey, Penna.

When a mechanical stimulus produces a constant displacement of raccoon glabrous skin, concommitant reactive force reaches its peak just as the controlled static displacement is achieved, and then declines rapidly to approach a steady level after 20-40 sec. In contrast, when a constant force is applied, skin displacement may continue to increase over a period of several minutes. Upon stimulus retraction, several minutes are also required for the skin to return to its original resting position. Skin displacement is adequately described as a power function of applied force, with exponents < 1.0, over the force range 1-20 g, both initially and 20 sec after static force onset. Within this force range, displacements of 50 to more than 2000 μ are generated.

To study the role of force in determining slowly adapting (SA) mechanoreceptive afferent fiber discharge, individual median nerve fibers were isolated by microdissection in 10 sodium pentobarbital anesthetized raccoons.

The median absolute force threshold was 560 mg (range = 70-1270mg), while the median absolute displacement threshold was 20 μ (range = 5-185 μ). Absolute force and displacement thresholds were positively correlated (N = 27; P < .01).

For most moderately slowly adapting (MSA) and very slowly adapting (VSA) units (see Pubols, B., and Pubols, L., J. Neurophysiol., 1976, 39, 773-787), the decline in discharge rate over time (adaptation) is less with constant static forces than with constant static displacements.

Static forces of 1-20 g were applied at a constant rate of 10 mg/msec, and discharge rate was analyzed during the measurement period 100-500 msec following static force onset. Linear, logarithmic, and power functions were fit to the stimulus-response data of 10 MSA and 6 VSA units. For a majority of MSA units a power function (exponents between 0.62 and 1.23) provided the best fit (highest r), while for a majority of VSA units, a logarithmic function provided the best fit.

Results of the present investigation indicate that static discharge of glabrous skin slowly adapting cutaneous mechanoreceptors is in part dependent upon the viscoelastic properties of skin, and suggest that force may be a more potent variable than displacement in determining the behavior of such receptors. (Supported in part by research grant NS-13418, USPHS).

2408 SYMPATHETIC MODULATION OF SENSITIVITY IN CAT CUTANEOUS MECHANORECEPTORS. William J. Roberts and Joseph P. Pierce*. Neurological Sciences Institute, Good Samaritan Hospital & Medical Center, Portland, Oregon 97209.

Sympathetic efferent control of afferent activity has been studied in two classes of cutaneous mechanoreceptors in anesthetized cats; "type II" receptors and G hair receptors. Single units were functionally identified and then stimulated mechanically with a solenoid-driven probe to establish a baseline response. During this mechanical stimulation the sympathetic trunk was stimulated electrically and any sympathetic influence recorded either as a change in activity unrelated to a constant stimulus amplitude or as a change in stimulus intensity required to maintain a constant level of activity.

Type II receptors most commonly showed a marked increase in tonic discharge but no change in sensitivity to mechanical stimuli. This increased activity was often maintained throughout 3 min of sympathetic stimulation, although some variation was seen between units. Sympathetic-induced changes in the sensitivity of G hair receptors were related to the velocity sensitivity of individual units. The most rapidly-adapting (G_1) were generally desensitized, while the least rapidly-adapting (G_2) were sensitized. Intermediate units (G_I) were least affected. The measured changes in sensitivity of affected units were commonly on the order of 10-30% of the unconditioned threshold, although some were much larger. Sympathetic activity thus appears to result in a shift in the sensibility of G hair receptors, enhancing the detection of slower hair deflections relative to rapid deflections. As with most of the "special" senses previously tested, cutaneous sensibility in mammals is now demonstrated to be subject to efferent control.

2409 DIENCEPHALIC PROJECTIONS OF THE PONTINE RETICULAR FORMATION. Richard T. Robertson. Department of Anatomy, College of Medicine, University of California, Irvine, CA., 92717.

Ascending projections of the pontine reticular formation were studied in the cat with autoradiographic techniques. Small injections of ^{3}H-leucine were placed in either the rostral (n. reticularis pontis oralis) or caudal (n. reticularis pontis caudalis) divisions of the large celled pontine reticular formation. Pathways of ascending fibers and patterns of termination were studied in sections cut in the transverse or sagittal planes.

Efferent projections from both rostral and caudal pontine regions ascend to the caudal diencephalon and appear to bifurcate into two branches, as has been described previously. A dorsal branch terminates in the intralaminar nuclei of the dorsal thalamus, and a ventral branch terminates in the subthalamic region. The relative densities of these two general regions of termination, however, vary with the injection site. Fibers from the rostral pons project primarily beneath the dorsal thalamus, and terminate throughout the zona incerta, the fields of Forel, and in the ventral part of the thalamic reticular complex. Substantially fewer labelled fibers appear to terminate in the dorsal thalamus, but terminal sites include the centre median, parafascicular, paracentral, central medial and central lateral nuclei. Termination is prominant along the dorsal border between the dorsal medial and central lateral nuclei; the terminal field includes both nuclei.

Projections ascending from caudal pons, in contrast, terminate relatively heavily in the dorsal thalamus and sparsely in the subthalamic region. Termination sites in the dorsal thalamus include the centre median, central lateral, paracentral, and central medial nuclei. Most prominant termination occurs in the dorsal border zone of the dorsal medial and central lateral nuclei. Relatively few fibers project beneath the dorsal thalamus, but light termination is seen in the zona incerta, fields of Forel, and the ventral part of the thalamic reticular complex.

These data demonstrate that both rostral and caudal pontine reticular nuclei project to the thalamic intralaminar nuclei and the subthalamic region, but the relative densities of termination in each of these regions differ. Thus, these data suggest that the classical dorsal and ventral branches of the ascending reticular projections have largely separate origins.

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2410 ULTRASTRUCTURAL CHARACTERIZATION OF NORADRENERGIC AXONAL ENDINGS IN LAYERS I AND II OF THE DORSAL HORN OF THE MEDULLA. M.A. Ruda, B. Allen* and S. Gobel. NAB, NIDR, NIH, Bethesda, MD 20205.

Recent studies have drawn attention to the role of monoamines in the modulation of the response of nociceptive neurons in the dorsal horn. Previous experiments in our lab have characterized serotonergic (5HT) axonal endings in layers I and II of the dorsal horn of the medulla (DHM). The purpose of the present experiments was to similarly identify noradrenergic endings. Our approach accesses the ability of neurons which use NE as a neurotransmitter, to take up tritiated norepinephrine ([^{3}H] NE) at their axonal endings. Adult cats, pretreated with a monoamine oxidase inhibitor, were anesthetized with sodium pentobarbital and [^{3}H] NE (10^{-5}M) topically applied onto the DHM for 1 hr. In one experiment, 2 hrs prior to the [^{3}H] NE application, a serotonin neurotoxin (10^{-5}M 5,6 dihydroxytryptamine (5,6 DHT)) was topically applied for 1 hr. 5,6 DHT causes degeneration of 5HT axonal endings. Thirty min after application of the [^{3}H] NE, the cats were perfused and the DHM processed for autoradiography. Analysis of light microscopic autoradiographs demonstrated an accumulation of [^{3}H] NE in layers I and II. In EM autoradiographs, two categories of labeled axonal endings were identified: dome-shaped endings which form synapses on a single postsynaptic structure and scalloped endings which form synapses on several postsynaptic structures. These categories were further divided into five types based on morphological criteria. The same five types of labeled endings were observed in the experiment combining 5,6 DHT and [^{3}H] NE and in the experiment in which only [^{3}H] NE was used as a marker. Three types of dome-shaped endings were found in both layers I and II and were distinguished by the presence of either pleomorphic, small oval or large oval agranular vesicles. They formed mainly asymmetrical synapses on dendritic spines and shafts and occasional axosomatic synapses. In several cases, the same dendrite received synapses from both a degenerating 5HT ending and an [^{3}H] NE labeled ending. One type of scalloped ending was found both in layers I and II and contained large oval agranular vesicles while a second type was found only in the deeper part of layer II and contained small oval agranular vesicles. Both scalloped endings formed asymmetrical synapses on dendritic shafts and spines. The presence of [^{3}H] NE labeled axonal endings in layers I and II of the DHM suggests that NE afferents have access to both the projection neurons in layer I and the interneurons in layer II. The observation of both 5HT and NE endings synapsing on the same dendrite further suggests that both monoaminergic systems modulate the output of layer I projection neurons through similar mechanisms.

2411 COLLATERAL BRANCHING OF CORTICAL NEURONS TO SPINAL CORD AND MEDULLA IN CAT AND RAT. Aldo Rustioni and Nancy L. Hayes, Depts. of Anat. and Physiol., and The Neurobiology Program, UNC, Chapel Hill, NC, 27514. (Spon: R.A. King)

Collateral branching of cortical neurons projecting to the dorsal column nuclei (DCN) and spinal cord has been investigated with a double-labeling technique, using HRP and ^{3}H-apo-HRP as distinguishable retrograde tracers (Hayes and Rustioni, 1978). In cats and rats, one tracer was injected in the brachial enlargement of the spinal cord, and the other tracer was injected in the ipsilateral dorsal medulla. In rats, cervical spinal injections through the dorsal columns, in which the corticospinal tract travels, presumably allowed retrograde labeling of corticospinal neurons projecting to both cervical and lumbar levels, while in the cat the same injection avoids damage to corticospinal fibers descending to lower levels in the dorsal part of the lateral funiculus. Therefore, in one cat the spinal injection was made in the lumbar enlargement. All animals were sacrificed by perfusion with mixed aldehydes after 48 hours. One mm thick slabs through the sensorimotor cortex were processed with Hanker-Yates substrate for histochemical visualization of HRP, embedded in paraffin, sectioned at 15 um, and processed for autoradiographic demonstration of ^{3}H-apo-HRP.

In the rat, corticospinal neurons were concentrated in layer V in a band which extended approximately 7 mm along the dorsomedial aspect of the contralateral hemisphere (areas 3,4,6 and 7 of Krieg). Neurons labeled by injections in the dorsal column and trigeminal nuclei were found throughout layer V along a similar rostro-caudal extent and were most numerous in more lateral parts of the hemisphere. While the locations of the cells of origin of these two descending pathways were, for the most part, segregated (in part due to the large face representation in the lateral hemisphere of the rodent), many neurons double-labeled by both injections were consistently found intermingled with corticospinal neurons throughout their extent. That is, in regions where corticospinal and corticomedullary neurons are intermixed, the majority of corticomedullary neurons also project to the spinal cord by way of collateral branching.

In the cat, neurons labeled by the medullary and spinal injections were layer V pyramids located throughout all cytoarchitectonic subdivisions of the somatosensory cortex, with a completely overlapping distribution. However, neurons with collateral projections to both spinal cord and medulla were relatively sparse and were observed only in areas 3 and 4.

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2412 CHANGES IN MOTOR-SENSORY CEREBRAL CORTEX CELLS FOLLOWING SYSTEMIC NALOXONE INJECTION. M.Y. Spiegelstein* and C.F. Tyner, Dept. Medical Neurosciences, Walter Reed Army Inst. Res., Wash DC 20012.

We studied single neurons extracellularly in forepaw postcruciate (4ℊ) cortex in chloralose-anesthetized cats and held blood gases normal. Cells were hunted in some cats with 30 mA, 1/sec shocks alternating between the central footpads of the forelimbs, and in others with innocuous mechanical forelimb stimuli. Cells were classed as S (small, contralateral forelimb [CF] receptive fields), M (whole-body fields), or I (responsive to body areas away from the CF topographic focus but either unresponsive to all CF stimuli or responsive to weak but not strong CF stimuli [Tyner and Miller, 1977. Neurosci. Abstr. 3:72]). S/M/I ratios in a reference sample of 650 cells were 9/8/3.

In a new cell sample of 90, S/M/I ratios of 1/17/2 were found after i.v. naloxone injection; 11 cells were studied before and after naloxone injection, and the remainder hunted and classified after injection. Within 0.5-2.0 min. of injection, electrically-determined CF thresholds of S neurons began to decrease, up to 50%, and responses to electrical or innocuous stimulation of other limbs began to emerge. S cells thus changed to "M" were held for up to 1.5 hrs. without reverting to the small-field configuration. I cells initially responsive to weak but not strong CF stimuli acquired responsiveness to strong CF stimuli after naloxone, becoming indistinguishable from M cells; when hunting in cats previously given naloxone, these I cells were not found. I cells initially unresponsive to all CF stimuli remained so after naloxone injection, but the powerful inhibition of other inputs to these cells, evoked by CF stimuli, was greatly reduced in amplitude and duration. We have used naloxone doses of 0.4-1.0 mg/kg principally, but have seen effects, including S to "M" conversion, with doses as low as 0.1 mg/kg.

After correcting for microelectrode sampling bias (Towe and Harding, 1970. Exp. Neurol. 29:366), a pericruciate 4ℊ cell sample contains mostly S neurons; we conclude that most 4ℊ S cells probably receive sensory input from the entire body surface. The mechanism for our result may be similar to that for the finding of Satterthwaite et al (1978. Exp. Neurol. 60:603) that 70% of pericruciate S cells receive some type of subthreshold influence from the rest of the body. We are unsure whether naloxone-induced S to "M" conversions will occur in post-dimple SI where those authors found subthreshold influences on only 25% of S cells. It is also unclear whether the unmasking of wide-field input by naloxone should be interpreted in the context of pain/stress and the antagonism of endorphins, or whether it must be regarded as a less specific release from inhibition, perhaps reflecting an interaction with chloralose.

2413 MAGNIFICATION, RECEPTIVE FIELD AREA AND "HYPERCOLUMN" SIZE IN SOMATOSENSORY CORTEX OF THE OWL MONKEY. M. Sur*, M.M. Merzenich*, and J.H. Kaas. Departments of Psychology and Anatomy, Vanderbilt University, Nashville, Tennessee and Coleman Memorial Laboratory, *University of California, San Francisco, California.*

Several features of the two complete and separate body surface representations within Areas 3b and 1 of owl monkeys (Merzenich et al., J. Comp. Neur., 181: 41, '78) have been quantified. First, magnification factors have been calculated for the two representations. The magnification factors for the same body part in both representations are similar, with somewhat greater magnifications for most body parts in Area 3b. Within each representation, cortical magnification varies greatly across different body regions. For example, both the glabrous hand and foot representations occupy nearly 100 times more cortical tissue per unit of body surface area than either the trunk or upper arm representations in both Areas 3b and 1. Within the representations of the hand digits inverse magnification is linearly related to distance from the digit tip. By interpreting the magnification function for the digits as the Jacobian of the mapping of the digits on cortex, an equation describing the mapping is derived. Second, receptive field size has been shown to be inversely related to magnification factor. Thus, mean receptive field sizes are nearly 100 times larger for the trunk than for the glabrous hand, and receptive fields are generally larger for the same body part for Area 1 than for Area 3b. Specifically, within the hand representations in Areas 3b and 1, receptive field areas over the digit phalanges vary linearly with inverse magnification. Thirdly, receptive field overlap has been related to cortical distance between recording sites. Measurements of the percentage of overlap of pairs of receptive fields and the surface distance between cortical recording sites were made for the glabrous hand and foot, the arm and the trunk. Amount of overlap of receptive fields is linearly related to cortical distance; receptive fields for recording sites more than 500-600 microns apart fail to overlap, regardless of position on the body surface. The independence of the receptive field overlap and cortical distance relation from body part is a consequence of the inverse relation between cortical magnification factor and receptive field size. Since receptive field overlap for neurons must be considered in all cortical directions, it follows that a surface area of cortex approximately 1-1.5mm in diameter would contain all neurons with receptive fields overlapping a particular receptive field any place on the body surface. It may be useful to consider such an area of tissue as a basic processing unit for somatosensory cortex, much like the "hypercolumn" proposed for primary visual cortex (Hubel and Wiesel, J. Comp. Neur., 158: 267, '74).

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2414 VISCOELASTIC PROPERTIES OF SKIN AND THE ADAPTATION OF THE TYPE I MECHANORECEPTOR IN THE CAT. Daniel N. Tapper, Thomas K. Baumann* and Leonard M. Smithline*. Sections of Physiology and of Neurobiology and Behavior, Division of Biological *Science and College of Veterinary* Medicine, Cornell University, Ithaca, N.Y. 14853.

The static and dynamic mechanical behavior of the Haarscheibe (Hs), the epithelial structure containing the Type I slowly adapting mechanoreceptor of hairy skin, and of the skin alone was studied in 14 anesthetized adult cats by recording the reactive force generated by the tissue in response to indentations by a stylus of a mechanical stimulator. A new mechanical stimulator with force monitoring, based on a galvanometer scanner and a highly sensitive force-measuring system, was built for this study. By use of this device the reactive forces of the tissues to the mechanical stimuli could be monitored, thus gaining a better estimate than previously available of the actual stimuli applied to the receptor. The Hs and the adjacent skin were found to have similar mechanical characteristics. They both displayed load relaxation, a property typical of viscoelastic materials, and a nonlinear load-deformation relationship including a hysteresis. The mechanical response of the tissue was found to depend on the previous history of loading. This appears to be a long lasting effort of the order of minutes. An attempt was made to relate the properties of the Hs, acting as a mechanical filter, to the characteristics of the neural discharge of the Type I receptor. Mechanical factors appear to contribute significantly to the adaptation of the Type I receptor. (Supported by USPHS Grant 07505).

2415 REINNERVATION OF GLABROUS SKIN IN THE HAND SUBSEQUENT TO NERVE TRANSECTION AND REPAIR. J.K. Terzis and R.W. Dykes. Microsurgical Research Laboratories, Royal Victoria Hospital, McGill University, Montreal, Quebec.

The left ulnar nerve was cut at the level of the wrist in five female baboons (Papio anubis). Using sterile surgical techniques the transected nerve was repaired under magnification and using microsurgical techniques. The contralateral unoperated ulnar nerve served as the control. The animals were allowed to survive from one to five months. At monthly intervals the functional properties of the regenerating mechanoreceptive afferent fibers reinnervation the glabrous skin on the ulnar side of the previously denervated hand were investigated. The data were compared to results from normal skin and from skin subsequent to nerve crush. The electrophysiological techniques employed in this study included electrical stimulation of the ulnar nerve at the wrist, and single fiber dissection of the ulnar nerve at the mid-arm level. This arrangement allowed rapid isolation and identification of a large number of single fibers. Once the latency of the afferent fiber was recorded, the hand was searched for its receptive field. Once identified, the receptive field was demarcated under magnification and drawn on enlarged photographs of the hand. Areas of highest sensitivity were outlined. Over 600 single fibers were isolated from the repaired ulnar nerve. The results demonstrated that on the side of the nerve transection, (i) conduction velocities of the nerve fibers were significantly slower than the controls, (ii) receptive field size and shape, and areas of highest sensitivity were abnormal and this was maintained throughout the study period (contrary to findings after nerve crush), (iii) multiple receptive fields subserved by a single axon were a more common finding after nerve cut than after nerve crush, (iv) the response threshold and stimulus-response relation were altered, (v) there was a slow progression of reinnervation with time occuring in a proximo-distal direction. Contrary to findings in the study of crush injuries, there was no measureable reinnervation of glabrous skin at one month following nerve cut and repair.

These results provide insight into the basic processes underlying cutaneous reinnervation in the hand and contribute to an explanation of our clinical observations of altered sensibility subsequent to nerve injury and repair.

2416 VISION AND RECOVERY FROM DORSAL COLUMN NUCLEI LESION. T.A. Tran,* D.E. Teodoru,* and A.J. Berman. Department of Neurosurgery, V.A. Hospital, Bronx, N.Y. 10468.

Gilman and Denny-Brown (1966) reported that after dorsal column medial lemniscal system (DCMLS) interruption, blindfolded monkeys would no longer reach into extrapersonal space. Reaching movements made when vision was unobstructed were viewed as visual in origin and not indicative of somatosensory recovery. Many authors have challenged this view.

Four monkeys were trained to perform a variety of tasks involving reaching and manipulating objects and grasping small morsels of food while blindfolded. All the monkeys were overtrained. Two monkeys were kept in total darkness throughout overtraining and postoperative survival. Two were not light deprived. After DCN lesion, all monkeys were retested for 30 sessions. The dark-maintained monkeys achieved preoperative performance within 15 sessions. The monkeys maintained in light, on the other hand, failed to perform on any of these tests until placed in total darkness for 3 weeks and then retested. They then exhibited the same pattern of recovery as the dark-maintained monkeys.

The pattern of recovery in both groups followed the sequence: (1) reaching forward, grasping, and retraction of the hand to the mouth, irrespective of whether or not the hand had contacted the food; (2) reaching, followed by groping and palpation until the target was touched, followed by retraction of the hand to the mouth, regardless of whether food had entered the hand or not; (3) reaching, followed by manipulation of the food until it was successfully grasped, followed by retraction of the hand to the mouth, even if the food had meanwhile been dropped; and (4) reaching and retrieval of the food, with palpatory searching by the hand if the food was dropped before reaching the mouth. We interpret this recovery sequence to consist of the gradual introduction of tactile feedback into a preprogrammed movement.

We propose that after DCN lesion the informative quality of the residual somatosensory input is degraded. The brain therefore blocks this input's access to higher centers and depends on other modalities, e.g., vision, to guide movement. Thus, when the monkeys which were permitted vision were tested, they were incapable of using residual somatosensory feedback. Chronic blockage of vision postoperatively may force the brain to relearn the informative qualities of residual somatosensory feedback. This would explain why the dark-maintained monkeys reached into extrapersonal space whereas the light maintained animals failed to do so as was the case in the study reported by Gilman and Denny-Brown.

2417 PATTERNS OF REINNERVATION FOLLOWING REPLANTATION OF THE RABBIT EAR. B.G. Turnbull* and J.K. Terzis. Microsurgical Research Laboratory, McGill University, Montreal, Quebec.

Refinements in microsurgical techniques over the past decade have improved the surgical management of peripheral nerve lesions and have permitted replantation of free composite tissues. However, cutaneous reinnervation following replantation or nerve injury and repair often remains abnormal. The aim of this work was to replant composite tissue and to study the characteristics of the reinnervated mechanoreceptors physiologically at successive time intervals. The rabbit ear was chosen because the size of the supplying neurovascular bundle is comparable to nerves supplying the digits in man.

The right ears of 30 New Zealand white rabbits were replanted with microsurgical anastomoses of the central artery, vein and the greater auricular nerve. At various post-operative periods ranging from two weeks to eight months, the greater auricular nerve was re-exposed proximal to the nerve repair. Single fibres were isolated by dissection and their signals were studied using standard electrophysiological equipment. Once a single active unit was isolated, the ear was searched for the receptive field (RF). The size, shape and relative proportions of the several types of RF's were studied as a function of innervation age. The number of active areas within each RF was recorded as were details of its response characteristics. Finally, the conduction latencies of the fibres were obtained by percutaneous electrical stimulation.

Reinnervated RF's differed from normal fields by (i) being smaller and more irregular in contour, (ii) having fewer hairs innervated by a single nerve fibre, (iii) exhibiting changes in the relative population of guard hair units versus down hair units and by displaying multiple skin areas served by one fibre. Although both the size of the RF's, and the number of hairs capable of activating the fibre increased with time, reinnervated RF's seldom achieved normal dimensions, and irregularities in shape and high thresholds persisted. The mean conduction velocity (CV) of the regenerated nerve fibres also approached, but did not attain normal value up to the end of the study period.

Despite these differences from normal fibres it was observed that (i) submodalities of the reinnervated mechanoreceptors (down hair units v.s. guard hair units) were recognized even at early stages of reinnervation, when RF's were small and threshold high and, (ii) the relative populations of hair units approached normal values with time.

2418 VISUAL INPUT TO NEURONS IN THE CAT'S POSTCRUCIATE CORTEX. Tom O. Videen* (SPON: A. L. Towe). Depts. of Psychology and Physiology/Biophysics, Univ. of Washington, Seattle, WA 98195.

The sensitivity of neurons in the lateral postcruciate gyrus (contralateral forepaw focus of motor cortex) of chloralose anesthetized cats to visual and somatic stimuli was compared. Excitable cells were located using electrical stimulation of either the contralateral forepaw or the medial lemniscus, alternately with brief flashes of light (strobe lamp subtending approximately 23° in the central visual field, average luminance 2.5 log cd/m^2, background about -1 log cd/m^2, 3 mm diam. pupils).

Previous studies have claimed that only about one-fifth of the lateral postcruciate neurons respond to visual stimulation; most of the pericruciate visual neurons are reported to be concentrated more medially and rostrally. Of 114 excitable neurons in this study, 59% responded to light-flashes. All but one also fired to somatic stimulation. Mean latency to light-flashes was 43 msec. When the flash intensity was reduced, the response latency increased and the probability of firing decreased, falling to zero when an opaque filter was used.

When neurons were classified according to the size of their excitatory receptive fields, only 13% of those neurons excited (response probability, P≥0.3) by a single paw with no excitation (P=0) from other paws (*sa* neurons, n=24) were also excited by light-flashes, whereas 92% of those cells excited by all four paws (*m* neurons, n=38) also fired to light-flashes. Most cells excited by 2 or 3 paws (n=11) also fired to light-flashes. Some cells excited by medial lemniscus stimulation did not fire to any paw (n=5); these cells had small receptive fields on the contralateral forearm and none were excited by light.

When neurons were tested for subthreshold interactions, a facilitatory input from other paws was revealed in 6 of 15 *sa* neurons and from light in 6 of 16 nonvisual neurons. When completely tested neurons were reclassified using sub- and suprathreshold excitatory input, 94% of *m* neurons (n=48) and 33% of *sa* neurons (n=9) had excitatory visual input. All cells excited by 2 or 3 paws (n=14) were excited by light-flashes.

While the majority of neurons with excitatory visual input have large excitatory receptive fields, usually including all four paws, some visual cells have small somatic receptive fields. Two of these cells that had natural receptive fields limited to parts of the contralateral forepaw had short latency suppression from all other paws. Another cell that fired to light was suppressed by all 4 paws and by an auditory click. It is conceivable that the visual input to *sa* cells differs from that to *m* cells in some way that would be apparent with more specific visual stimuli.

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2419 LOCALIZATION OF TOUCH ON GLABROUS SKIN FOLLOWING DORSAL COLUMN LESION IN A PRIMATE. Charles J. Vierck, Jr.[1] and Ruth Rand*. Dept. Physiol., Sch. Med., Univ. North Carolina, Chapel Hill, N.C. 27514.

Two Macaca nemestrina monkeys have been trained to identify the location of a tactile stimulus delivered to the glabrous skin of either foot. The monkeys initiate trials by pressing a lever and holding until a pulsatile tactile stimulus (10Hz) begins. Release of the lever within 1.5 sec of stimulus onset starts a response period in which a press of one of 6 buttons on a panel produces liquid reinforcement. On the panel surface facing the animal is an artist's rendering of the glabrous surfaces of a monkey's feet. One of several panels is selected for a daily session; the panels differ in the location of the six response buttons. Panel A, presented during preoperative training and early postoperative testing, has 3 buttons on each foot, located over the distal phalanx of the 4th toes, the middle of the soles and the heels. During blocks of 100 trials within sessions, three stimulus probes (1.5 mm diameter) are positioned over different combinations of locations on the feet that are represented by buttons on the panel, and the correct button response on each trial is defined by the location of the stimulator driven. For the combinations used with panel A, 2 monkeys have performed preoperatively at better than 95% correct button responses, and a left dorsal column (DC) lesion in one animal has deteriorated performance slightly at only one of the points (85% correct responses to stimulation of the ipsilateral mid-sole location). In addition, bar release latencies and button response latencies are unchanged by the lesion. The lesioned monkey has been trained postoperatively, using panel B, to respond accurately to stimulation of the distal pads of toes 2 or 4 or the base of toe 4 on either foot. There is no detectable ipsilateral deficit in performance on this panel. Thus, despite severe deafferentation of the distal extremity portion of SI cortex in monkeys by DC lesions, single stimuli to the feet can be quite accurately localized following DC section. Supported by grants NS 11132 and NS 14899.

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2420 ACETYLCHOLINESTERASE STAINING OF BARRELS IN SOMATOSENSORY CORTEX: VIBRISSEAL COAGULATION AND COMPARISONS BETWEEN RAT AND MOUSE. Jacqueline V. Waldman* and Donald A. Kristt. Dept. Path., Johns Hopkins Univ. Sch. Med., Baltimore, Md. 21205.

There are unique neuronal clusters in primary somatosensory cortex (SmI) of rodent that receive the specific thalamocortical projection from the ventral basal complex. In previous work (Neurosci. Lett. 12, '79), it was shown that the barrel neuropil of postnatal rat (< 21 DPN) stained deeply for AchE. This staining consists of a "blush" presumed to be AchE positive fibers, which disappeared following cortical undercutting. In the present series of studies, answers to the following questions were sought. First, is this staining pattern present in the mouse (where the barrel centers are relatively cell sparse in contrast to the rat)? Using an AchE method described previously (J. Comp. Neurol. 1979), we noted conspicuous central staining of the barrels in postnatal mice without staining of the septae (and possibly the walls too). However, barrel staining was first observed at 14-16 days postnatally, in contrast to the rat, where it is first detectable at 3 days DPN. It is noteworthy that in mouse, the period 14-16 DPN corresponds to the onset of functional thalamocortical synapses in the barrels. The second question was: what effect would neonatal vibrisseal coagulation have on barrel staining? Previous workers have shown that this procedure produces fusion of the neurons from the cortical row of barrels which corresponds to the coagulated row of vibrissae. We found that following coagulation of row C vibrissea unilaterally, row C in PMBSF has become an oval mass of cells in Nissl stained sections; AchE staining is also altered. The third question was: what happens to the staining in adult rodent (in rat the blush disappears between 16-21 DPN)? In contrast to younger mice, animals 21 DPN and older show marked staining of the septae with a blush occupying the top 75-100 μm of the barrel. In adult rat, barrel centers are less well stained than the septae. In conclusion, although there are differences in barrel staining between rats and mice, the observations continue to support the hypothesis that the AchE-rich projection is derived from VBC thalamus. If this hypothesis can be verified, then it seems that in mouse VBC afferents do not possess sufficient AchE until synaptogenesis begins. This, in turn, would suggest that the AchE is, at least partially, related to neurotransmission. It would also suggest that in the mouse, the AchE is not related to catecholaminergic afferents, which appear in the barrel centers by 8 days PND. (The cell bodies and their processes in locus coeruleus contain considerable AchE in addition to their usual neurotransmitter, norepinephrine).

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SPINAL CORD

2421 BENEFICIAL EFFECTS OF HIGH DOSE METHYLPREDNISOLONE ON RECOVERY FROM EXPERIMENTAL SPINAL CORD COMPRESSION. Douglas K. Anderson, Eugene D. Means, & Thomas R. Waters*. VA Hosp. & Depts. Physiol. & Neuro., Univ. Cinn., Col. Med., Cincinnati, OH. 45220

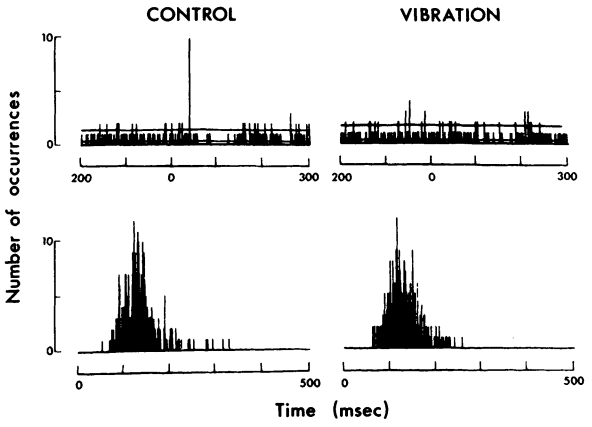
The efficacy of steroids in protecting the spinal cord (SC) from blunt trauma is problematic. Disparity in results may exist due to differences in (a) experimental animals (b) severity of injury (c) doses of steroids administered (d) techniques for evaluating neurological recovery, or (e) length of recovery period. The purpose of the present study was to determine the effectiveness of long-term, high dose methylprednisolone sodium succinate in ameloriating the effects of SC compression trauma. For this study immunized & conditioned female mongrel cats ranging in weight from 2.5 to 4.0 kg were anesthetized with pentobarbital sodium (30mg/kg ip). All cats were immobilized in a stereotoxic frame, a one-segment laminectomy performed at L2, injured & post-operatively treated as described previously. (J. Neurosurg. 44:715-722, 1976). The SC's of 23 cats were compressed with 170gms for 5 minutes. Starting 2 hrs post injury, 16 of these cats were given methylprednisolone (30mg/kg body wt) for 3 days, 15mg/kg body wt for the next 3 days & 7.5mg/kg body wt. for the final 3 days of a total 9 day regimen. The remaining 7 cats served as injured but untreated controls. Neurological function was evaluated in all cats bi-monthly for a 2 month recovery period after which they were sacrificed by intracardiac perfusion of 10% formalin & the SC's removed for histologic examination. Our neurologic evaluation procedure is based on a 16 point scale with 16 being normal. Methylprednisolone treated cats showed significantly ($p<0.001$) earlier recovery (total score: 5.9 ± 0.3 & 11.4 ± 0.3 at 2 weeks and 1 mo. respectively vs 3.7 ± 0.4 & 8.0 ± 1.0 at the same time intervals for controls) & more complete recovery (total score: 14.3 ± 0.3 at 2 mo. vs 11.1 ± 0.5 at 2 mo. for controls) than control animals. Histologically, SC's from steroid treated animals consistently showed greater tissue preservation than those from controls. Our data clearly shows that long term high dose methylprednisolone treatment is effective in protecting feline SC tissue from post injury auto-destruction. However, its potency in this regard may depend on the extent to which methylprednisolone reaches the injury site. We suggest that this is a function of the level of post injury SC blood flow which has been shown to be inversly related to the magnitude of compression (J. Neurosurg. 45:660-676, 1976). Supported in part by the VA and grants from the Paralyzed Veterans of America & the Upjohn Co.

2422 PRESYNAPTIC INHIBITION IN THE HUMAN SPINAL CORD. Peter Ashby and Molly Verrier*. Playfair Neuroscience Institute, Toronto Western Hospital, Toronto, Canada. Dept. Med., University of Toronto, Toronto, Canada.

Single, repetitively discharging, human motoneurons show a brief increase in firing probability in response to homonymous group 1 volleys. This is of appropriate latency to represent the monosynaptic Ia EPSP.

This effect of the group volleys can be blocked by continuous 60 Hz vibration of the limb even though excitability of the neuron, estimated from its mean firing rate, is unchanged.

This may be an example of presynaptic inhibition in man.



2423 DISTRIBUTION OF MET-ENKEPHALIN, SUBSTANCE-P AND SOMATOSTATIN IMMUNOREACTIVITY IN THE SPINAL CORD DORSAL HORN AFTER HEMISECTION IN THE RAT. M.S. Beattie, J.C. Bresnahan, R. Ho and F. Liuzzi*, Dept. Anat., Sch. Med., The Ohio State University, Columbus, O., 43210.

Met-enkephalin (ENK), substance-P (SP) and somatostatin (SOM) -like immunoreactivities have been described in the dorsal horn of a number of species, and a role for these peptides in the transmission and modulation of nociceptive information has been postulated. In a preliminary attempt to assess the effects of chronic disruption of the descending components of the pain modulating system, we have evaluated the effects of thoracic spinal hemisection on the distribution of these peptides in laminae I and II of the rat spinal cord dorsal horn.

The indirect immunofluorescent and modifications of the indirect PAP techniques were employed to demonstrate ENK, SP and SOM immunoreactivities on semi-adjacent sections in animals allowed to survive 1, 3, and 5 days, and 3, 5 or 20 weeks following spinal hemisection. Cross reactivity tests and absorption controls demonstrated specificity of immunostaining for each of the antisera used.

The pattern of distribution in the superficial laminae of the dorsal horn was comparable to that reported by other authors for each of the three peptides. ENK, SP, and SOM immunoreactivities were observed in varicosities and terminals within both laminae I and II. Overlap between the distributions was substantial with ENK exhibiting the deepest penetration into the dorsal horn. Although consistent assymetries were observed between the two sides of the spinal cord, these differences were not consistently related to the surgical treatment. These results suggest first, that the majority of ENK, SP, and SOM immunoreactivities in laminae I and II is related to segmental systems in the spinal cord (i.e. dorsal roots and interneurons). Secondly, the results suggest that in the rat, unilateral disruption of descending systems does not produce a reorganization of these peptidergic systems of sufficient magnitude to produce obvious changes in their distribution as revealed by these immunohistochemical techniques. A report by Naftchi et al. (Brain Res., 153: 515-528, 1978) showing fairly dramatic changes in SP distribution following spinal transection in the cat, may be indicative of a species difference in this regard. Studies are currently underway to examine this possibility. (Supported by N.I.H. Grants NS-14457 and NS-10165.)

2424 SURAL NERVE INPUT TO THE DORSAL HORN IN NORMAL AND ACUTELY HEMISECTED CATS. Gene L. Brenowitz* and Lillian M. Pubols. Department of Anatomy, The Medical College of Pennsylvania, Philadelphia, PA 19129.

Axonal sprouting of dorsal root afferents is known to occur following partial hemisection of the spinal cord (Murray and Goldberger, J. Comp. Neur. 158:19-36, 1974). Our long term goal is to examine changes in cutaneous input to the dorsal horn correlated with this type of sprouting.

This report describes the distribution of dorsal horn neurons responding to electrical stimulation of the sural nerve in normal and acutely hemisected cats. The responses of single units and unit clusters in the L7 segment to electrical stimulation of the sural nerves and to natural tactile stimulation were studied in adult cats maintained on methoxyfluorane anesthesia, using platinum iridium microelectrodes. The dorsal horn was mapped bilaterally in a transverse, 2 dimensional grid of recording loci 0.2 mm apart mediolaterally and 100 μ or 200 μ apart dorsoventrally. Some cats received right (low thoracic or high lumbar) hemisections, sparing the dorsal columns, 2 or 3 days prior to recording.

Sites yielding reliable short latency ($\leq$ 5msec) responses to electrical pulses were clustered in the lateral one half of the dorsal horn, largely in laminae IV-VI. Natural receptive fields for penetrations containing loci responsive to nerve stimulation always included some part of the region extending from the heel to the ventral foot just above the base of digit 4. The region of the dorsal horn yielding longer latency (> 5msec) responses to sural nerve stimulation overlapped with that described above but was slightly larger.

In acutely hemisected cats it was more difficult to delineate natural receptive fields on the lesioned side due to an increase in spontaneous activity in the laminae responding to tactile stimuli. Our preliminary findings indicate, however, that there are no striking changes in somatotopy or in the location and extent of the region responsive to sural nerve stimulation as a result of hemisection.

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2425 ULTRASTRUCTURE OF SYNAPTIC TERMINALS IN LAMINAE I AND II OF THE CAT SPINAL CORD AFTER ANTEROGRADE LABELLING OF DORSAL LATERAL FUNICULUS AXONS WITH HRP. J.C. Bresnahan and M.S. Beattie, Dept. Anat., Sch. Med., The Ohio State University, Columbus, O., 43210.

Axons descending from the brainstem raphe nuclei and adjacent reticular formation probably participate in the modulation of neuronal activity in the marginal layer and substantia gelatinosa of the spinal cord (laminae I and II). These fibers have further been shown to travel in the dorsal lateral funiculus (DLF) of the cord in rat, cat, monkey, and opossum. We have labelled axons in the DLF using anterograde injury-filling with HRP and examined the synaptic terminals of these fibers in laminae I and II.

Adult cats were anesthetized with sodium pentobarbital and maintained as described previously (Beattie et al., Brain Res., 153: 127-134, 1978). Microinjections of HRP were made in the DLF either hydraulically or iontophoretically; the damage done by the injection pipette was sufficient to produce considerable injury filling of axons which were confined to the DLF with little or no contamination of Lissauer's tract. Alternatively, a small cut in the DLF was made and dried pellets of HRP were introduced into the cut. Tissue was processed using DAB as the reaction substrate with cobalt chloride intensification. Survival time was from 18 to 24 hours.

With the light microscope, labelled axons within the DLF could be traced for 5-10mm caudal to the injection site. Many entered laminae IV-VIII in a dense projection presumably representing cortico-spinal, rubro-spinal, and other descending systems. A smaller number of relatively fine-caliber axons could be traced to laminae I and II with many exhibiting a transverse orientation capping the dorsal horn. Terminal and en passant swellings were easily visible. With the electron microscope, both small myelinated and unmyelinated axons were labelled with HRP in laminae I and II. The majority of synaptic terminals labelled with HRP contained round vesicles and a few dense core vesicles per profile. Additionally, small terminals containing only round vesicles were observed. Synaptic contacts were usually on dendritic shafts, but contacts with vesicle-containing profiles were also seen. A small number of terminals with similar morphology were degenerating caudal to the region of HRP labelling. Both the HRP-labelled and the degenerating terminals were comparable to the types of degenerating terminals observed by Goode et al. (1977) after lesions of raphe magnus in the opossum. The relationship of these terminals to those of primary afferent origin (Beattie et al., 1978) will be discussed. (Supported by N.I.H. Grants NS-14457 and NS-10165.)

2426 THE USE OF FLUORESCENT DYES FOR STUDIES OF BRAINSTEM-SPINAL CONNECTIONS IN THE RAT. A COMPARISON WITH THE HRP METHOD AND THE POTENTIAL FOR COMBINING WITH THE GLYOXYLIC ACID METHOD. Walter R. Buck, Albert O. Humbertson, Jr. and George F. Martin, Dept. Anat., Sch. Med., The Ohio State University, Columbus, O., 43210.

HRP as a retrograde marker has done much to revolutionize the way we visualize neuronal connectivity in the central nervous system. Although extremely useful, the technique is time consuming and not easy to use in double labelling experiments (i.e., tritiated but inactivated HRP-regular HRP). Recently several authors have reported success using fluorescent dyes as retrograde markers and because of the potential use of dyes for double labelling we have tested their effectiveness on systems of interest to us. The fluorescent dye 4,6-diamino-2-phenylindole (DAPI) appears to be particularly good. Following injection of DAPI into the cord we have seen evidence for brainstem labelling which appears comparable to that present in other animals processed via the HRP method. The animals with dye injections were sacrificed two to four days after surgery and the unfixed brains were removed for cryostat sectioning. The sections were examined with a fluorescence microscope and labelled cells could be seen using a 360nm. filter system. Such cells were bright blue in color and often surrounded by similarly labelled satellite cells. Alternate sections were stained for Nissl substance for orientation. Some sections from the dye injected cases were processed by the glyoxylic acid technique before viewing with the microscope and by using the appropriate filters the examiner could determine whether or not labelled neurons contained monoamines. Other fluorescent dyes have been tried (e.g., Evans Blue, Primuline), but to date the labelling is not as crisp as that seen with DAPI. The technique will be described and the results obtained on brainstem-spinal connections using this method will be compared with those derived with the HRP technique. (Supported by U.S.P.H.S. Grant NS-07410.)

2427 FUNCTIONAL ORGANIZATION OF ELECTRICAL CONNECTIONS IN THE FROG SPINAL CORD. William F. Collins, III* and Solomon D. Erulkar. Dept. of Pharmacology, University of Pennsylvania Medical School, Philadelphia, PA 19104.

Since Grinnell[1] first described the electrical nature of the interaction between frog motoneurones, the presence of electrical connections between frog motoneuron dendrites has become widely accepted. All electrophysiological studies have involved stimulation of ventral roots usually VR_9 and VR_{10}. Since the motoneurones innervating a given muscle in the hind limb are typically spread out over more than one segment[2], these studies yield little information as to the functional organization of the electrical connections. In order to determine the functional organization of the electrical connections, the isolated, hemisected frog spinal cord (Rana pipiens) was used. The dorsal roots were sectioned and the ventral roots were left intact to the sciatic nerve. The cord was pinned down on sylgard (Dow Corning) medial side up and was perfused with cold ($14 \pm 1^{o}C$) oxygenated normal ringers. The ventral roots were led out through a greased slit to a pool of oil where the sciatic nerve (for identification of motoneurons), the muscle nerve to the Gracilis Major (knee flexor and hip extensor) and the muscle nerve to the Iliacus Internus (knee extensor and hip flexor) were each placed on bipolar platnum electrodes for stimulation. Intracellular recordings from motoneurons in the rostral part of the lumbar enlargement were made using standard techniques. To date, 93 motoneurons have been studied. Of these motoneurons, 36 responded to stimulation of the Iliacus Internus muscle nerve, 27 responded to stimulation of the Gracilis Major muscle nerve and 7 responded to both. These ventral root excitatory post synaptic potentials (VR-epsps) were virtually always subthreshold (0.5 - 2.0 mV). Current injection had no effect on the amplitude of the VR-epsps. When muscle nerve stimulation was preceeded by stimulation of the sciatic nerve (antidromic response) or a dorsal root, the size of the VR-epsp was often increased. This increase in VR-epsp amplitude was associated with an increase in the extracellular field potential indicating increased antidromic invasion of motoneurons presynaptic to the motoneuron from which recordings were made. This procedure did not reveal VR-epsps which were not present following muscle nerve stimulation alone. These results indicate functional specificity in the electrical connections of lumbar motoneurons in the frog such that motoneurons innervating synergistic muscles are much more likely to be coupled than motoneurons innervating antagonistic muscles.

1. Grinnell, A.D. J. Physiol. (Lond.) 182:612-648, 1966.
2. Cruce, W.L.R. J. Comp. Neur. 153:59-76, 1974.
(Supported by NS 12211 and USPHS GM 07302)

2428 CYCLOBENZAPRINE: ACTION ON THE DESCENDING COERULOSPINAL NORADRENERGIC PROJECTION. John W. Commissiong†, Farouk Karoum* and Norton H. Neff. LPP and LCP, NIMH, WAW Bldg., St. Elizabeths Hospital, Washington, D.C. 20032.

The locus coeruleus (LC) provides a massive noradrenergic projection to the ventral horn (VH) of the spinal cord (Commissiong et al., 1978). Cyclobenzaprine (CBZ), a centrally-acting muscle relaxant, caused a dose-dependent depletion of norepinephrine (NE) in the VH of the lumbar spinal cord. However, noradrenergic transmission in the cord, as assessed by the production of methoxyhydroxyphenylglycol (MHPG), the major metabolite of NE, remained intact, even when NE was depleted by greater than 80%. All analyses were done by a gas chromatographic-mass fragmentographic method. CBZ (1.5 mg/kg i.v.) completely abolished muscular rigidity of the gastrocnemius-soleus muscle group in the intercollicular decerebrate rat. Muscular rigidity was assessed by an electromyographic method. However, in rats in which the LC had been lesioned bilaterally 8 days or more previously, CBZ, even at high doses (10 mg/kg i.v.) had only minimal effects on muscular rigidity. It is suggested that the clinical beneficial effects of CBZ could be mediated by an interaction with the descending coerulospinal noradrenergic inhibitory projection.

Commissiong, J., Hellstrom, S., and Neff, N. Brain Res. 148, 207, 1978.

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2429 SYNAPTIC COVERAGE OF THE MOTONEURONAL MEMBRANE FOLLOWING AXOTOMY. William L.R. Cruce and Bruce L. Kinney*. Neurobiology Dept., Northeastern Ohio Univs. Coll. of Medicine, Rootstown, OH 44272.

The ninth ventral root (mid-lumbar) of bullfrogs (Rana catesbiana) was transected extradurally through the intervertebral foramen thus severing axons of motoneurons but minimizing damage to dorsal root afferents. Animals were allowed to survive 5, 10, 15, 20, 25, 30, 45, and 60 days then were perfused transcardially with a mixed aldehyde fixative in cacodylate buffer, postfixed in osmium, and prepared for electron microscopy. Light microscopy of 1 μ thick epon sections was used to orient the trimming of tissue blocks around large motoneurons in the ventral horn. Three to five motoneuron somas were photographed from each side of the ninth segment of each animal and three to five animals were used for each time point. Additional cells were photographed from sham-operated and unoperated control animals. Negatives at a magnification of 4500X were projected to a final magnification of 22,500X onto a Summagraphics digitizing tablet and microprocessor where membrane lengths and lengths of membrane covered by synaptic boutons were measured and tabulated by hand or collected on-line for statistical analysis by a PDP 11/34 minicomputer. Approximately 100 μ of membrane length was sampled for each motoneuron.

Cytoplasm of the swollen chromatolytic cells showed the characteristic increase in neurofilaments and neurotubules which form channels through the aggregates of granular endoplasmic reticulum, free ribosomes, and polysomes. The motoneuron soma membrane changed from its normal smooth contour to a rough or vacuolated appearance. Numerous spaces appeared in the neuropil immediately outside the cell membrane even though nearby regions of neuropil and nearby small neurons were well fixed. Occasionally, "free boutons" making no contact to any recognizable neuronal structure were seen near the cell membrane and were surrounded by glial elements. These glial elements were usually astrocytic rather than microglial in appearance. Microglial type cells proliferated in the region near motoneurons, but rarely did they appear to surround a bouton, to be in contact with a motoneuron, or to be lodged between a bouton and a motoneuron. Preliminary measurements indicated that synaptic coverage of the motoneuron membrane dropped from 26% in controls to 20% at 5 days and to 12% at 25 days (peak of the chromatolytic reaction) following axotomy.

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2430 EFFECTS OF L-DOPA ON DORSAL HORN UNIT RESPONSES TO NOXIOUS THERMAL STIMULATION. Jonathan Delatizky, Charles J. Hodge and Charles I. Woods*. Dept. of Neurosurgery, Upstate Medical Center, Syracuse, N.Y. 13210.

Descending noradrenergic pathways are believed to participate in modulation of spinal nociception. Stimulation of locus coeruleus has been reported to cause inhibition of spinal nociceptive cells, although interactions with known inhibitory serotonergic pathways may contribute to this effect. Direct iontophoretic application of noradrenaline (NA) or serotonin (5-HT) on spinal nociceptive cells is inhibitory. In contrast, nociceptive thresholds have been shown to increase following NA depletion, suggesting that NA may enhance nociception. We previously described a facilitatory role for NA on cells responding exclusively to innocuous stimuli. The contribution of NA to spinal sensory modulation is consequently uncertain.

We have investigated the effects of L-DOPA induced terminal NA overflow on responses of lumbar dorsal horn units to noxious thermal stimuli. NA actions have been separated from those of other transmitters and metabolites. Unit responses to noxious thermal stimuli were recorded extracellularly in acute spinal cats. A Peltier-effect thermode with feedback control was used to vary the skin temperature between 25 and 56°C in a standard stepped sequence. Responses to this sequence were recorded every 5 to 10 minutes at least twice before, and for at least an hour after, IV injection of L-DOPA (15 mg/Kg). Average unit activity at each temperature was determined and peristimulus time histograms were constructed by a PDP-11 computer. The following groups of acute spinal animals were used: a) no pretreatment; b) p-chlorophenylalanine (PCPA) (a 5-HT depletor); c) 6-hydroxydopamine (6-OHDA)(twice intracisternally); d) fusaric acid (FA)(dopamine B-hydroxylase inhibitor).

L-DOPA caused marked inhibition of the unit response to noxious thermal stimulation (maximum average inhibition was to 35% of the control response). This inhibition was dependent on intact 5-HT stores and was not abolished by destruction of catecholamine tracts by 6-OHDA or by blocking the conversion of dopamine to NA by fusaric acid.

The results suggest that NA alone has minimal inhibitory effect on dorsal horn nociceptive units. Most of the inhibition could be ascribed to L-DOPA triggered 5-HT release. Thus direct NA influences on segmental nociceptive mechanisms appear to be minimal, and previously reported inhibition (Anden et.al., Acta Physiol. Scand. 67:373, 1966; Pearson, Proc. Soc. Neurosci. 4:570, 1978) is possibly caused by noradrenergic interactions with serotonergic or other descending systems.

2431 SUBSTANCE-P (SP),SOMATOSTATIN (SOM) AND METHIONINE-ENKEPHALIN (ENK) IMMUNOREACTIVITY IN THE SPINAL CORD OF THE NORTH AMERICAN OPOSSUM. Frank J. DiTirro*, Raymond H. Ho and George F. Martin (SPON: D.L. Clark), Dept. Anat., Sch. Med., The Ohio State University, Columbus, O., 43210.

The rat spinal cord receives SP and SOM containing projections from the dorsal root ganglia. These projections as well as some ENK containing intrinsic elements are thought to play a role in nociception. To date, SP and SOM immunoreactivity has been identified only in selected mammals and it is premature to conclude that the results from such studies can be extended to all species. The occurrence and distribution of SP, SOM and ENK was studied in the North American opossum. Antibodies against the three peptides were raised in rabbits. The indirect immunofluorescent method of Coons was used to localize the three peptides on 10μm cryostat cut sections of spinal cords that were fixed by intracardiac perfusion with 4% paraformaldehyde in phosphate buffer. SOM immunoreactivity was undetectable in the spinal cord. Only SP immunoreactive fibers were present in the tract of Lissauer and the lateral funiculus. SP and ENK immunoreactivities were localized in the gray matter. SP immunoreactivity in terminals and fibers visible as fluorescent dots and occasional lines were present throughout the gray matter. These structures were most numerous in laminae I and II of the dorsal horn, whereas the ventral horn exhibited a very sparse staining. The distribution of ENK immunoreactive elements paralleled closely that of the SP containing structures. ENK immunoreactive elements were densest in the substantia gelatinosa and around the central canal while all other regions of the gray matter exhibited relatively sparse distribution. There were more ENK immunoreactive elements in the ventral horn than SP immunoreactive elements. The specificity of immunostaining was established in control experiments in which the primary antisera for each peptide, pretreated with an excess of the corresponding synthetic antigen failed to demonstrate the aforementioned structures on semiadjacent sections. We conclude that as in the other mammalian species reported, SP and ENK immunoreactivities are present in the opossum spinal cord. However, SOM immunoreactivity was undetectable in the spinal cord of this species. (Supported by the Snyder Fund, The Graduate School of The Ohio State University and U.S.P.H.S. Grant NS-07410.)

2432 BICUCULLINE INDUCES STEPPING IN DECEREBRATE CATS. S. H. Duenas* E. Eidelberg. Division of Neurosurgery, University of Texas Health Science Center, San Antonio, TX 78284.

It has been proposed that active inhibition has a role in the control of stepping by gating the bursts of motoneuron discharge and by modulating the overall rate of stepping. It seems likely that GABA is a major central inhibitory transmitter. We tested the possibility that GABA may be involved in stepping by injecting animals with a potent GABA agonist (muscimol) and an antagonist (bicuculline).

Bicuculline (100-300 μg/Kg,i.v.) initiated stepping in decerebrate cats, either on a treadmill or as fictive locomotion under neuromuscular block. Muscimol (1.0-2.0 mg/Kg,i.v.) abolished this stepping. Picrotoxin (0.75-1.5 mg/Kg), a putative GABA antagonist, also initiated stepping but this effect was contaminated by seizure-like discharges. The bicuculline effect could not be elicited in spinalized preparations. We conclude that these results support the hypothesis that stepping may be under GABA-mediated active inhibitory control.

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2433 AXON COLLATERALS OF DORSAL HORN CELLS RESPONDING TO CUTANEOUS STIMULATION. M. David Egger, Natalie C. G. Freeman* and Eric Proshansky*. Dept. Anat., CMDNJ - Rutgers Medical School, Piscataway, N.J. 08854.

Intracellular injections of horseradish peroxidase into dorsal horn cells in lumbar segments of the cat's spinal cord revealed distinctive patterns of axonal projections from cells responding to low threshold cutaneous stimulation of the hind foot. Axonal projections have been examined for 17 cells: 10 in lamina IV, 4 in lamina V, and 3 in lamina VI. In general, cells responding to stimulation of the central foot pad were located most medially, those responding to stimulation of the proximal foot and dorsal surface of the toes were most lateral.

A striking characteristic of the axonal projections of many of these dorsal horn cells was a dense arborization of collaterals in the laminar region ventral to the cell body.

In addition, all cells contributed axons to the ipsilateral white matter, many with two separate branches that either both ascended, or ascended and descended within the same white column, or entered entirely separate columns. In lamina IV, two cells that contributed axons to the dorsolateral funiculus were probably cells of the spinocervical tract. Of the other 8, the 3 most medial contributed fibers to the medial dorsal column, the 3 most lateral contributed fibers to the lateral column or to the lateral fascicles of the dorsal intracornual tract (DIT). One of these also sent an axon collateral to the dorsal portion of the dorsal column. Of the two lamina IV cells lying in an intermediate position, one sent a collateral to the medial dorsal column, the other contributed to the lateral portion of the DIT, as well as a collateral to the dorsal portion of the dorsal column. This same medial-lateral organization of axon collaterals tended to hold for the 4 cells of lamina V, but in lamina VI, all 3 cells, including two quite medially placed, sent long axon collaterals to the lateral column only.

In summary, 1) medial cells in lamina IV tended to contribute axons to the medial dorsal column, while lateral cells in lamina IV tended to send axons to the lateral column, and 2) most of the dorsal horn cells studied had, in addition to at least one long projection axon, from one to 4 collaterals arising from the main axon and distributing within the dorsal horn in approximately the same transverse level as the cell body. The pattern of distribution of these collaterals varied widely, but a prominent feature was that the collaterals were distributed ventral to the cell body, often most heavily in the lamina just ventral to the one in which the cell body was found.

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2434 STEPPING, WALKING, AND INTERLIMB COORDINATION AFTER COMPLETE AND PARTIAL CORD LESIONS. E. Eidelberg, B.L. Meyer* and J. Nystel*. Research Program, Veterans Administration Hospital, and Division of Neurosurgery, University of Texas Health Science Center, S.A., TX 78284.

Midthoracic level spinal cord lesions were produced in adult cats. Their gait was studied in a treadmill; sparing of descending neurones was defined by retrograde HRP labeling from the lumbar cord. Completely transected animals stepped with their hindlimbs but did not support or propel effectively with them, and fore-hindlimb coordination was absent. Cats with incomplete lesions, even severe ones, walked albeit not normally; some interlimb coordination was clearly present even when a small fraction of cervical long propriospinal neurones had escaped section. There was no clear and consistent correlation between sparing of specific brain stem-lumbar cord pathways and residual walking ability. Ablation of the motor cortex or corticospinal tract section in the cord did not produce significant, persistent, gait deficits.

It appears possible that one of the roles of descending connections may be to facilitate the engagement of alpha motoneurones by the spinal step generators, at least in cats.

2435 CHARACTERISTICS OF INDIVIDUAL MEDIAL GASTROCNEMIUS Ia AFFERENT PROJECTIONS TO TYPE-IDENTIFIED TRICEPS SURAE MOTONEURONS. James W. Fleshman, John B. Munson, and George W. Sypert. Depts. Neurosci. & Surg., Col. Med., U. Fla.& VA Med. Ctr., Gainesville, FL. 32610

Motoneurons may be divided into more or less discrete motor unit groups based on the properties of the muscle fibers they innervate. Burke and colleagues (see J. Neurophysiol. 39: 447, 1976) have shown that several input pathways to cat triceps surae motoneurons (TSMs) exert differential effects as a function of motor unit type. We are studying the projection frequency of individual Ia afferent fibers and the characteristics of single fiber Ia EPSPs in type-identified TSMs using the spike-triggered averaging technique.

TSM intracellular potentials, obtained by conventional techniques, are led to a signal analyzer, which is triggered by action potentials in a single medial gastrocnemius Ia fiber. The EPSP generated from a single fiber's terminals is accumulated by the analyzer. Intracellular current pulses are then injected to generated specific discharge patterns in the TSM. Resulting single and repetitive muscle unit contractions are measured with a strain gauge. Motor units are classified as FF, FR, or S based on the shape of an unfused tetanus tension envelope and fatigue resistance, using Burke's criteria.

To date, EPSPs have been studied in 45 type-identified TSMs in 4 cats. Although the sample size makes statistical evaluation inappropriate, these preliminary data show a trend toward higher projection frequency and larger single-fiber EPSPs in FR units than FF. More detailed comparisons will require a considerable increase in sample size. Characteristics of the single fiber projection will be discussed in relation to data obtained with whole nerve stimulation.

2436 ANTEROGRADE TRANSPORT OF HORSERADISH PEROXIDASE IN THE FROG SPINAL CORD. Fe Glanzman*, Patricia L. Mensah, Dennis Glanzman and Richard F. Thompson. Department of Psychology, Arizona State University, Tempe, AZ, Department of Anatomy, School of Medicine, University of Southern California, Los Angeles, CA, and Department of Psychobiology, University of California, Irvine, CA.

Twenty adult frogs (Rana catesbeiana) measuring three to four inches in length were used in this study. Horseradish peroxidase (Sigma type VI) was injected into caudal medulla or cervical, thoracic and lumbar spinal cord. Large injections were accomplished under MS 222 (Tricaine) anesthesia by delivering 0.4 to 0.6 microliters of a 20% solution of HRP dissolved in distilled water. After a survival period of one to four days at room temperature, the animals were perfused intracardially with a fixative containing 1% paraformaldehyde and 1.25% glutaraldehyde in 0.1M phosphate buffer. The brain and spinal cord were then removed, post-fixed in the same fixative for 2 to 3 hours, stored in 0.1M phosphate buffer and 5% sucrose for 3 days, and sectioned at 40 micra. Sections were washed in buffer and processed for the blue reaction product according to the technique described by Mesulam (J. Histochem. Cytochem., 24: 1273, 1976). Sections were counterstained with 1% neutral red. Significant amounts of anterograde transport occurred at all times examined. After caudal medulla injections, spinal projecting fibers were seen in the lateral funiculus and in the area of the ventral funiculus occupied by the vestibulospinal system. These fibers entered the central gray and established terminal fields as described previously in lesioned Fink-Heimer stained material by Mensah and Thompson (J. Anat., 125:1, 1978) for the lateral column fibers and by Corvaja and Grofova (Prog. Brain Res., 37:297, 1972) for the vestibulospinal system. Extensive pericellular networks were common and bouton-like enlargements were seen along the course of individual axons. These results indicate that anterograde transport did occur over long distances. In addition, this study suggests that HRP histochemistry may be a reliable method for marking the terminal endings of individual afferent fiber systems. (Supported by NIMH grant number MH-25127 to R.F.T.).

2437 ENKEPHALIN PERIKARYA IN THE MARGINAL LAYER AND SACRAL AUTONOMIC NUCLEUS OF THE CAT SPINAL CORD. Ellyn J. Glazer AND Allan I. Basbaum. Dept. Anat., Sch. Med., UCSF, San Francisco, Ca. 94143.

Although several studies have reported the CNS distribution of immunoreactive leucine-enkephalin, there are no detailed descriptions of the location and morphology of enkephalin somata. In the present study, enkephalin-containing perikarya of the cat spinal cord were studied by immunocytochemistry. The cats were administered either an intraventricular (100ug in 5 ul) or an intraspinal (10ug in 1ul) injection of colchicine. Forty-eight hours after colchicine administration, the cats were perfused with 4% paraformaldehyde. Spinal segments were embedded in paraffin, sectioned at 20u and processed by PAP immunocytochemistry.

The majority of enkephalin-containing perikarya were concentrated in the marginal layer (Lamina 1) of the superficial dorsal horn. Surprisingly few enkephalin cell bodies were observed in the substantia gelatinosa. The marginal neurons, when viewed in transverse sections, varied in size and included a population of large, bipolar, fusiform neurons. The dendritic processes of these neurons extended along the outer curvature of the dorsal grey. Sections taken tangential to the marginal zone revealed a class of enkephalin-containing Lamina 1 neurons with a dendritic morphology reminiscient of the layer 1 pyramidal neurons described by Gobel in Golgi stained material (J. Comp. Neurol. 180: 375, 1978). Large enkephalin neurons were also observed more ventrally in Lamina 4/5 of the dorsal horn and medially, near the central canal. The prescence of numerous enkephalin neurons in the marginal zone raises the possibility that both local and projection neurons of the superficial dorsal horn contain enkephalin.

Of particular interest, was the presence of numerous enkephalin-containing cell bodies in the second sacral segment of the spinal cord. These cells were clustered around the lateral edge of the intermediate and ventral grey and continued dorsomedially in a narrow band towards the central canal. The distribution of these neurons corresponds to that of sacral parasympathetic preganglionic nuclei demonstrated by retrograde transport of HRP (de Groat et al; Neuroscience Letters 10: 103, 1978). Ligation of the S2 ventral root in noncolchicine-treated cats produced a comparable distribution of enkephalin perikarya in S2 and a "damming" of enkephalin-immunoreactivity in the ventral root proximal to the ligature. These results indicate that parasympathetic preganglionic neurons in the cat synthesize and transport enkephalin or enkephalin-like material to the periphery.

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2438 PRIMARY AFFERENT TERMINAL DEFECT IN CATS WITH ACRYLAMIDE NEUROPATHY. Barry D. Goldstein and Herbert E. Lowndes. Dept. of Pharmacology, CMDNJ, N.J. Medical School, Newark, N.J. 07103.

Major clinical manifestations of toxic axonopathies include ataxia and loss of deep tendon reflexes. A defect in the unconditioned spinal monosynaptic reflex (MSR) of cats intoxicated with acrylamide occurs (Goldstein and Lowndes, Neurotoxicol. 1: 75, 1979) and may contribute to the neurological signs of the neuropathy. This defect could result from alterations in primary afferent terminal (PAT) function, changes in the post-synaptic membrane, or both. Spinal monosynaptic input-output characteristics and PAT function were determined in soleus (SOL) and medial gastrocnemious (MG) monosynaptic motoneuron pools in normal cats and cats with subclinical, moderate, and severe degrees of acrylamide neuropathy (total administered doses of 75, 150, and 300 mg/kg, respectively). The primary afferent discharge relative to maximal afferent input necessary to produce a liminal monosynaptic response in the ventral roots (critical input) was 29.7% in normal SOL PAT and 23.5% in normal MG PAT. The critical input increased to 52.7%-57.2% in SOL PAT and to 43.7%-49.7% in MG PAT after the administration of acrylamide. Repetitive stimulation of the MSR at low frequencies (2-10 Hz) causes a rundown to a plateau (homosynaptic depression). The plateau attained following the rundown was lower in response to all stimulating frequencies in cats treated with acrylamide. The plateau was reduced by 22%-62% in the SOL MSR and by 5%-65% in the MG MSR as a function of dose of acrylamide. Analysis of the apparent transmitter turnover parameters determined from homosynaptic depression (Capek and Esplin, J. Neurophysiol. 40: 95, 1977) indicated that the constant fraction of neurotransmitter released (P) was unchanged in either SOL or MG PAT regardless of the dose of acrylamide. However, the rate of replenishment of neurotransmitter (R) decreased in the SOL PAT by 12%-46% as a function of dose of acrylamide. In the MG PAT, R was depressed by 60% but only at the highest dose of acrylamide. It appears that fewer PAT are functional in cats with acrylamide neuropathy, even at doses which produce no clinical symptoms (75 mg/kg). Also, reduced rate of neurotransmitter replenishment and enhanced homosynaptic depression in cats with acrylamide neuropathy suggest a defect in the mobilization process of neurotransmitter to the ready releasable pool in the PAT. Further, SOL and MG appear to be differentially sensitive to the neurotoxic actions of acrylamide.

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2439 LOCALIZATION AND CHARACTERIZATION OF PHRENIC MOTONEURONES IN THE SPINAL CORD OF THE ADULT RAT. Harry G. Goshgarian and Jose A. Rafols. Dept. Anat., Sch. Med., Wayne State Univ., Detroit, MI 48201

The motoneurones which innervate the diaphragm of the adult rat were localized and morphologically characterized in the spinal cord by using the method for the retrograde transport of horseradish peroxidase and the zinc chromate modification of the Golgi technique. In order to distinguish between those motoneurones which innervate most of the diaphragm and those which give rise to the accessory phrenic nerve, peroxidase was applied to crushed phrenic axons either in the neck (rostral to the union of the accessory phrenic and phrenic nerves) or within the thorax (after both nerves have joined into a single trunk). Peroxidase labelled cells were studied in frontal and sagittal sections of cervical spinal cord. In sagittal sections pinholes were inserted perpendicularly to the long axis of the cord through the center of the C3-C7 dorsal roots to identify spinal cord levels. The spinal cord was notched at C2 to distinguish rostral from caudal ends of each section. When peroxidase was applied in the neck, labelled cells were found at the C3-C6 levels of the spinal cord. However, when the application of peroxidase included accessory phrenic fibers, labelled cells were found at the C3-C6 levels of the cord. The labelled neurons were mostly polygonal or fusiform in shape and peroxidase activity ranged from lightly labelled to heavily labelled cells. The long axes of the heavily labelled cell bodies ranged from 32-46 μm while the short axes ranged from 19-26 μm. In most cases clusters of 2-6 closely apposed cell bodies were observed, but isolated cells were occasionally found. In frontal sections, the location of peroxidase labelled cell bodies was determined from measurements along mid-sagittal and horizontal coordinates using the center of the spinal canal as a zero reference point. The greatest number of labelled cells were seen at C4. At this level labelled somata occupied a cross-sectional area of approximately 150 μm in diameter with the center of the area lying 600 μm lateral to the mid-sagittal plane and 400 μm anterior to the horizontal plane. The center of the area shifted posterolaterally at C3 and anteromedially at C5 and C6. Phrenic motoneurones were identified on Golgi impregnated sections by utilizing the above coordinate system. Examination of Golgi sections showed that phrenic neurons issued several thick dendrites which branched repeatedly and radiated in all directions. Small somata were noted at the periphery of the larger phrenic cell bodies. These small neurons displayed delicate, beaded dendrites which overlapped with the dendritic fields of the phrenic neurons. Dendritic overlap between phrenic neurons and other adjacent neurones also occurred. Supported by the Paralyzed Veterans of America and NINCDS grant NS 06925-13.

2440 SYNAPTIC STIMULATION OF FROG AFFERENT TERMINALS ACTIVATES AN ELECTROGENIC SODIUM PUMP. J.C. Hackman and R.A. Davidoff. Neurology Service, V.A. Hospital and Dept. of Neurology, University of Miami School of Medicine, Miami, Florida 33101.

The dorsal root potential (DRP) reflects changes in the membrane potential of the presynaptic terminals of primary afferent fibers. In the cat, positive DRPs--which indicate hyperpolarization of afferent terminals--have been recorded from dorsal roots (DR) following single volleys in adjacent DRs; this has not been demonstrated in the amphibian (with DC recording). Our present experiments, however, show that following repetitive activity of adjacent DRs, hyperpolarization of the terminals of afferent fibers occurs in the frog.

We used the hemisected frog spinal cord continuously superfused with HCO_3^-buffered Ringer's solution maintained at 15°C. DRPs were recorded using the sucrose gap technique.

Tetanic electrical stimulation of a lumbar DR at frequencies ranging from 5 to 1000 Hz for durations of 0.05 to 30 sec produced a sustained negative shift of the potential of fibers in an adjacent DR. Such a sustained potential was consistently succeeded by a positive potential of up to 6mV in amplitude and 5 min. in duration. A single supramaximum DR volley only evoked a negative DRP but as few as 5 DR volleys evoked a detectable positive response provided that the DR was stimulated with voltages 25x the threshold for producing a detectable negative DRP. Both the amplitude and duration of the hyperpolarization were monotonically related to the frequency and duration of stimulation. Tetanic stimulation of ventral roots elicited only a negative DRP.

The hyperpolarization of the afferent terminals was clearly dependent upon metabolic processes, since it was significantly, but reversibly, reduced by cooling (Q_{10}, 2.6) and exposure to metabolic inhibitors (DNP, $5x10^{-5}$M; NaCN, $2x10^{-3}$M).

Such metabolically dependent hyperpolarizations usually reflect active transport of ions. Since there is evidence that the negative DRP results from a selective efflux of Cl^-, afferent terminal hyperpolarization could result from activation of an inward Cl pump. But compounds known to block Cl^- transport (SITS,10^{-4}M; furosemide,10^{-3}M) did not affect the potential change.

On the other hand, a variety of procedures used to inhibit Na^+ pumps did significantly reduce the hyperpolarization. These included application of ouabain (10^{-5}M); elimination of external K^+ and partial substitution of Na^+ in the superfusate by Li^+ (57mM).

These observations show that repetitive synaptic depolarization of afferent terminals is followed by a hyperpolarizing change in membrane potential which reflects an increased rate of active Na^+ extrusion from these terminals. This Na^+ pumping may be activated by the elevated $[K^+]_e$ known to occur during tetanic DR stimulation. (Supported by V.A. Hospital Funds, MRIS 1769)

2441 GLUCOCORTICOID INTERACTIONS WITH BIOGENIC AMINE AGONISTS AND ANTAGONISTS ON CAT SPINAL REFLEXES. Edward D. Hall. Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272.

A short-term triamcinolone diacetate regimen (8 mg/kg i.m./7 days) has been demonstrated in acute spinal (C-1) cats to enhance excitatory spinal monosynaptic (2N) and polysynaptic reflex function (Hall et al., J. Pharmacol. Exp. Ther. 206:361, 1978; Hall and Baker, J. Pharmacol. Exp. Ther. in press). In the present work, the effects of intensive triamcinolone dosing have been examined on the 2N actions of alpha adrenergic and serotonergic agonists and antagonists. In all experiments, 1.2X supramaximal stimuli were applied to the triceps surae nerves of one leg every 5 sec. and the evoked reflex responses recorded at the ipsilateral L7 or S1 ventral root.

In one series of experiments, the 2N reflex effects of a 1 mg/kg dose of the centrally-active noradrenergic agonist methoxamine (MX) given as a 20 minute infusion was studied. As described by Vaupel and Martin (J. Pharmacol. Exp. Ther. 196:87, 1976), MX produced a progressive increase in the 2N response amplitude in untreated cats with a maximum of 34.5%. In the glucocorticoid treated animals, however, no increase by MX was seen; rather a 10-15% depression in response amplitude occurred. One hour after the MX infusion, 20 mg/kg phenoxybenzamine (PBZ) was infused over 20 minutes. In the untreated cats, alpha adrenergic receptor blockade by PBZ produced a 24.2% decrease in the 2N response while in the treated animals, PBZ caused an increase of 21.4%.

In a second series of experiments, the experimental protocol of Clineschmidt et al (J. Pharmacol. Exp. Ther. 179:312, 1971) was employed to assess glucocorticoid effects on the 2N reflex actions of serotonin manipulation. Fifty mg/kg 5-hydroxytryptophan (5-HTP) was infused over 10 min.; followed at 60 min. by 5 mg/kg amitryptyline (AMT) over 5 min.; followed 25 min. later by 1 mg/kg methysergide (MS) over 5 min. In the untreated animals, 5-HTP produced a moderate decline (20-40%) in 2N amplitude. When the serotonin reuptake inhibitor AMT was then given, an immediate elevation (40-50% above control) in the response was observed which was rapidly and completely blocked by subsequent administration of the serotonin antagonist MS. In the treated preparations, 5-HTP produced the same 2N depression, but subsequent AMT produced a 3-fold greater increase in the response that was refractory to antagonism by MS.

These data may have relevance to the pathophysiology of psychotic depression in man. For instance, in many individuals with depression, there is a significant elevation in plasma levels of the endogenous glucocorticoid cortisol (Prange et al., Life Sci. 20:1305, 1977) which could as the present results suggest, have an effect on central biogenic amine transmission. (Supported by NIMH Small Grant MH 31887-01).

2442 TERMINAL DISTRIBUTION OF IA AND GROUP II SPINDLE AFFERENT FIBERS IN THE SPINAL CORD AS REVEALED BY POSTSYNAPTIC POPULATION POTENTIALS RECORDED FROM TWO ADJACENT SPINAL SEGMENTS. Elwood Henneman, Hans-R. Lüscher*[1] and Paul Ruenzel*. Spon. R. B. Szamier). Dept. of Physiology, Harvard Medical School, Boston.

Postsynaptic population potentials (PSPPs) were elicited by stretch evoked impulses in single Ia and group II fibers from the medial and lateral gastrocnemius muscle and were recorded simultaneously from L_7 and S_1 ventral roots perfused with isotonic sucrose, as described in Neurosci. Abst. 4:568, 1978. The amplitudes of the two PSPPs were correlated with the precise entry point of the dorsal root filament containing the afferent unit and with the conduction velocity of the afferent impulse. In general, PSPPs were significantly larger when recorded from the spinal segment which the afferent fiber entered than from the neighboring segment. Only 15 exceptions to this rule were found in 165 pairs of PSPPs. Data from individual experiments were used to construct 3-dimensional plots for the two recording levels, illustrating the precise and systematic relationship between the amplitude of the PSPP, the level of entry of the afferent fiber and the conduction velocity of its impulses. These results suggest that the density of active endings given off by primary afferent fibers is highest around its entry point and diminishes with increasing distance from it.

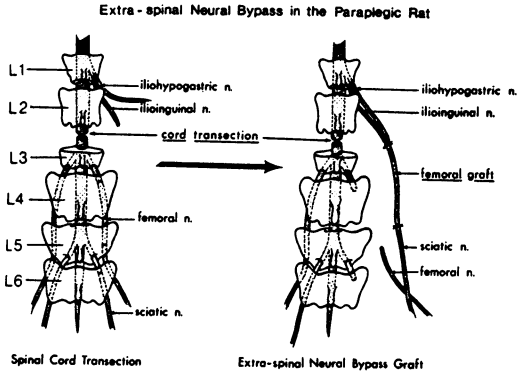
The findings indicate that the projection which each Ia fiber sends to its pool consists not only of a diffuse component going to all homonymous motoneurons (Mendell and Henneman, J. Neurophys. 34:171, 1971) but also a localized component that is topographically organized. The latter may account for the phenomenon of "localized stretch reflexes" within a circumscribed part of a muscle reported by Cohen (J. Neurophys. 17:443, 1954).

Supported by a grant from the National Institutes of Health.

[1]Supported by the Swiss National Science Foundation.

2443 FUNCTIONAL RETURN IN PARAPLEGIC RATS USING EXTRA-SPINAL NEURAL BY-PASS. Pamela K. Hill and Glenn R. Carwell.* Depts. Anatomy and Plast. Surg., East. Va. Med. Sch., Norfolk, VA 23510.

Extra spinal neural by-pass (ESNB) was performed in the right hand limb of 20 rats subjected to spinal cord transection. The ESNB utilized a graft from the right femoral nerve to connect the iliohypogastric and ilioinguinal nerves to the right sciatic nerve (see figure below). Three months post-operatively, discrete flexor movements of the toes were noted in 4 of 9 surviving animals and were synchronous with respiration. These movements were filmed for documentation. Histological examination of the nerve grafts demonstrated continuity without fibrosis through proximal and distal suture lines in 5 out of 9 animals. Collateralization of motor end-plates were present in the right gastrocnemius muscle. These neurological and morphological findings substantiate the re-establishment of communications between the cerebrum and the denervated limb.

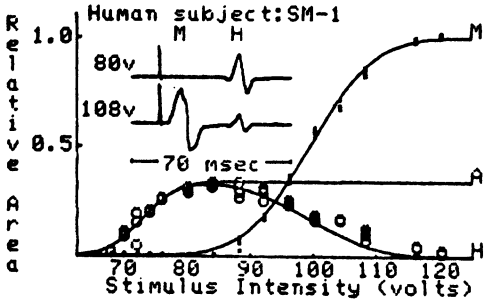


2444 A QUANTITATIVE MODEL OF THE H REFLEX. F. K. Hoehler* and A. A. Buerger. Dept. of Physical Med. & Rehab. and Physiology, Calif. Coll. of Med., UC Irvine, CA 92717.

Transcutaneous electrical stimulation of posterior tibial nerve usually elicits two separate EMG responses in gastrocnemius or soleus. The first of these responses results from the direct activation of motor axons and is termed the M response while the second is believed to be monosynaptic and is termed the Hoffmann (H) reflex. At high stimulus intensities, the H reflex is greatly reduced, presumably by collision with antidromic impulses elicited in the motor nerve. The figure below shows (a) EMG responses to two different stimulus intensities and (b) the overall effect of stimulus intensity on the size of the M response and the H reflex. If the collisions are essentially random, then the occlusion of H can be modeled by the equation

$$H = A - AM$$

where H is the size of the H reflex, A is the size of the monosynaptic reflex volley prior to collision and M is the size of the antidromic volley in motor axons (estimated by the size of the M response) with all response magnitudes expressed as proportions of the entire motoneuron pool (estimated by the size of the maximum M response). A computer program was used to generate theoretical curves which best fit the observed data and, in the majority of subjects, the fit (as shown in the figure) was quite close. "Failures" of the model resulted when subjects continued to produce H reflexes at high stimulus intensities at which complete occlusion would be predicted. Modeling of these residual H reflexes may require the assumption that, in some subjects, either (a) direct electrical stimulation of motor axons cannot activate the entire motoneuron pool or (b) antidromic impulses in motor axons may reach the motoneurons before those cells are orthodromically activated by the afferent volley.



2445 AN ANALYSIS OF MOTONEURON INTERSPIKE INTERVALS IN CATS DURING TREADMILL AND FICTIVE LOCOMOTION. Larry M. Jordan, Carol A. Pratt* and John E. Menzies*. (Spon: R.M. Jell) Dept. Physiology, Univ. of Manitoba, Winnipeg, Manitoba, Canada, R3E 0W3.

Muscle tension resulting from intracellular current injection in alpha motoneurons (MNs) was found to be substantially enhanced when the stimulus train (8-12 Hz) contained an initial doublet (interpulse interval 10msec) (Burke, et al., Brain Res., 109, 1976). It was subsequently shown that the discharge of all flexor and 86% of the extensor MNs studied during controlled treadmill locomotion (evoked by brainstem stimulation) was characterized by an initial short interspike interval (ISI) or doublet, while the intervals separating the spikes in the remainder of the burst were longer and relatively constant (Zajak and Young, In: Neural Control of Locomotion, 1976, pp.789-793). It was suggested that this discharge pattern reflected the most efficient method of maximizing tension production. Evidence is now available (R.B. Stein, personal communication) that the pattern of MN discharge which maximizes the force per impulse is one in which a short initial ISI is followed by a relatively long ISI with the remaining ISIs being of an intermediate and fairly constant duration.

The discharge of MNs which were active during controlled treadmill locomotion or fictive locomotion was analysed to determine whether the ISIs within a burst conformed to the optimal pattern which has been derived experimentally. In one series of experiments, cats were decerebrated and walked on a treadmill in response to stimulation of the mesencephalic locomotor region (MLR). Rhythmic activity in motor axons was recorded from ventral root filaments. In other experiments, decerebrate cats were paralyzed (Flaxedil, i.v.), and MNs were recorded intracellularly as well as from ventral root filaments during MLR-evoked "fictive" locomotion.

Although some MNs were found to fire during both types of locomotion according to the pattern proposed by Stein, the pattern most commonly observed consisted of a fairly regularly spaced spike train with mean ISIs in the range of 40 - 50msec, with a shorter initial ISI (mean = 24 msec) separating the first two spikes. This pattern was also observed during both types of locomotion. The mean initial ISI seen in MNs active during treadmill locomotion was much shorter and less variable(7±2 msec) than that observed during fictive locomotion (29 ± 22 msec). With one exception, MNs which produced less than 10 spikes/burst did not display an initial doublet, whereas the mean initial ISI for those with more than 10 spikes/burst was 11.2 msec. These data suggest that the details of MN firing patterns during locomotion are centrally determined.(Supported by M.R.C. of Canada)

2446 REGIONAL DISTRIBUTION OF MUSCARINIC RECEPTORS IN SPINAL CORD. S. Oguz Kayaalp* and Norton H. Neff. (SPON: D. L. Cheney). Lab. Preclin. Pharmacol., NIMH, St. Elizabeths Hosp., Washington, D.C. 20032

The distribution of muscarinic cholinergic receptors was studied in the major areas of the rat spinal cord at different levels by using the tritiated ligand [^{3}H]quinuclidinyl benzilate (QNB) as a marker. Binding sites were found in both gray and white matter. The binding capacity of the former, however, was about 3-4 times higher than the latter. The area richest in muscarinic receptors was the anterior horn, with a Bmax ranging from 62 to 122 fmol/mg protein which is significantly less than found in brain. Binding was lowest in the thoracic cord when compared with the other areas of the cord. The affinity of the binding sites for QNB seemed to be comparable at different levels with Kd values ranging from 0.06 to 0.09 nM. The binding capacity of the posterior horn was about half the anterior horn. A section of the spinal cord at the thoracic level did not change the binding parameters to a significant extent in gray or white matter. The order of potency of the cholinergic agents in inhibiting QNB binding was as follows: methscopolamine ($IC50=6x10^{-9}$ M)> atropine>oxotremorine>carbamylcholine>nicotine>hexamethonium ($IC50=3x10^{-3}$ M). The results suggest that 1) muscarinic receptor density is lower in spinal cord than in brain, 2) binding sites are quite homogenous with respect to their affinity for the ligand, 3) the binding sites in white matter may be located, at least in part, on glial cells as a section of the axons did not alter binding.

2447 SPATIAL ARRANGEMENT OF ANKLE EXTENSOR SYNERGISTIC MOTOR NUCLEI IN THE CAT LUMBAR SPINAL CORD. William D. Letbetter. Dept. Anat., Emory Univ., Atlanta, GA 30322.

Retrograde labeling with horseradish peroxidase (HRP) was used to identify all the motoneurons belonging to muscles contributing to the Achilles tendon. Medial gastrocnemius (MG), lateral gastrocnemius (LG), soleus (S), and plantaris (PL) muscles of ipsi- and contralateral hindlimbs in each preparation were injected with HRP, both singly and in various combinations, so that a detailed analysis of their spatial organization could be made from 3-dimensional serial reconstructions of the spinal cord segments containing the labeled cells. It was determined that the particular techniques employed in this study yielded quantitative results in agreement with the proposition that all motoneurons (both alpha and gamma) projecting to an injected muscle were labeled.

It was found that in the rostro-caudal dimension, MG motor nucleus is shifted by a fixed relative distance caudally to the LG nucleus with the soleus motoneurons lying in the middle of the region of overlap between the two. Furthermore, in the rostral half of the soleus motor nucleus, both the number of cells and their relative distribution are congruent with those of the rostral part of MG nucleus; likewise, in the caudal half of the soleus motor nucleus, both the number of cells and their relative distribution are congruent with those of the caudal part of LG nucleus. Although these three motor nuclei account for the vast majority of the motoneurons in their particular column (column 5 of Romanes), there are some other large neurons present which were not labeled. That they all contribute to the innervation of tibialis posterior and popliteus muscles as suggested by Romanes has yet to be determined. The number of these unlabelled cells was greatest at the rostralmost and caudalmost limits of the distribution of the ankle synergistic nuclei. The plantaris motor nucleus was found to be layered dorsal to the rostralmost portion of the distribution of the LG motor nucleus in what appeared to be the caudal part of Romanes' column 4. As the rostro-caudal overlap between LG and PL motor nuclei was not very extensive, PL motor nucleus is most aptly described as being spatially "detached" from the MG-LG-S motor nuclear complex.

This work demonstrates clearly that it is now possible to map accurately and in quantitative detail the spatial organization and interrelationships of motor nuclei in the spinal cord. It offers new hope of discovering at least the architectural, and even perhaps the functional organizational scheme of the spinal motor apparatus.

(Supported by NS 11949 from NINCDS)

2448 SPINAL TERMINATIONS OF SUBCUTANEOUS HIGH THRESHOLD MECHANORECEPTORS. A. R. Light, S. Mense* and E. R. Perl. Dept. Physiology, Univ. North Carolina, Chapel Hill, NC 27514 and Physiologisches Institut d. Univ., 2300 Kiel, West Germany.

The spinal terminations of cutaneous receptors with fine myelinated fibers have recently been described in detail (Light and Perl, J. Comp. Neurol. 186, 1979). To compare the terminal distributions of slowly conducting afferent units originating in deep tissues, identified units innervating the tail of anesthetized cats were stained with horseradish peroxidase (HRP). After laminectomy, impulses from fibers close to the dorsal root entry zone were recorded extracellularly with a glass micropipette containing HRP solution. Once a presumed deep unit was found, the overlying skin was carefully removed and the subcutaneous tissues stimulated directly. Upon satisfactory physiological characterization of a unit, HRP was injected intraaxonally by iontophoresis. The tissue was processed histologically with diaminobenzidine.

The deep sensory receptors stained had dorsal root fibers conducting at 46.0-13.1 m/s. Most responded like cutaneous high threshold mechanosensitive (HTM) receptors, in that they required strong pressure to be activated. Their receptive fields were discontinuous and were located in muscles, tendons, joint capsules, or connective tissue of the tail. Vigorous bending of the tail was not excitatory.

The central terminals of deep HTM receptors branched extensively in the dorsal spinal gray matter. Very many en passant enlargements and terminal boutons indicative of synaptic contacts were found ipsilaterally in lamina I and in a region dorsal to the central canal; a few were in lamina II. Less numerous boutons and enlargements were also present in ipsilateral lamina V and in contralateral laminae I and V. In contrast, secondary muscle spindle fibers that conducted at velocities comparable to rapid HTM receptors terminated principally in the intermediate zone of the spinal gray matter and in the ventral horn.

These findings suggest no substantial difference between the spinal terminations of deep and cutaneous high threshold mechanoreceptors. Both end in lamina I and (to a lesser extent) in lamina V, regions implicated in nociception. The presence of numerous terminations from both deep and cutaneous nociceptors in the superficial layers of the dorsal horn underlines the importance of this region as a site for integration of afferent information.

This investigation was supported by a research grant (NS10321) and general facilities were supported by a Program Project grant (NS14899), both from the NINCDS of the USPHS. S.M. received a grant (Me 492/5) from the Deutsche Forschungsgemeinschaft.

2449 HOW THE SIZE OF MOTONEURONS DETERMINES THEIR SUSCEPTIBILITY TO DISCHARGE. Hans-R. Lüscher*[1], Paul RUENZEL* and Elwood Henneman. Dept. of Physiology, Harvard Medical School, Boston.

Volleys of Ia impulses evoke aggregate EPSPs in motoneurons (MNs), whose amplitudes correlate inversely with cell size and directly with susceptibility to discharge. The following hypothesis is proposed to explain the basis for these relationships. (1) Ia fibers approaching a MN branch more frequently and send more terminals to cells with larger surface areas; (2) the more branching there is, the greater is the possibility that impulses in these fibers will fail to invade all the terminals; (3) the percentage of synaptic knobs activated by Ia impulses is, therefor, determined by the branching pattern in the terminal arborization and by factors that influence invasion of terminals. To test this hypothesis, the sizes of aggregate EPSPs in MNs before and after post-tetanic potentiation (PTP) were related to the input resistance (IR) of the cells, which was measured by the "spike height" method. Ventral roots of L_7 and S_1 were severed to eliminate backfiring of MNs, which interferes with recordings of EPSPs. Hindlimb muscle nerves in cats were stimulated at 1/sec with shocks that set up afferent volleys in all their Ia fibers. A series of 16 monosynaptic aggregate EPSPs was recorded from the MN and averaged electronically. The muscle nerve was then tetanized with similar shocks at 500/sec for 10 sec. Thereafter another series of 16 EPSPs was recorded and averaged. As our hypothesis predicts, the smallest control EPSPs and the greatest % potentiations occurred in MNs with the lowest IRs, i.e., in the largest cells. It has generally been assumed that PTP is due to release of more transmitter from a fixed number of active endings. This theory predicts that the magnitude of PTP is independent of cell size and now appears untenable. With few exceptions PTP increased the rise-times and half widths of EPSPs, effects consistent with invasion of additional terminals. The findings suggest that invasion of Ia terminals is a graded process that is more complete in arborizations on small cells, which explains their greater susceptibility to discharge.

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[1]Supported by the Swiss National Science Foundation.

2450 EVIDENCE FOR FOUR DIRECT BRAINSTEM PROJECTIONS TO THE INTERMEDIOLATERAL CELL COLUMN WITH NOTES ON THEIR DEVELOPMENT. George F. Martin, Dept. Anat., Sch. Med., The Ohio State University, Columbus, Ohio, 43210.

The horseradish peroxidase method was used to identify those areas of the brainstem which project to the thoracic cord and would thus be possible sources for direct projections to the intermediolateral cell column. Injections of ^{3}H-leucine were made into all of the areas identified by the HRP technique and the resulting autoradiograms show that the intermediolateral cell column is innervated by at least four areas. Injections which include the nucleus coeruleus (Coe) and closely adjacent areas elicit labelling of the ipsilateral intermediolateral cell column. Bilateral labelling of the same region is present after injections of the nucleus reticularis gigantocellularis pars ventralis (RGcv), the nucleus obscurus raphae (RaO) and the rostral part of the lateral reticular nucleus (RL). Fluorescence microscopy reveals that each of the above areas contain neurons of either the catecholamine (Coe, LR) or indolamine (RGcv, RaM, RaO) type. Since fluorescent varicosities of both types pack the intermediolateral cell column and it is known that they arise within suprasegmental centers, it seems reasonable to conclude that they take origin from the areas referred to above. Developmental studies of pouch-young opossums show that fluorescence appears in sympathetic ganglia of the peripheral nervous systems very early (11 days after conception) and that the intermediolateral cell column is one of the first targets of brainstem-spinal neurites. Supported by U.S.P.H.S. Grants NS-07410 and NS-10165.

2451 CONDUCTION STUDIES IN ISOLATED PERFUSED SPINAL CORD AND IMPLICATIONS FOR THE CAUSES OF IRREVERSIBLE SPINAL INJURY. H.F. Martin, S. Katz, and J.G. Blackburn. Department of Physiology, Medical University of South C.rolina, Charleston, South Carolina 29403.

Irreversible effects of spinal cord trauma are thought to be due to either direct damage to neural structures (i.e. membrane disruption) or to necrosis secondary to anoxia following microvascular injury. The latter hypothesis has been investigated by subjecting the spinal cord to anoxia separate from trauma. We have developed a model to investigate the former hypothesis by allowing traumatic damage without anoxia.

Excised spinal cord strips from cats or rats were placed in a chamber perfused by oxygenated Ringer's solution. Stimulating and recording electrodes were positioned to measure axonal conduction along the length of the strip. Results of these experiments have shown that conduction can be maintained for an excess of four hours provided adequately oxygenated fluid remains in contact with the tissue. Raising the cord segment into a layer of mineral oil attenuates conduction within 30 minutes, however, conduction can be restored by renewed perfusion by oxygenated Ringer's solution.

This model forms the basis for continuing investigations into the temporal sequence of changes in neural conduction following trauma. (Supported by NINDS grant #P-5P81-NS-11066).

2452 THE EFFECTS OF EXPERIMENTAL SPINAL CORD INJURY ON FIBER DENSITIES & DIAMETER DISTRIBUTION IN FELINE SPINAL CORD. Eugene D. Means & Douglas K. Anderson, Dept. Neuro. & Physiol. VA Hosp., & Univ. Cinn. Sch. Med., Cincinnati, OH. 45220

The differential effects of compression & ischemia on nerve fibers have been incompletely studied. Most data has been derived from work on the peripheral nervous system. To determine the effect of experimental spinal cord compression injury on feline spinal cord white matter, fiber densities & the fiber spectrum (diameter distribution) was computed for the dorsal column of normal & injured animals. Adult, female, mongrel cats were subjected to laminectomy at the upper lumbar area & the wound was either closed or the spinal cord was compressed using a 170gm/wt for 5 minutes. At 30, 45 & 60 days post-compression, the animals were sacrificed under pentobarbital anesthesia by intra-aortic perfusion fixation using buffered 5% gluteraldehyde. Transverse sections of SC were removed from the T_8 & C_3 levels, post-fixed in osmium & embedded in epon. Whole transverse sections of spinal cord were cut at 1 micron, stained with toluidine blue & mounted on glass slides. Using a Zeiss photomicroscope, every 6th microscopic field was systematically sampled beginning initially with any field between 0 & 9 chosen ramdomly. The photographs were enlarged to approximately 2100 x. Using a MOP-3 (Kontron) image analyzer the fiber diameter distribution was recorded from the photographic enlargements using a logarithmic mode. The dorsal columns of normal (laminectomy alone) spinal cord contained from 39,000 to 56,000 myelinated fibers/mm^2. Fiber diameters ranged from 0.5-15 micra, the majority falling in the 2-5 micron range. Injured animals showed an average fiber density in the dorsal columns of 8000 myelinated fibers mm^2. Myelinated fibers of all diameters showed a reduction in number but there was a statistically significant differential loss of fibers in the 2-4 micron range. Previous studies on peripheral nerve have shown a susceptibility of large myelinated fibers to compression injury while smaller myelinated fibers have been more susceptible to ischemia. The pathogenesis of experimental spinal cord injury is unclear but ischemia plays a significant role in the auto-destructive process following blunt trauma. Although fibers were decreased over the entire fiber spectrum, the disproportionate loss of small myelinated fibers suggests that ischemia may play a more significant role than compression in experimental spinal cord injury. Moreover, the almost total absence of degenerating fibers at the C_3 level at 60 days post-compression suggests that Wallerian degeneration was complete by this time. Supported in part by the VA & a grant from the Paralyzed Veterans of America & the Upjohn Co.

2453 A MORPHOLOGICAL STUDY OF CAT DORSAL SPINOCEREBELLAR TRACT NEURONS AFTER INTRACELLULAR INJECTION OF HORSERADISH PEROXIDASE. Vjekoslav Miletić, Mirjana Randić and Arthur D. Loewy. Dept. Vet. Physiol. & Pharmacol., Iowa State University, Ames, IA 50011 and Dept. Anat. & Neurobiol., Wash. Univ. Sch. Med., St. Louis, MO 63110.

This work represents an attempt to elucidate structural features of electrophysiologically characterized, individual cat dorsal spinocerebellar tract (DSCT) neurons by using intracellular application of horseradish peroxidase (HRP).

The experiments were performed on thirty adult spinalized cats. After a lumbar laminectomy (L2 - L3 Level), intracellular recordings and HRP injections were made in DSCT neurons of the Clarke's column, which were identified by antidromic invasion following electrical stimulation of the ipsilateral dorsolateral funiculus. In addition, sensory inputs to DSCT neurons were determined by natural (adequate) stimuli applied to the left hind limb with intact innervation. Horseradish peroxidase was injected intracellularly into the DSCT neurons by passing a positive current of 16-18nA for 5-10 minutes through the microelectrode filled with 25% HRP (Boehringer-Mannheim) in 0.5M KCl. After a survival period of 1-6 hours the animals were perfused with a solution of 2.5% paraformaldehyde and 1% glutaraldehyde in 0.1M phosphate buffer at pH 7.4. The frozen spinal cord was cut horizontally in 100μm thick sections with a microtome and the sections processed for HRP visualization under the light microscope using the diaminobenzidine (DAB) cytochemical technique.

The morphological analysis is based on data obtained from 13 successfully labeled DSCT neurons. Ten of these units received excitatory sensory inputs from muscle receptors, while the remaining three units had either cutaneous or convergent (muscle + cutaneous) inputs. The DSCT neurons that received excitatory muscle receptor inputs are equivalent to the large Clarke cells (class C of Loewy, J. Comp. Neur., 139: 53, 1970). Their dendrites were oriented primarily in the rostrocaudal direction and generally smooth except for some branchlets. In two of these cells, the axon was traced into the lateral funiculus. There was no evidence that axonal collaterals arose from these axons during the initial trajectory through the spinal gray matter. One of the three cells that received cutaneous inputs had the morphological characteristics of a B' cell while the other two were similar in appearance to the Clarke cells.

Supported by NSF Grant BNS 23871 and USPHS Grant NS12751.

2454 A CELLULAR SOURCE FOR SPONTANEOUS SPINAL CORD SLOW WAVES. J.T. Molt, D.A. Poulos and R.S. Bourke, Division of Neurosurgery, Albany Medical College, Albany, New York 12208.

Spontaneous, negative-going, slow waves are the characteristic components of the spontaneous spinal electrogram (SEG) as recorded from the dorsal surface of the spinal cord. It has been demonstrated that spontaneous slow waves increase in frequency of occurrence in the intact cord caudal to a site subjected to blunt trauma. The degree of change in frequency of occurrence of slow waves appears to correlate well with the extent of the lesion that results from blunt trauma. Because of this the SEG may be an important diagnostic tool in the evaluation of spinal cord injury and in the determination of effectiveness of treatment modalities. It has been shown by mapping the amplitude of slow waves as a function of depth within the cord that neurons in the dorsal gray matter may be a source of the waves. This experiment was designed to establish the cellular source of the slow potentials.

In anesthetic-free, decerebrate cats with a spinal transection at the T12 level, slow waves were recorded from a silver ball electrode on the exposed surface of the cord 2mm lateral to the midline at the L6 segment. Less than 1mm from this site a microelectrode was inserted into the cord and the spontaneous discharges of single neurons were recorded. Many neurons were sampled within a dorsal-ventral track through the gray matter, while simultaneously recording the SEG. Time intervals between successive unit firing and between successive peaks of slow potentials were measured using a PDP-12 computer. Statistical tests were performed on the time intervals.

The results show that the intervals between successive slow waves are not Poisson distributed; time interval histograms show significant bimodality. The great majority of spontaneously active cells have time intervals that show a Poisson distribution. However, in all tracks cells were encountered that showed histograms with definite bimodality. Cross correlation analyses indicate that firings in these cells correlate with the occurrence of slow waves. Cross expectation density histograms show the correlation to occur with delays of less than 10 msec regardless of whether the slow waves or the units are treated as the generating source. Electrolytic lesions placed at sites where non-Poisson unit activity was recorded show these units to be located in lamina IV of the dorsal horn.

These results suggest that a population of units in the dorsal gray matter show spontaneous activity that is reflected in a surface slow wave. That the occurrence of the slow wave can slightly precede, coincide with, or slightly follow the occurrence of unit firings indicates that the slow wave may result from summated excitatory post-synaptic potentials in these cells. Supported by NINCDS grant 13042.

2455 DEXAMETHASONE PREVENTS LOSS OF AXONAL CONDUCTION AND REFLEX ACTIVITY, AND REDUCES SPREAD OF STRUCTURAL DAMAGE IN ACUTE SPINAL CORD TRAUMA. A. C. Nacimiento, M. Bartels*, and H. D. Herrmann*. Departments of Physiology I and Neurosurgery. Saarland University, School of Medicine, 6650 Homburg (Saar) F.R.G.

A short-lasting compression of spinal L7 segment in the cat under conditions allowing quantitative monitoring of ensuing functional and structural changes (cf. Soc. f. Neurosci. Abstr., 1978, 4, 1828) was used to assess the effects of dexamethasone in CNS trauma. In control experiments a compression of 3 mm brought about immediate loss of polysynaptic reflex discharge and axonal conduction across the damaged segment. Some partial recovery of both functions took place about 2 hrs following trauma, with further decline and disappearance within the 6 hrs observation period. Main structural changes at this point were hemorrhages, edema and axonal disruption and swelling. The i.v. administration of dexamethasone (4 mg/kg) 30 min after compression brought about the following changes: a) the phase of partial recovery of polysynaptic reflex activity was prolonged throughout the 6 hrs period; b) similarly, decline and loss of axonal conduction following improvement was largely prevented; c) histopathological changes were circumscribed to the point of striking discreetness. It is suggested that dexamethasone counteracts the typically progressive tendency of functional and structural derangement following localized spinal cord trauma. Dexamethasone may exert these effects upon both functionally damaged and surrounding undamaged neuronal elements and axonal bundles in the lesioned segment. As a result improvement in synaptic transmission as well as in axonal excitability may be different expressions of a direct effect of dexamethasone on defined neuronal membrane properties.

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2456 IMMUNOFLUORESCENCE STUDIES WITH GFA AND NEUROFILAMENT ANTISERA IN RATS WITH SPINAL CORD TRANSECTION. Chi Nguyen*, Doris Dahl, Bich Nguyen* and Amico Bignami. Spinal Cord Injury Service, West Roxbury Veterans Administration Medical Center and Harvard Medical School, Boston, Ma. 02132.

Antisera to neurofilament (NF) and gliofilament (GFA) proteins were used to examine with specific staining procedures the axonal and glial changes in rats with spinal cord transection. The NF antisera, raised against urea-soluble chicken brain antigen (J. Comp. Neurol. 176, 645, 1977), have recently allowed the isolation of the ∿ 70K polypeptide in bovine brain filament preparations by immunoaffinity chromatography (Trans. Am. Soc. Neurochem. 10, 141, 1979). Preliminary experiments showed the ability of the NF antiserum to stain regenerating peripheral nerve fibers by immunofluorescence. Following transection of the sciatic nerve and disappearance of the degenerated fibers, thin regenerating axons stood out prominently on a dark background in the distal stump. Transection of the posterior columns in the rat spinal cord at the low thoracic level resulted in the loss of immunofluorescence with NF antisera together with the appearance with GFA antisera of elongated hypertrophic astrocytes (isomorphic gliosis) in the fasciculus gracilis at the cervical level. With NF antisera the areas of tissue necrosis at the site of transection were characterized by the accumulation of brightly immunofluorescent round structures (axonal spheroids) often collected in clusters. With GFA antisera there was a mesh of glial fibers surrounding the areas of necrosis and diffuse astrocytic hypertrophy in the surrounding tissues. In all operated animals a few regenerating axons were seen. Although the posterior roots were not directly involved by the surgical lesion, regenerating fibers were invariably associated with cells similar to fibroblasts. In order to confirm the ability of centrally cut axons to undergo regeneration, autogenous sciatic nerve grafts and centrally connected posterior root stumps were inserted in the spinal cord. Vigorous axonal growth was observed not only in the graft but also in the surrounding spinal cord. In the spinal cord, axonal growth was associated with bundles of fibroblast-like cells. Supported by USPHS grant NS 13034 and by the Veterans Administration.

2457 THE PHRENIC NUCLEUS OF THE GUINEA PIG: SEGMENTAL LOCALIZATION OF AFFERENT AND EFFERENT CELL BODIES. E.S. Nylen*, L.S. Sigmund*, D.D. Rigamonti, C.C. Kao*. Depts. of Anat. and Neurosurg., Georgetown Med. Sch. and Veterans Admin., Washington, D.C. 20007.

Although the peripheral contributions of the phrenic nerve in the guinea pig(C4, C5, C6) have been documented by Cooper and Schiller(1975), knowledge regarding its central origin is unknown. The extensive body of information that exists for other mammals, including man, suggests that the phrenic nucleus constitutes a discrete, intermediately placed column of cells in the ventral horn. The longitudinal distribution has been observed rostrally to the third and caudally to the seventh cervical segments. Furthermore, the longitudinal placement of motor neurons does not always correspond to the ventral rami contributing to the phrenic nerve(Ullah, 1978). The ratio of afferent to efferent fibers of the phrenic nerve has not been reported in the guinea pig.

The present study exploited the HRP technique to segmentally localize the sensory and motor cell bodies giving rise to the phrenic nerve. Thoracotomies were performed aseptically on animals anesthetized with a combination of chloral hydrate and Inovar. HRP pellets were placed on crushed portions of the exposed right phrenic nerve and the enzyme isolated from the adjacent tissues by a silicone cuff. Allowing sufficient time for HRP transport, the animals were sacrificed and the cervical cord along with the right dorsal root ganglia were removed. Histological preparation follows Mesulam's technique. Longitudinal and transverse sections were made defining rostral to caudal and medial to lateral extensions of the motor cell bodies. The number of cells stained in the dorsal root ganglia were counted and segmentally defined.

Our data indicates that the longitudinal distribution of the motor cell bodies extends further rostral than the previously reported peripheral contributions would suggest. The morphological aspects of this study will be discussed in connection with data existing from other mammals. (Supported by the Veterans Administration and NIH Grant NS14413-02).

2458 SPINAL CORD AMINO ACID LEVELS IN NORMAL AND DIABETIC RATS. James T. Patrick*, David L. Felten, Michael A. Rea* and William J. McBride (SPON: Francis J. Fry). Departments of Anatomy, Psychiatry and Biochemistry and Institute of Psychiatric Research, Indiana University School of Medicine, Indianapolis, IN 46223.

Levels of the amino acids alanine (Ala), glycine (Gly), gamma-aminobutyrate (GABA), aspartate (Asp) and glutamate (Glu) were determined in thoracic spinal cord gray areas of normal and streptozotocin-induced diabetic rats. Using a dissecting microscope mounted on the outside of a specially constructed cold-box, microdisks of tissue were punched out from the dorsal gray (DG), intermediate gray (IG) and ventral gray (VG) using a modified needle (i.d. 330μm) with stylet. In the spinal cords from normal rats, an uneven distribution of amino acids was observed (Table): the level of GABA was significantly higher in the DG than in the IG and VG; the levels of Asp were significantly higher in the IG and VG than in the DG; the level of Glu was significantly higher in the IG than in the DG and VG; although there was no significant difference in the levels of glycine, there appeared to be a trend towards higher levels in the VG and IG.

Distribution of Amino Acids in Rat Thoracic Spinal Cord

Area	Ala	Gly	GABA	Asp	Glu
DG	7.4±0.9	48.7±3.8	18.1±0.8*	22.8±2.3*	85.1±6.1*
IG	6.8±0.7	67.7±8.1	12.2±1.2***	34.2±3.7	103.1±7.8***
VG	5.5±0.4	64.6±7.4	6.2±0.5**	32.4±0.9**	80.7±2.8

Levels of amino acids are expressed as nanomoles/mg protein ± SE; significance ($p<0.05$) for DG vs IG (*), DG vs VG (**) and VG vs IG (***).

In the diabetic rats, neuronal counts revealed a decrease of alpha motoneurons by 42% in the VG and a decrease of interneurons by 11% in the IG and VG (D.L. Felten, Clin. Res. 26: 67A, 1978). A comparison of the levels of the five amino acids in the DG, VG and IG of the normal and diabetic rats showed no significant difference. This suggests a nonselective diabetogenic loss of neurons. (Supported by NIH Grant P60 AM 20542 and Alfred P. Sloan Foundation Fellowship (DLF)).

2459 PHASE RELATIONSHIPS OF MOTONEURON, RENSHAW CELL AND Ia INHIBITORY INTERNEURON ACTIVITY PERIODS DURING FICTIVE LOCOMOTION IN MESENCEPHALIC CATS. Carol A. Pratt* and Larry M. Jordan, Dept. of Physiology, Univ. of Manitoba, Winnipeg, Canada, R3E OW3.

Our understanding of the neuronal circuits which comprise the spinal locomotion pattern generator in mammals has been handicapped by a lack of information on the activity patterns of identified lumbosacral interneurons (INs) during locomotion. Renshaw cells (RCs) (McCrea and Jordan, Can. Fed. Biol. Soc., 146, 1976) and Ia inhibitory interneurons (IaINs) (Feldman and Orlovsky, Brain Res., 84, 1975) have been shown to be rhythmically active during locomotion in the absence of cyclic afferent input, suggesting that they might operate as part of a central locomotion program. The role of these inhibitory INs in the control of locomotion is unknown, however. Attempts have been made in the present study to determine the function of these INs by analysing the discharge patterns of IaINs, RCs and MNs and the phase relationships of their activity periods during locomotion.

Locomotion was evoked by stimulation of the mesencephalic locomotor region (MLR) in decerebrate (precollicular-post-mammillary) cats. Rhythmic motor axon activity in an L6 ventral root filament was identified as being either flexor or extensor coupled, and then the cat was paralyzed (Flaxedil, i.v.) and artificially respired. MLR-evoked rhythmic activity in the filament provided a monitor of "fictive" locomotion. Neurons in the lumbar enlargement were recorded via glass micropipettes and identified by their responses to stimulation of various hindlimb nerves and to antidromic stimulation of cut ventral roots (L5-S1). All three cell types were recorded in the same preparation which allowed the activity of each cell to be compared to a common ventral root filament. By normalizing each step cycle, the phase relationships among cells recorded throughout an individual experiment could be determined.

RCs fired in phase with the MNs to which they were coupled with their periods of peak discharge tending to coincide with the onset of hyperpolarization of these MNs. Extensor-coupled IaINs were found to exhibit maximal firing rates in phase with the hyperpolarization of antagonist MNs. These data suggest that RCs and IaINs may function to curtail spurious MN firing by contributing to MN hyperpolarization during the appropriate phases of the step cycle. Examples were found which suggest that RC inhibition of IaINs may be a determinant of IaIN rhythmic activity during locomotion. (Supported by M.R.C. of Canada).

2460 ULTRASTRUCTURE OF SYNAPTIC TERMINATIONS OF FUNCTIONALLY IDENTIFIED FINE MYELINATED AFFERENT FIBERS. M. Réthelyi*, A. R. Light and E. R. Perl. Department of Physiology, University of North Carolina, Chapel Hill, NC 27514.

Physiologically identified axons from cutaneous high threshold (nociceptive) and "D-hair" mechanoreceptors of cat were stained intracellularly by iontophoresis of horseradish peroxidase. Histochemical reaction produced an electron-dense staining of the fibers and their terminations, traceable within the spinal dorsal horn. Ultra-thin serial sections were made of axonal branches selected with light microscopy.

Synaptic enlargements of high threshold mechanoreceptors are found principally in the ipsilateral marginal zone and the neck of the dorsal horn; boutons in the marginal zone contain round synaptic vesicles and, occasionally, dense-core vesicles (90 to 100 nm in diameter). The terminals often partially enclose dendritic profiles, greatly enlarging the contact area.

Most "D-hair" terminals are found in lamina III, with some extensions into adjacent parts of laminae II (inner substantia gelatinosa) and IV. The boutons are round, lack enclosing processes, and are filled with clear, round vesicles. Often, a bouton makes synaptic contacts with several dendritic structures. Some "D-hair" boutons are contacted by profiles containing flattened vesicles.

Thus, even though "D-hair" and high threshold mechanoreceptors have axons with overlapping (slow) conduction velocities, they differ not only in their regions of termination, but also in the morphology and ultrastructure of their central synaptic terminations.

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2461 SPINAL CORD RECONSTRUCTION: ELECTRICAL ADJUNCTIVE TECHNIQUES. D.D. Rigamonti, C.C. Kao*, R.O. Becker*, J.R. Wrathall*, M.R. Braford. Veterans Admin. Medical Centers (Syracuse and Washington, D.C.) and Depts. of Anat. and Neurosurg., Georgetown Medical School, Washington, D.C. 20007.

The delayed nerve graft technique is an experimental procedure in which viable nervous tissue is transplanted into the gap of a contused or transected spinal cord in an attempt to restore functional continuity between the injured cord stumps (Kao, 1974). This technique is routinely used in our laboratory in an attempt to restore function to paralyzed adult mammals. An adjunctive technique now under investigation is the use of electrical devices to enhance the wound healing process and subsequently promote axonal elongation into the nerve graft.

A brief description of the surgical procedure is as follows: Adult cats anesthetized with Ketamine are injured either by contusion or subpial transection and a forelimb nerve (future nerve graft) is transected in the axilla. Reconstructive surgery follows at periods varying from one to two weeks at which time "non-neuronal cells" (Wrathall, et al, 1978) are placed in the proximal and distal interface of the cord-nerve graft site. D.C. field electrodes with an output of 100 and 500 nA have been implanted during reconstructive surgery. The device, measuring 2 by 0.8 cm encapsulated in medical grade silicone, was placed between the paraspinous muscles and the spinous processes rostral to the implant site. The cathode was a 0.5 mm diameter silver wire insulated with Teflon with an exposed tip of 2.0 mm. The cathode was placed in the interface between the proximal cord stump and the nerve graft perpendicular to the long axis of the cord at a depth of 3.0 mm. The anode consisted of a loop of uninsulated silver wire with a length of 1.5 cm and a diameter of 0.5 mm. Histological reconstruction of the repaired spinal cord suggests that wound healing was improved. A second prototype is currently being constructed using platinum electrodes closely applied to the outer surface of the dura and completely encircling the cord and nerve graft. The electrode designs and significance of the histological findings will be discussed. (Supported by Veterans Administration, NIH,PCR, and NASA).

2462 VENTRAL HORN CELLS OF THE CERVICAL CORD PROJECT TO BRAINSTEM. Martine J. RoBards, Mark Stritzel,* and Richard T. Robertson. Dept. Anat., Calif. College of Med., Univ. of Calif., Irvine, Calif., 92717.

Large injections of horseradish peroxidase (HRP) into the upper brainstem and thalamus of adult opossums and hooded rats produce retrogradely labelled neurons deep in the intermediate gray zone and ventral horn of the spinal cord between C1 and C4. When the sensitive chromogen tetramethylbenzidine (TMB) is used in the HRP reaction, the fine processes of the filled neurons are visible. Several of the reactive neurons clearly show two axons; one exiting the cord in the ventral root, and another, crossing in the anterior gray commissure, ascending to the upper brainstem or thalamus.

Injections of HRP into trapezius, sternocleidomastoid, and splenius capitus muscles were used to identify the cervical ventral horn neurons projecting to neck muscles in the hooded rat. Filled cells were found throughout the ventral horns of C1 through C4, generally in a stratum deeper than that occupied by brainstem-projecting cells, but nonetheless showing spatial overlap and morphological similarity. Again, in TMB-reacted sections, cells were seen which had two axonal processes, one exiting in the ventral root and another crossing in the anterior gray commissure, the latter's destination uncertain.

Double-labelling experiments will be required to define with certainty the proportion of motor neurons in the upper cervical cord possessing both ascending and peripheral projections; and the precise loci of termination in brainstem or thalamus of these ventral horn cells remains to be disclosed.

2463 Detection of Sub-clinical Spinal Tract Sensory Dysfunction. Richard J. Schneider and Ronald Burke*. Div. Neurosurg., Dept. Surg., Univ. Md. Sch. Med., Baltimore, MD 21201.

The ability of humans and rhesus monkeys to discriminate between two somatosensory stimuli was examined in the context of signal detection theory (TSD). The subjects were seated with one leg restrained as a discrete group of hair shafts were stimulated in an oscillatory fashion. Two different frequencies of stimulation were used. The discrimination task required that the subject push a button in response to the "go" stimulus and refrain from pushing it in response to the "nogo" stimulus. Response latencies were recorded in 100 msec. time bins which formed the basis of a confidence rating scale and allowed the sensitivity of each subject to be characterized in the form of a receiver operating curve (ROC). A population of normal human subjects was compared with a population diagnosed to have multiple sclerosis. Monkeys were tested prior to and following surgically produced lesions of the spinal cord.

Normal subjects were found to generate reliable ROCs with duplicable d's regardless of response bias (β). These subjects responses indicated an ability to make the discrimination even when their subjective assessment of their performance was poor. Moreover, a varying discriminative ability, from adequate to extremely acute was shown. There was some evidence that younger subjects made the discrimination with greater facility than older ones. In comparison, the group of MS patients showed a diminished ability to make the same discrimination. Their d's were reduced, and sometimes the ROC showed that they were discriminating at random. Additionally, there appeared to be two sensory thresholds. One, seen at low frequencies and amplitudes, and another as both or either variable increased. The data from the MS population also indicated that sensory discriminative ability may diminish with repeated trials or with time.

We conclude that TSD may be utilized to produce a high-resolution, objective method for detecting spinal cord sensory injury. These results, as depicted in ROCs, clearly showed a difference between human populations. Moreover, as motor fatigue is well-documented in MS, there may be a sensory nervous system fatigue reflected by diminishing discriminative ability of the MS population as the test went on. Finally, we believe the first threshold to be related exclusively to hair-follicle stimulation and from our previous studies we think it reflects only dorsal funiculous function. We believe the second threshold is a skin or touch receptor threshold which involves other sensory pathways in addition to the dorsal funiculi.

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2464 THE PHRENIC NUCLEUS OF THE DOG: SEGMENTAL LOCALIZATION OF AFFERENT AND EFFERENT CELL BODIES. L.S. Sigmund*, E. Nylen*, D.D. Rigamonti, C.C. Kao*. Depts. of Anat. and Neurosurg., Georgetown Med. Sch. and Veterans Admin., Washington, D.C. 20007.

Our earlier studies on the activation of the crossed phrenic pathway have prompted this present investigation to define the anatomical location of the phrenic nucleus in the dog and to determine the ratio of afferent to efferent nerve fibers supplying the diaphragm. Identification of the roots of origin and peripheral distribution of the phrenic nerve is well documented. However, the literature is noticeably lacking in conclusive evidence of the exact segmental location and extent of the phrenic nucleus. There appears to be almost complete agreement that the phrenic cell bodies lie in the fourth cervical segment, irrespective of species(Warwick et al.). Marinesco(1898) proposed that the distribution of these cell bodies further extended thru the fifth and sixth cervical segments in the dog. In regard to the ratio of afferent to efferent fibers, Landau, using the deafferented dog, found that efferent fibers comprised 55-65% of the fibers supplying the diaphragm.

The HRP technique was used to further define the afferent and efferent nerve supply and the segmental extent of the phrenic nucleus. The phrenic nerve was exposed in the thoracic cavity of anesthetized dogs. HRP pellets were placed on crushed portions of the phrenic nerve and subsequently isolated from surrounding tissue. Following sacrifice, the cervical spinal cord and accompanying dorsal root ganglia ipsilateral to the operated phrenic nerve were removed and histological processing was performed utilizing Mesulam's technique. Longitudinal and transverse sections were made to define both the rostral to caudal and medial to lateral extent of the nucleus. Dorsal root ganglia were also processed. The comparison of reactive dorsal root ganglia cells to phrenic nuclear cells in the corresponding spinal cord segment determined the ratio of afferent to efferent fibers. The significance of this study will be discussed as it relates to the surgical manipulations(phrenicotomy, phrenectomy, and dorsal rhizotomy) currently used to activate the latent crossed phrenic pathway. (Supported by the Veterans Administration and NIH Grant NS14413-02).

2465 RESPONSES OF EXTRACELLULAR CALCIUM ACTIVITY $[Ca^{2+}]_o$ DURING AFFERENT NERVE STIMULATION AND DURING SEIZURES IN SPINAL CORD OF CATS. G. Somjen, Dept. Physiol. Duke Univ. Med. Center, Durham, N.C. 27710.

It has been reported that $[Ca^{2+}]_o$ declines during stimulation and during seizures in cerebral and cerebellar cortex and in sympathetic ganglia[1,2,3]. I used Ca-selective microelectrodes to measure $[Ca^{2+}]_o$ in gray matter of unanesthetized decapitate spinal cords. Electric potential was recorded DC coupled from the reference barrel of the microelectrode. VRs were cut and the sciatic nerve stimulated repetitively. Changes of $[Ca^{2+}]_o$ were small and often required electronic averaging for detection. In some preparations repetitive stimulation was consistently associated with decreases of $[Ca^{2+}]_o$. Sustained potential (SP shifts of -1.5 to -6.0 mV were then associated with $\Delta[Ca^{2+}]_o$, between -10 and -50 μM. The timecourse of $\Delta[Ca^{2+}]_o$ was slower than that of the SP, and sometimes the minimum of $[Ca^{2+}]_o$ was reached only 1 or 2 sec after cessation of stimulation. In some preparations SP shifts up to-12 mV were not associated with any detectable change of $[Ca^{2+}]_o$ (limit of detection: ± 10 μM). Sometimes very prolonged repetitive stimulation evoked a very slow increase of $[Ca^{2+}]_o$ which could reach, in 35 to 45 sec, up to +120 to +150 μM, was then maintained at a plateau level, and after cessation of stimulation declined equally slowly. Responses of $[Ca^{2+}]_o$ in spinal cord are smaller than those reported for other tissues[1,2,3]. The difference cannot be explained by different total "packing density" of neural elements, for changes of $[K^+]_o$ in spinal cord are comparable to those elsewhere[4]. Opposing processes appear at work, releasing Ca^{2+} into ECF and removing it at the same time but at different rates, either by the same, or by different cellular elements. During seizures induced by i.v. injection of penicillin, decrements of $[Ca^{2+}]_o$ were also quite small and sometimes not detectable. However, in cord as in cortex[1] the decline of $[Ca^{2+}]_o$, if detectable, began before the onset of the SP shift related to a seizure. This is compatible with the concept that penicillin-induced spinal seizures are initiated by inward current in presynaptic terminals which is carried in part by Ca^{2+} (ref. 5). (Supported by NINCDS grant NS 11933)

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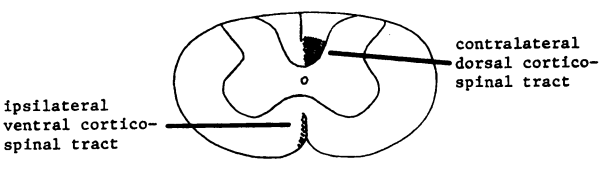
2466 EFFECT OF PRESYNAPTIC INHIBITION ON AXONAL POTENTIALS, TERMINAL POTENTIALS, FOCAL SYNAPTIC POTENTIALS AND EPSPS IN CAT SPINAL CORD. George W. Sypert, John B. Munson and James W. Fleshman. Dept. Neurosci.& Surg., Col. Med., U. Fla.& VA Med. Ctr., Gainesville, FL. 32610

Electrical test stimulation of triceps surae Ia afferents produces terminal potentials (TPs), focal synaptic potentials (FSPs) and excitatory postsynaptic potentials (EPSPs) in L_7 spinal cord laminae VI (interneuronal pool), VII (inhibitory interneuronal pool) and IX (triceps surae motoneuronal pool) and axonal potentials (APs) dorsal to these neuronal pools. Electrical conditioning stimulation (CS) of posterior biceps-semitendinosus (PBST) muscle nerve (4 shocks @ 300 Hz, max. Ia + Ib) reduces the amplitude of MG Ia EPSPs in triceps surae motoneurons (presynaptic inhibition). The present study demonstrates alterations in the latency and amplitude of MG Ia-elicited APs, TPs, FSPs, and EPSPs following CS of PBST in barbiturate-anesthetized normothermic cats. The data were averaged and digitized, using 10 μsec bin widths. In laminae VI, VII, and IX, CS reduces TP latency (20-40 μsec) and TP amplitude (15-25%) associated with reductions in FSP and EPSP amplitudes (15-25%). Similar but progressively diminishing temporal and amplitude changes in APs were observed with increasing distance of the recording microelectrode from these neuronal pools. These data suggest that CS to PBST depolarizes triceps surae Ia terminals in laminae VI, VII, and IX resulting in a shorter latency and reduced amplitude of MG Ia axonal and terminal potentials and, hence, smaller EPSPs due to reduced transmitter release (presynaptic inhibition). Supported by MRS, Veterans Administration.

2467 THE AUTORADIOGRAPHIC DEMONSTRATION OF AN UNCROSSED VENTRAL CORTICOSPINAL TRACT IN THE RAT. H. Lee Vahlsing* and Earl R. Feringa. Depts. Neuro. and Path., V.A. and Univ. of Mich. Med. Ctrs., Ann Arbor, MI 48105.

Twelve-week-old female albino rats were ether anesthetized and immobilized in a stereotactic apparatus. The skull was exposed and a 1 mm burr hole was made 2.5 mm posterior to the coronal suture and 2.5 mm lateral to the sagittal suture directly above the area of cerebral cortex containing the cell bodies that give rise to the corticospinal tract in the rat. Using a micromanipulator and a short beveled, 30-gauge needle, 50 μCi of tritiated proline in 2.5 μl lactated Ringers solution was slowly injected at a depth of 1 mm beneath the pial surface. After a 1 minute delay, the needle was withdrawn and the scalp sutured. After slow axoplasmic transport had labeled the corticospinal tract, each animal was killed by formalin perfusion and the brain and spinal cord removed. Cross sections of the brain and cord were sampled every 2 mm and 10 mm respectively. Sections were cut at 6 μm, mounted on slides, and dipped in NTB2 emulsion. They were exposed for 3 weeks in a light tight box with a CO_2 atmosphere and developed for 30 seconds in developer diluted 1:2 with water. The autoradiograms were examined by dark field microscopy.

The cross-sectional autoradiograms at the level of the injection site showed dense silver grains directly above the region of the injection site and adjacent cerebral cortex. Exiting cortical axons were labeled in the corpus callosum, the ipsilateral internal capsule and the ipsilateral ventral thalamic nucleus. At the level of the mesencephalic-metencephalic junction, only the ipsilateral corticospinal tract was labeled. In the posterior medulla oblongata, most of this tract decussated dorsally and medially to the ventral part of the dorsal white funiculus. However, roughly 10% of the tract continued uncrossed lateral to the ventral median fissure. This uncrossed tract was visible as a discrete fiber bundle in the high cervical region. It became more diffuse and contained fewer fibers in the lower cervical segments and in the thoracic spinal cord. The tract could not be traced below the mid-thoracic level.



2468 REFLEX SPECIFICITY IN THE FROG'S SPINAL CORD. M. Westerfield* and E. Frank. Dept. Neurobiology, Harvard Medical School, Boston, MA 02115.

How specific are synaptic contacts between sensory afferents and motoneurons in the bullfrog's spinal cord? We investigated this question with intracellular recordings from motoneurons innervating forelimb muscles in isolated decerebellate spinal cords. Antidromic activation by stimulation of muscle nerves identified the motoneurons.

The results suggest that the synaptic connections of sensory and motor neurons innervating the triceps muscles of bullfrogs are specific. Triceps motoneurons appear to receive short latency afferent synaptic potentials only from their own muscles. Similarly, triceps sensory neurons make strong, direct connections only with their own motoneurons.

Stimulation of the whole triceps nerve produced short latency EPSP's in motoneurons innervating triceps muscles. These EPSP's were often of sufficient size to orthodromically activate the motoneurons. Most neurons innervating the medial triceps muscle were not antidromically activated by stimulation of the other two branches of the triceps nerve. However, these motoneurons often did receive short latency EPSP's from the other two heads of triceps. Motoneurons innervating the external head of triceps almost always innervated the internal head too. Some motoneurons could be antidromically activated by stimulation of all three branches of the triceps nerve. Stimulation of other nerves in the arm produced longer latency EPSP's and IPSP's and in some cases, orthodromic activation of triceps motoneurons.

Motoneurons innervating other muscles of the front legs received short latency EPSP's from their own sensory afferents and longer latency EPSP's and IPSP's from other nerves. In many cases, single EPSP's could produce orthodromic activation of these motoneurons. Stimulation of triceps nerves produced no observable synaptic potentials in these motoneurons.

The development of this reflex specificity is being investigated.

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2469 EFFECTS OF L-DOPA ON DORSAL HORN UNIT RESPONSES TO INNOCUOUS STIMULATION FOLLOWING PRETREATMENT WITH (1) 6-OHDA (2) FUSARIC ACID. Charles I. Woods*, Charles J. Hodge and Jonathan Delatizky. Dept. of Neurosurgery, Upstate Medical Center, Syracuse, NY 13210.

Studies of L-DOPA effects on sensory transmission suggest that L-DOPA modulates sensory input by releasing noradrenalin (NA) from noradrenergic nerve terminals in the spinal cord. Descending noradrenergic pathways from the locus coeruleus and other ponto-medullary reticular areas are the only source of spinal cord NA. In a previous study we demonstrated that L-DOPA increases the responsiveness of cells which respond only to innocuous stimuli, and depresses the activity of cells which respond to noxious stimuli, and showed that the inhibitory effects of L-DOPA are abolished by pretreatment with the serotonin depletor, p-chlorophenylalanine (PCPA). The present study was undertaken to determine if the effects of L-DOPA are specifically dependent on intact bulbospinal noradrenergic pathways and if these effects are due to NA rather than other metabolites of L-DOPA.

6-hydroxydopamine (6-OHDA), which causes selective degeneration of catecholamine fibers, was administered intracisternally to one group of animals (Breese & Taylor, Br. J. Pharmac. 42:88,1971), and fusaric acid, an inhibitor of dopamine-B-hydroxylase, was administered to another group (Nagatsu et.al., Biochem. Pharmac. 19:35, 1970). Cells were classified on the basis of their response characteristics, group I (cells responding to innocuous stimuli only), and group II (cells responding to noxious stimuli only or both noxious and innocuous stimuli). Cell responsiveness was evaluated using repeated identical deflection of hind limb skin or hair. The stimulus was presented at 5 sec. intervals and recorded for 15 min. prior to and at least 60 min. following I.V. injection of L-DOPA (15 mg/Kg). Average spikes per stimulus and average spontaneous activity were determined for each 5 min block.

Results following injection of L-DOPA: (1) 6-OHDA pretreatment: Most group I cells showed no change or a small decrease in responsiveness, most group II cells showed a decrease in responsiveness, (2) Fusaric acid pretreatment: (only group I cells were studied), most cells showed no change or a decrease in responsiveness.

The results suggest (1) the facilitatory effects of L-DOPA are specifically dependent on intact bulbospinal noradrenergic pathways, (2) the inhibitory effects caused by overflow of 5-HT are not mediated through noradrenergic pathways. The results of the fusaric acid experiments suggest that the facilitatory effects of L-DOPA are due to release of NA and not other L-DOPA metabolites, such as dopamine.

2470 EXPERIMENTAL TETHERED CORD. Shokei Yamada, David Zinke*, Delmar Sanders*. Dept. Neurosurg., Sch. Med., Loma Linda University, Loma Linda, CA 92350.

The syndrome "tethered cord" has been recently recognized as a neurological entity in children manifesting progessive signs, such as: incontinence and motor and sensory changes in the legs. This disorder is often associated with spinal anomalies and an elongated spinal cord. Neurosurgeons have experienced that untethering of the spinal cord often results in improvement of neurological signs.

Since neuronal cells rely absolutely on energy derived from intramitochondrial ADP phosphorylation, we postulated that oxidative metabolism of the tethered cord may be reduced below a critical level, thereby resulting in progressive neuronal injury. We applied the dual wave-length reflection spectrophotometer to the experimental tethered cord in vivo, and monitored changes in the reduction/oxidation ratio of cytochrome a,a_3, the terminal oxidase of the respiratory chain.

Twenty cats were anesthetized initially with ketamine and then with N_2O and O_2, and the lumbosacral spinal cord was exposed. Optical measurements were made at the junction of the lumbar and sacral cord. Tethering was produced by traction applied with 2-0 silk suture, one end tied around the filum terminale, the other passed over a pulley and attached to weights (1-5g). In addition, the animals were subjected to anoxic stress with 100% N_2O inhalation for 2.5 min. Spinal cord potentials were taken from its dorsal surface in response to the L6 nerve root.

Results. 1) The Traction Without Anoxic Stress. No significant changes occurred in redox level of cytochrome a,a_3 with traction 1-3g, whereas 5g traction resulted in a mild reduction. However, this reduction level is far less than the maximum reduction level obtained by anoxia.

2) Traction Under Anoxic Stress. As traction weight was increased, increases in the reduction level of cytochrome a,a_3 during anoxia became slow. Under 4 or 5g traction, changes in reduction levels failed to reach the maximum obtained with traction 0-3g; the interneuron potentials failed more rapidly under 5g traction than under 0-3g traction.

Conclusion. The experimental cord with 5g traction responds to anoxia with only minimum redox change. We believe the spinal cord under this circumstance resembles traumatized or anoxic cord with cytochromes highly reduced, probably due to vascular insufficiency. Untethering improves the oxidative metabolism of the cord, if neuronal mitochondria are not irreversibly damaged.

2471 FUNCTIONAL CHARACTERISTICS OF LARGE AND SMALL NEURONS IN SUPERFICIAL LAMINAE (I-III) OF THE CAT DORSAL HORN. Robert P. Yezierski, Dept. of Physiology and Biophysics, West Virginia University Medical Center, Morgantown, WV 26506.

Adequate stimuli and receptive field (RF) characteristics were determined for single neurons in laminae I-III of urethane anesthesized, spinal cats. Units were characterized while tracking small amplitude changes of waveforms with common functional properties using a real-time waveform recognition technique previously described (Yezierski et. al., 1978). Locations of maximum spike amplitudes were marked with ferric ions and Prussion-blue dots reconstructed using camera-lucida. Recordings were obtained from neurons across the full mediolateral extent of the gray in L6 and L7. Data from amplitude profiles were used in calculating source voltages; these voltages were used as an index to approximate size of neurons from which recordings were made. Results of this study confirm anatomical findings that neurons in these laminae constitute a heterogenous population with respect to size. Seventy-five percent of the neurons in laminae I-III had source voltages in the smallest calculated range; 30% of the neurons in deeper laminae were found in this range. Forty-two percent of the intermediate to large cells in laminae I-III were driven by light touch and noxious cutaneous stimulation; seven percent were driven only by noxious stimuli. Nineteen percent of the small neurons were driven by light touch, pressure, temperature, and noxious inputs. Very few small cells (<8%) responded exclusively to noxious stimuli. The relationships between RF characteristics (length/width ratios, RF area) and distance from toes were similar for both large and small neurons to those reported in deeper laminae (Brown et. al., 1973).

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SYNAPTIC TRANSMISSION

2472 BIPHASIC IPSPS IN RAT HIPPOCAMPAL SLICE. Bradley E. Alger and Roger A. Nicoll. Depts. of Pharm. and Physiol., Sch. of Med., Univ. of Calif., San Francisco, CA 94143.

Iontophoretically applied GABA has been reported to have a biphasic (negative-positive) effect on membrane potential on certain CNS neurons. The depolarizing response has been attributed to the activation of dendritic receptors. It is not known, however, if the biphasic response can be produced synaptically, nor if the postulated dendritic site of mediation is correct.

Using the rat hippocampal slice preparation we have found that a biphasic IPSP in CA1 pyramidal cells results from orthodromic (s. radiatum) stimulation in the presence of 10^{-4} M pentobarbital. The intracellular records were obtained from CA1 cells in slices held fully submerged between two nylon meshes. We have confirmed that iontophoretic application of GABA to the soma produces a negative-positive sequence while iontophoresis in the dendritic region yields primarily a depolarization. Moreover, GABA antagonists block the electrically stimulated biphasic response, further supporting the possibility that GABA is the neurotransmitter involved in it. We found also that the dendritic depolarizing GABA response could be elicited in the presence of a low Ca/high Mg saline which blocked synaptic transmission, implying that these iontophoretic responses were not indirectly produced. These results suggested the depolarizing aspect of the electrically stimulated response was mediated by dendritic receptors. We tested this hypothesis with localized pressure injections of TTX (10^{-5}M) and found TTX could selectively abolish either hyper- or depolarization of the electrically evoked response depending on whether it was applied to the soma or dendritic regions, spectively. Blockage of the depolarizing phase occurred while was still possible to elicit full-sized somatic action potent ls with direct current pulses, indicating the TTX injections w e localized and supporting our hypothesis of a dendritic origin oi the depolarizing phase.

Both negative and positive phases of the stimulated response were associated with a large conductance increase and had inhibitory effects on action potential generation, which were similar to those found to occur with iontophoretic GABA. The depolarizing phase of the synaptic response was readily produced with orthodromic activation, but only rarely and with high stimulus intensities antidromically. This suggests the dendritic receptors may be activated preferentially as part of a feedforward inhibitory network distinct from the classical recurrent one.

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2473 IMPLIED LOCUS OF HABITUATION OF THE MAUTHNER FIBER MEDIATED STARTLE REFLEX IN THE HATCHETFISH, GASTEROPELECUS. E. Aljure, J.W. Day and M.V.L. Bennett, Division of Cellular Neurobiology, Department of Neuroscience, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

Habituation of the Mauthner fiber (MF) mediated startle reflex is a common observation (J. Diamond in Fish Physiology, Vol. V, p. 265, 1971) and "fatigue" of responses of axial motoneurons (MNs) following activation of the ipsilateral MF by spinal stimulation was reported to occur at frequencies of 1/sec or less. We have confirmed EMG depression with intracellular stimulation of single MFs in uncurarized unanesthetized but restrained hatchetfish. EMGs due to activation of axial MNs were recorded from the caudal region. MF action potentials were present in the EMG record with an amplitude of about 0.1 mV, and conduction occurred at frequencies of stimulation much higher than those required for EMG depression. Recovery from depression could occur after rest periods of several seconds. During depression EMG amplitude varied, and maximal and markedly reduced responses were sometimes interspersed during stimulation at constant frequency. Since MFs activate axial motoneurons monosynaptically (cf. Diamond) and PSP amplitude at most synapses declines monotonically during depression (particularly when quantal variations are averaged over many synapses as would be expected for an EMG recording), the variability suggests a coordinated change in MN excitability (or a coordinated presynaptic inhibition or facilitation). Pectoral fin MNs participate in the startle reflex bilaterally. Either MF activates all of a group of interneurons, giant fibers (GFs), by chemical synapses and GFs activate fin MNs by electrotonic synapses. Fin movements show similar depression to axial EMGs with low frequency MF stimulation. Since MF to GF transmission remains one to one at these frequencies and electrotonic PSPs in fin MNs should be unaffected, a postsynaptic excitability change is likely to be responsible for fin MN depression and response habituation. If MF to axial MN transmission is like that from MF to GF, a postsynaptic locus would be indicated for habituation of axial movements as well. Our hypothesis is that in this system removal of a tonic excitatory input or addition of an inhibitory one causes depressed MN responsiveness which underlies behavioral habituation.

E. Aljure is a Fulbright-Hays Scholar on leave from Universidad del Valle, Cali, Colombia, S.A.

2474 A NORADRENERGIC S-IPSP IN MAMMALIAN SYMPATHETIC GANGLION, ELICITED BY A NON-MUSCARINIC ACTION OF PREGANGLIONIC VOLLEYS. John H. Ashe and B. Libet, Department of Physiology, School of Medicine, University of California, San Francisco, CA. 94143.

The atropinized superior cervical ganglion of the rabbit at 22°C exhibits a surface-positive (hyperpolarizing) potential, lasting 1-2 min, during and following a train of ganglionic spikes elicited by preganglionic nerve volleys (e.g., 5/sec for 8 sec). A considerable portion of this hyperpolarization is removed by incubation (60-90 min) with the α-blocker phenoxybenzamine (9 μM-12 μM), but not with the β-blocker sotalol (10 μM), indicating the apparent contribution of an α-adrenergic component. The remaining hyperpolarization is then "pure" posttetanic hyperpolarization (PT-HP), due to activity of the electrogenic Na-K pump (see Libet et al., Life Sci. 20: 1863-1870, 1977). PT-HP recorded at non-synaptic postganglionic axons is not affected by phenoxybenzamine.

The apparently adrenergic component of the hyperpolarization can also be eliminated by incubation with methacholine. This loss is presumably due to cholinergic action on an intraganglionic presynaptic store of catecholamine resulting in excessive release and depletion; subsequent incubation with NE or DA can in fact restore the hyperpolarization, with NE more effective. This contrasts with muscarinic depletion of SIF cells and loss of the DA-mediated s-IPSP, in which only DA is effective for restoration (Libet and Owman, J. Physiol. 237: 635-662, 1974). Conversely, incubation with the COMT inhibitor U-0521 resulted in a small but consistent increase in the amplitude and duration of ganglionic afterhyperpolarization.

These findings suggest that the phenoxybenzamine-sensitive component of hyperpolarization in the atropinized ganglion represents a second, parallel slow IPSP mediated at an "alpha" receptor by NE, and that the NE is released by a non-muscarinic action of ACh on an intraganglionic structure. Recurrent axon collaterals of ganglion cells do not appear to provide the presynaptic NE; the ganglionic hyperpolarization following a train of antidromic, postganglionic stimuli is insensitive to phenoxybenzamine. However, dendro-dendritic junctions between ganglion cells are not excluded as the synaptic mediators. (Supported by U.S.P.H.S. grant NS-00884 from NINCDS.)

2475 EVENTS UNDERLYING SYNAPTIC TRANSMISSION BETWEEN CONES AND BIPOLAR CELLS IN THE TURTLE RETINA. J.F. Ashmore* and D.R. Copenhagen* (SPON: R.H. Steinberg). Dept. Ophth., Sch. Med., UCSF, San Francisco, CA 94143.

The signal path between the cone photoreceptors and ganglion cells in the retina is formed by the bipolar cells. Two types can be distinguished by whether they depolarize or hyperpolarize to small centered spots of light. We have studied the kinetics of synaptic transmission and the voltage noise spectra from recordings in both types of bipolars in the turtle eyecup. For both cell types, the time to peak of linear range responses to flashes of light is 180-200 msec, which is slightly greater than that of the cones which drive these cells. The depolarizing bipolar cells exhibit a longer latency than the hyperpolarizing bipolar cells. In addition, the synaptic voltage gain from the cones to the depolarizing bipolar cells is greater than from cones to the hyperpolarizing bipolar cells. If it is assumed that the same transmitter is released from the photoreceptors a maximal rate in the dark and suppressed by light, then the differences in the light responses must be due to differences in postsynaptic transmitter action.

The voltage noise spectra from both types of bipolar cell has contributions from two components. Although the hyperpolarizing bipolar cells show a reduction in total noise with light (cf. Simon, Lamb, and Hodgkin, Nature 256:661, 1975), at all light levels the spectra can be fitted by the sum of a low frequency component which rolls off at 6-8Hz and a high frequency component which rolls off at 20-26Hz. The high frequency component corresponds to an event of approxmiately 20 msec in duration with an amplitude in the range 50-200 μV. It is most probable that this event is the miniature PSP whose frequency of occurrence is modulated by the dark noise fluctuations in the cones.

The depolarizing bipolar cells also have spectra which can be separated into two components. There is a low frequency component exhibiting a roll off at 6-8Hz. However the high frequency component corresponds to an event 35-40 msec in duration and is not completely suppressed by light. In both cell types, the power in the low frequency component is reduced by light, consistent with experiment data that light suppresses presynaptic voltage fluctuations. However it must be inferred that some special mechanism of transmitter action is required to explain the characteristics of the depolarizing bipolar cell response.

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2476 A BIPHASIC IPSP IN PYRAMIDAL NEURONS OF HIPPOCAMPAL SLICES IN THE PRESENCE OF PENTOBARBITAL. G. F. Ayala and R. H. Thalmann*. Dept. Neur. and Cell Bio., Baylor Coll. of Med., Houston, TX 77030.

Elsewhere in this meeting we report that iontophoretic application of GABA to the somatic region of pyramidal neuron of the hippocampus produced a chloride and picrotoxin-sensitive biphasic response: a hyperpolarization followed by a depolarization. We now describe a similar biphasic response which can be elicited by synaptic rather than iontophoretic release of the inhibitory neurotransmitter if pentobarbital is present in the bathing solution. The response of CA1 pyramidal neuron to orthodromic stimulation was recorded by intrasomatic electrodes in hippocampal slices maintained at 34-35°C and bathed in CSF with a potassium concentration of 5 mM. Slices were incubated in 125 mM sodium pentobarbital for at least one hour before the experiment began. Under these conditions, low intensity stimulation elicited a hyperpolarizing IPSP which lasted from one to two seconds, and which was associated with a marked increase in membrane conductance. Increased stimulus intensities usually evoked a depolarizing component which was evident following the early hyperpolarizing peak of IPSP. A marked increase and prolongation of the membrane conductance also occurred. Topical application of a low chloride solution increased the size of the depolarizing component while shifting its reversal potential to a less negative value. Application of the GABA antagonists picrotoxin and bicucculine methiodide reduced the depolarizing component more readily than the hyperpolarizing phase. Repetitive stimulation at a rate of 1 Hz produced a summation of the depolarizing components to a plateau which could also be reduced by picrotoxin or by bicucculine. Although most responses were recorded with potassium-acetate filled electrodes, similar biphasic responses were also recorded with K-acetate and K-sulphate electrodes.

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2477 **VOLTAGE CLAMP ANALYSIS OF PHENYTOIN AND PICROTOXIN ACTION IN CRAYFISH STRETCH RECEPTORS.** Floyd W. Banks and Paul R. Adams. Dept. Physiol. and Biophys. UTMB, Galveston, TX 77550.

Fast and slowly adapting abdominal stretch receptor neurones were voltage clamped using two potassium chloride filled microelectrodes and a Ringer solution containing 21 mM calcium. Inhibitory postsynaptic currents (ipscs) were set up by eliciting an axon reflex with a coarse fire polished Ringer-filled micropipette. At -100 mV and 23°C the ipsc had a rise time of ∿ 1.5 msec and an exponential decay phase with time constant (τ_{ipsc}) about 4 msec. τ_{ipsc} was somewhat increased by depolarizing the cell, the e-folding voltage being about 300 mV. The peak I-V relation was linear. In the presence of 50 μM phenytoin the ipsc decay remained a single exponential, but was about 6 times slower at all potentials. This slowing factor was proportional to phenytoin concentration in the range 10-100 μM. The ipsc amplitude was slightly increased. 10 μM picrotoxinin reduced the ipsc amplitude about 4-fold. The ipsc decay phase remained a single exponential, though slightly faster than the controls. In the presence of 50 μM phenytoin, picrotoxinin decreased the ipsc amplitude and τ_{ipsc} by about the same factor as in controls. 50 μM phenytoin shifted the log dose-conductance curve to bath applied GABA to the left, with no clear change in the maximum response. The leftward shift corresponded to a 3 to 4 fold increase in GABA potency. Formally, the results suggest that phenytoin and picrotoxinin bind to sites that control the rates of opening and closing of the GABA channel. It is not yet clear whether these sites are identical, and whether they are physically within the channel. (Supported by NIH grants NS-14920 and NS-14986).

2478 APOMORPHINE-INDUCED PHOSPHORYLATION OF A SPECIFIC SYNAPTIC MEMBRANE PROTEIN IN STRIATAL SLICES. M. Baudry* and G. Lynch Dept. of Psychobiology, Univ. of California, Irvine, CA 92717

The phosphorylation of synaptic membrane proteins has been postulated to mediate the effects of dopamine in the striatum via the stimulation of a dopamine-sensitive adenylate cyclase. However, until now, there have been no reports that dopamine or its agonists can produce a change in the phosphorylation of specific brain proteins, either in vivo or in vitro. We now report that, in rat striatal slices, apomorphine, a specific dopamine agonist, changes the phosphorylated state of a specific synaptic plasma membrane (SPM) protein. Further, this protein appears to be the same as that which has been previously reported to undergo phosphorylation after induction of long-term potentiation in the hippocampal slice preparation.

Striatal slices were incubated with or without apomorphine (100 μM) for 2 minutes, and either crude mitochondrial fraction or synaptic plasma membranes were prepared. These fractions were then assayed for endogenous phosphorylation, and the samples were then run on an exponential polyacrylamide gel system.

Apomorphine induced a marked and specific decrease in the incorporation of ^{32}P into a specific protein with a molecular weight of about 40,000 daltons (40K) in both the crude mitochondrial and the SPM fractions. This effect of apomorphine was totally reversed by incubating the striatal slices with the dopamine antagonist haloperidol (100 μM). In addition, phosphorylase B kinase (PBK), which has been shown to specifically phosphorylate the 40K protein in hippocampal SPMs, was also found to phosphorylate the 40K protein in striatal SPMs. However, this effect was smaller in SPMs prepared from apomorphine-treated slices than in SPMs from control slices.

These findings suggest the following conclusions:

1. Apomorphine, a specific dopamine agonist, increases the endogenous phosphorylation of a specific SPM protein in the striatal slice.
2. The change in the phosphorylated state of the 40K protein induced by apomorphine in striatal slices and by electrical stimulation in hippocampal slices may be mediated by phosphorylase B kinase.
3. Since dopamine agonists are known to induce long-term behavioral changes, and since repetitive electrical stimulation in the hippocampal slice induces long-term potentiation, the initial change in the 40K protein phosphorylation may well represent a common mechanism by which enduring changes in neuronal transmission are initiated.

2479 PRE-SYNAPTIC INHIBITION OF FACILITATION AT CRAYFISH NEUROMUSCULAR JUNCTIONS. Douglas A. Baxter* and George D. Bittner (Spon: Bob Grossfeld). Univ. of Texas, Austin, Tx. 78712.

The effect of pre-synaptic inhibition on facilitation was studied at the crayfish neuromuscular junction. Appropriately timed stimulation of the inhibitory motorneuron with respect to stimulation of the excitor motorneuron pre-synaptically reduced the excitatory transmitter output, presumably by decreasing the amplitude of each of the pre-synaptic action potentials in the excitor axon. When inhibitor stimulation was suddenly stopped in a train of excitor and inhibitor pulses the excitor axon at the next pulse in the train released the same amount of transmitter it would have released if only the excitor alone had previously been stimulated.

The release of excitatory transmitter was monitored via intracellular recordings of excitatory post-synaptic potentials (EPSP's) from opener muscle fibers in the cheliped of the crayfish Procambarus clarkii. A 100 Hz stimulus train having anywhere from 8 to 17 equal interval pulses was given to the excitatory axon. The train was repeated every 5 seconds and 25 successive trains were averaged with a signal-averaging computer. Then the excitatory train was repeated and the inhibitory axon was stimulated at 100 Hz at a phased angle which produced maximum pre-synaptic inhibition. After 4-14 excitatory stimuli in this second stimulus train, the inhibitor was no longer stimulated so as to release the remaining excitatory action potentials from inhibition.

In 18 of the 27 muscle fibers studied, pre-synaptic inhibition reduced the EPSP amplitude by 40-72% during the inhibited stimulus train (mean = 55%). Post-synaptic inhibition was found to have contributed only 2-9% (mean = 3.5%) of the observed reduction in EPSP amplitudes. In these fibers the amplitude for the EPSP which immediately followed the release from pre-synaptic inhibition suddenly increased to within 15% above or below the amplitude calculated for the corresponding EPSP in the non-inhibited train (mean = 8% below the EPSP amplitude observed in the non-inhibited train). The increase in EPSP amplitude following inhibition represented a far greater increase in transmitter release than is ever seen during the facilitation of transmitter release in the non-inhibited train and this increase probably resulted in part from the accumulation of facilitation during the inhibition of transmitter release.

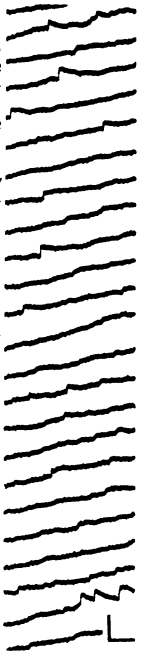
The results of this study indicates that the facilitation process can develop normally during the pre-synaptic inhibition of transmitter release. This result implies that the facilitation process is much more dependent upon the pattern than the amplitude of the pre-synaptic stimulation.

2480 PRESYNAPTIC MECHANISMS FOR CAPACITY-COMPENSATION IN A NON-IMPULSIVE NEURON. A.R.Blight and R.Llinás. Dept.Physiology and Biophysics, N.Y.Univ.Med.Ctr.,New York, N.Y.10016.

Action potentials mediate information transfer in nerve cells with low distortion from membrane capacitance,whereas graded, "analog" electrical signals are attenuated even over short distances, with much capacitative distortion. We have investigated the problem of how a non-impulsive cell is adapted nonetheless for the transmission of information, using the promotor stretch receptor fibre of the crab and its monosynaptic input to motoneurons (Blight and Llinás, Soc.Neurosci.Abst.4, 577, 1978). We have found a number of properties of the presynaptic fibre tending to compensate for the capacitative distortion of the receptor potential, conducted decrementally 12-15mm from the periphery. Injection of square current pulses into the presynaptic T-fibre revealed a graded, spike-like transient voltage response to depolarization of the membrane. The amplitude of this transient, which was previously thought to be sodium-dependent, was found relatively insensitive to TTX or replacement of external sodium, but to be directly dependent on external calcium concentration. This "graded spike", apparently based on a voltage-dependent calcium conductance with a tendency to become regenerative, increased the rate of depolarization, effectively producing a more "square" voltage response to a step current. It was found additionally to compensate lower frequency signals. Evidence was also obtained that the calcium transient induces a pulse of transmitter-release, giving a postsynaptic transient with a similar capacity-compensating effect on the motoneuron dendritic membrane, which is also non-impulsive.The form of the receptor potential itself was shown to be such that it tends to "voltage-clamp" the distant synaptic terminal, and the depletion characteristics of the synaptic transmitter store were found to be capable of re-introducing some of the "predictive", derivative properties of the receptor potential to the synaptically transmitted signal (e.g.producing a postsynaptic phase-lead in the transmission of sinusoidal signals). Though transmission in the stretch receptor is non-impulsive, it is not purely passive but rather complexly evolved to reconstruct an apparently faithful output (motor response) from an otherwise distorted input (decrementally conducted receptor potential). Similar and further mechanisms are to be expected in other "non-spiking" neurons.

2481 PROPERTIES OF SPONTANEOUS MINIATURE SYNAPTIC POTENTIALS IN HIPPOCAMPAL NEURONS. Thomas H. Brown, Robert K. S. Wong and David A. Prince. Neurology Dept., Stanford Univ. Med. Sch., Stanford, CA 94305.

The quantum hypothesis of synaptic transmission holds that neurotransmitter substances are released from nerve terminals in the form of integral numbers of multimolecular packets or quanta. In the peripheral nervous system, quanta can be liberated spontaneously (without nerve impulses) giving rise in the postsynaptic cell to the random occurrence of brief depolarizing events termed spontaneous miniature synaptic potentials. So far there has been no demonstration of spontaneous miniature potentials in the mammalian brain. We now report that miniature potentials can be recorded in guinea pig hippocampal nerve cells under conditions in which regenerative spiking activity and evokable (impulse-dependent) transmitter release have been blocked by TTX (1 µg/ml) and manganese (2 mM). This phenomenon was observed regularly in neurons of the CA3 region in the in vitro slice preparation (calibration in example at right: 5 mV and 50 msec).



The properties of these miniature potentials were analyzed in 8 cells. In each case the frequency distribution of miniature potential amplitudes was positively skewed, with the smallest events usually merging into the background noise. The mean amplitude of those events emerging above the background noise was 2.1 ± 0.3 mV. The large size of these events was not unexpected, given the high input resistance (about 40 Mohms) of these cells. The approximate net inward charge transfer associated with a miniature potential was estimated from the relation $Q = G_N \int V(t)dt$, where G_N is the input conductance measured at the soma (about 25 nS) and V(t) is the miniature potential amplitude as a function of time. For the larger events with fast rise and fall times, which presumably originate near the recording site, Q averaged 2.9 ± 1.1 pC, which is not exceptionally large. The timing of occurrence of miniature potentials in these cells suggested a random process, in that the intervals between successive events conformed approximately to an exponential probability density function $f(t) = 1/T \exp(-t/T)$, where T is the mean time interval. The mean value of T was 1.3 ± 0.9 sec.

This ability to resolve quantal events should aid our understanding of synaptic transmission between well defined neuronal populations within the hippocampus (Supported by NS 06161 & 06477).

2482 SYNAPTIC VESICLES HAVE SPECIFIC ANTIGENS, ABSENT FROM OTHER MEMBRANES. Steven S. Carlson & Regis B. Kelly. Dept. Biochem. Biophys. Univ. Calif. San Francisco, CA 94143.

The molecular constituents of synaptic vesicles may be unique or they may be shared by other cellular structures. We have examined this question immunologically by raising rabbit antibodies to cholinergic synaptic vesicles purified from the electric organs of Narcine brasiliensis. We have shown that a unique population of synaptic vesicle antigens exists. This conclusion was based upon two immunological assays; a solid phase assay and a solution assay which utilized inactivated S. aureus cells as an immuno-adsorbent. With the solid phase assay, antiserum could be shown to bind to pure synaptic vesicles which had been immobilized on a plastic microtiter well. The antibody bound was linear with antigen concentration at low antigen concentrations. To test whether the serum contained antigens which were specific for vesicles, the serum was adsorbed with a membrane fraction from the electric organ which did not contain synaptic vesicles. After extensive adsorption with non-vesicular membrane, 40% of the antigenic sites could still be recognized by the antiserum and are presumably specific to vesicles. The S. aureus radioimmune assay utilized precipitation of antibody-labeled antigen complexes with protein A coated S. aureus cells. This assay, which was linear with antigen concentration in the range of 0.05 to 1 µg of synaptic vesicle protein, could be used to detect synaptic vesicle antigen in crude homogenates of electric organ. Although the synaptic vesicle protein amounted to less than 1% of the total protein of electric organ, fractionation of crude homogenates of electric organ by differential centrifugation revealed that acetylcholine containing membranes and synaptic vesicle antigenicity co-purified. When the 100,000g membrane pellet was further fractionated on a glycerol density gradient, the antigenicity measured by both solid phase and S. aureus assays had the same density (1.20 g/cc) as vesicles detected by their acetylcholine content. No other electric organ membranes on the glycerol gradient were antigenic. Because synaptic vesicles contain specific antigens, anti-sera directed towards them will be valuable reagents in the study of nerve terminals. The vesicle-specific antigens are conserved during evolution since the anti-vesicle serum binds to nerve terminals of the frog, rat and chicken neuromuscular junction. This work supported by NIH grant NS09878 (to RBK) and a Postdoctoral fellowship from the Muscular Dystrophy Association (to SSC).

2483 EVIDENCE THAT ONE POOL OF CHOLINERGIC VESICLES IN MOUSE BRAIN CAN EMPTY AND REFILL WITH NEWLY SYNTHESIZED ACETYLCHOLINE AND ACETYLHOMOCHOLINE INDEPENDENTLY OF THE MONODISPERSE POOL OF CHOLINERGIC VESICLES. Paul T. Carroll* and Christina G. Benishin* Dept. Pharmacology, Univ. Rhode Island, Kingston, RI 02881. (SPON: J.T. McIlwain, Brown Med. School, Prov., RI 02912).

Previously we have reported that incubation of mouse forebrain minces in a lithium Krebs (L.K.) solution reduces the acetylcholine (ACh) content of a crude vesicular fraction (P_3) independently of the cytoplasm; subsequent incubation of minces in Krebs (K.) with labelled precursor, either ^{14}C choline or ^{14}C homocholine, refills the crude vesicular fraction with ^{14}C ACh or ^{14}C acetylhomocholine, respectively (Carroll & Nelson, Fed. Proc., 1979). Also, some choline o-acetyltransferase (E.C.2.3.1.6.-ChAT) cannot be solubilized from this crude vesicular fraction by high ionic strength washes (Smith & Carroll, Fed. Proc., 1979). The crude vesicular fraction contains at least two pools of cholinergic vesicles which can be separated by sucrose density gradient centrifugation, a monodisperse pool and another pool believed to be associated with neuronal membranes which sediments in a higher density of sucrose than the monodisperse pool (Barker & Whittaker, 1972). The objective of the present investigation was to determine if incubation of mouse forebrain minces in L.K. selectively reduces the ACh content of the denser vesicle fraction (D.V.F.) independently of the monodisperse vesicles (M.V.). Minces of mouse forebrain were incubated in K. or L.K. solution at 37° for 30 min, subcellular fractions prepared (Saleghmoghaddam & Collier, 1976) and the levels of ACh determined in the M.V. pellet and the D.V.F. Electron micrographs showed the presence of vesicles in both fractions. Also some non-solubilized ChAT activity was associated with the D.V.F. L.K. reduces the ACh content of the D.V.F. 86% without altering that of the M.V. with respect to K.; an acetylcholinesterase inhibitor is not required to demonstrate this reduction or maintain ACh levels in the D.V.F. Subsequent incubation of both sets of pretreated minces for 30 min in K. containing either ^{14}C choline (0.1mM) or ^{14}C homocholine (0.1mM) and paraoxon facilitates accumulation of ^{14}C choline (108%) and ^{14}C homocholine (109%) by the D.V.F. but not the M.V. of minces pre-incubated in L.K. with respect to those pre-incubated in K. The D.V.F. depleted of its ACh content by lithium pre-incubation can be refilled with either ^{14}C ACh formed from extracellular ^{14}C choline or ^{14}C acetylhomocholine formed from extracellular ^{14}C homocholine. These results suggest that a dense vesicle fraction contains non-hydrolyzable ACh which can be emptied and refilled with either newly synthesized ACh or acetylhomocholine independently of the monodisperse vesicle fraction. (Supported in part by NSF grant #BNS 78-05160).

2484 FREQUENCY DEPENDENCE OF GANGLIONIC BLOCKADE BY d-AMPHETAMINE. Daryl Christ* (SPON: D.L. Avery). Dept. of Pharmacology, Univ. of Arkansas for Medical Sciences, Little Rock, AR 72201.

The magnitude of ganglionic blockade by d-amphetamine is dependent on the frequency of preganglionic stimulation (Downing, Br. J. Pharmac. 44:71, 1972). The mechanism underlying the frequency-dependent blockade was explored in the isolated stellate ganglion of the hamster using extracellular recording techniques. d-Amphetamine reduced the compound action potential of the postganglionic nerve produced by supramaximal stimulation of the preganglionic nerve at 0.2 Hz. As the frequency of stimulation was increased, the magnitude of ganglionic blockade was decreased. 10^{-4}M d-amphetamine reduced the postganglionic potential by 86% at 0.2 Hz, and 41% at 2 Hz. The frequency-dependent block by d-amphetamine was not affected by propranolol (10^{-6}M), was slightly reduced by phentolamine (10^{-5}M), and was considerably reduced by atropine (10^{-6}M), suggesting that a muscarinic mechanism is involved.

d-Amphetamine produced spontaneous discharges in the postganglionic nerve at 10^{-3} and 10^{-4}M. This is indicative of a depolarizing action, and this depolarizing action may potentiate muscarinic transmission in the ganglion. d-Amphetamine potentiated discharges from the application of McN-A-343, a muscarinic cholinergic agonist, however this effect was not specific because discharges from the application of DMPP, a nicotinic cholinergic agonist, were also potentiated. Furthermore, the discharges by d-amphetamine and potentiation of discharges from cholinergic agonists lasted only for 10 to 20 minutes in the continuous presence of d-amphetamine. The frequency dependent effects of d-amphetamine occurred after much longer durations of exposure to d-amphetamine.

In the presence of 10^{-4}M d-amphetamine, repetitive stimulation (30 Hz, 5 sec) produced asynchronous afterdischarges. The afterdischarges were blocked by atropine, thus they were probably produced by muscarinic actions of the neurotransmitter. This indicates d-amphetamine does not depress muscarinic transmission as effectively as nicotinic transmission.

These results suggest that at low stimulation frequencies, the nicotinic blocking action of d-amphetamine will predominate and result in blockade. At high frequencies, the muscarinic actions of the neurotransmitter can potentiate the nicotinic actions of the neurotransmitter, thereby reducing the magnitude of ganglionic blockade by d-amphetamine.

2485 CHARACTERIZATION OF A POST-GANGLIONIC CATECHOLAMINE RECEPTOR IN THE RABBIT SUPERIOR CERVICAL GANGLION (RSCG), Alison E. Cole* and Patricia Shinnick-Gallagher, Univ. of Tex. Med. Br., Galveston, TX 77550.

Catecholamines (CA) have long been known to have potent inhibitory effects on ganglionic transmission (Marrazzi, 1938). Evidence indicates that a disynaptic pathway which involves a CA-releasing interneuron may mediate the inhibitory action via hyperpolarization of the post-synaptic membrane. Dopamine has been proposed as the primary neurotransmitter of this hyperpolarizing ganglionic response in the RSCG (Libet, 1970). This study will characterize the receptor involved in the inhibitory response by comparing the relative potencies of three CA's (epinephrine (EPI), norepinephrine (NE) and dopamine (DA)) in producing a hyperpolarization of the RSCG.

To examine this post-synaptic effect of CA on ganglionic transmission a concentration-response analysis was performed using an extracellular recording technique, the sucrose-gap. EPI, NE and DA in varying concentrations were administered by superfusing the ganglion.

All CA were consistently found to hyperpolarize the preparation in a clear concentration dependent relationship (N=30) with EPI>NE>>DA. DA was about ten times less potent than EPI in producing this response. The EC_{50}'s for EPI, NE and DA were 9 x 10^{-7}M, 2 x 10^{-6}M and 10^{-5}M respectively. In addition, apomorphine (10^{-3}M) had no effect on the ganglionic membrane potential (N=9), whereas clonidine (10^{-5}M), phenylephrine (10^{-4}M) and isoproterenol (10^{-3}M) produced a hyperpolarization. Phentolamine (10^{-6}M) was found to block the hyperpolarizing response of EPI, NE and DA, and shifted the concentration response curve to the right. The IC_{50} of phentolamine was 10^{-6}M. Haloperidol (10^{-6}M) was found to have no effect on the DA-induced hyperpolarization.

These results suggest that the CA induced hyperpolarizing response was due to activation of an α-adrenoceptor.

2486 FLUCTUATIONS IN LATENCY OF EPSP ONSETS: LARGE EPSPs EXHIBIT SMALLER FLUCTUATIONS THAN SMALL EPSPs. T.C. Collatos, S.G. Nelson and L.M. Mendell. Duke Med. Ctr., Durham, N.C. 27710

A recent paper from this laboratory (Collatos et al, Brain Res. 160:514, 1979) has demonstrated that EPSPs produced in a given motoneuron by a given Ia fiber exhibit fluctuating synaptic delays. The purpose of this communication is to explore the relationship between the statistical properties of the fluctuations and the resultant averaged EPSP. In cats we studied relatively large EPSPs (171-1129 μV) with brief rise times (0.3-0.6 msec, 10-90%). We have found that the variance of the latency distribution tends to be smaller at synapses which generate large EPSPs. One problem in the interpretation of these results is that large EPSPs might be easier to pick out of synaptic noise in single sweeps. This would be expected to reduce the variance of the onset latency distribution for large EPSPs artifactually. However, if this were so, it would be expected that the distributions would be skewed to the right (long latencies) since we have almost invariably observed that the population of EPSPs with short latencies at a given synapse is larger in amplitude on the average than the population with long latencies (as at the neuromuscular junction - Barrett and Stevens, J. Physiol. 227:665, 1972). In fact, about one-half the distributions were skewed, but in the opposite direction, i.e. in the direction of the larger EPSPs with shorter onset. This suggests that the variance of EPSP delays is not significantly influenced by difficulties in detection of their onsets at least for this population of relatively large EPSPs. Furthermore, since the distributions which were skewed were restricted almost entirely to synapses at which relatively large EPSPs were generated, it is unlikely that skewness was a consequence of an unfavourable signal to noise ratio. As expected from the above findings, skewed distributions tended to exhibit the smallest variances; the larger variance of some distributions was not associated with skewness. We conclude that at synapses at which large EPSPs are produced, the average time variability in the release process is less than at synapses where EPSPs are smaller. The alternative hypothesis, that the fluctuations are the result of increased variability in conduction time through the presynaptic terminals, seems unlikely since a small potential recorded from inside the motoneuron and identified as a terminal potential in the presynaptic Ia fiber (Munson and Sypert, Neuroscience Abst. 4, 1978) exhibits no latency fluctuations despite variability in onset of the subsequent synaptic potentials. It remains to be seen whether these fluctuations can be affected by stimuli which cause presynaptic inhibition at this synapse. (Supported by NIH).

2487 FAST EXCITATORY POSTSYNAPTIC CURRENTS IN SYMPATHETIC GANGLIA: ALTERATION BY PROCAINE. Elizabeth Connor*, Amy MacDermott* and Rodney L. Parsons*. (SPON: G.D. Webb). Dept. of Physiol. & Biophys., School of Med., University of Vermont, Burlington, VT 05405 and Laboratory of Preclinical Studies, NIAAA Intramural Research, Rockville, MD 20852.

Procaine and many other local anesthetics alter the decay kinetics of end-plate currents in skeletal muscle. In the presence of these drugs, end-plate currents no longer decay exponentially but have a decay time course which is the sum of at least two exponential components. The simplest kinetic scheme which accounts for this drug action is one in which drug molecules block open end-plate channels. We thought it of interest to determine whether a similar mechanism of drug action occurs at other vertebrate cholinergic synaptic membranes. Consequently, we have analyzed, with voltage clamp techniques, the alteration in fast excitatory postsynaptic currents (EPSCs) produced by procaine in bullfrog sympathetic postganglionic B cells. Synaptic currents were recorded from control and procaine treated cells from the same ganglion preparation maintained in a HEPES-buffered solution (mM: NaCl 117, KCl 1.8, $CaCl_2$ 1.8, HEPES 1.0; pH 7.3, 12-20°C). A two microelectrode voltage clamp system was used to clamp membrane voltage between +30 to -100 mV. In the absence of procaine, the EPSC rises to a peak value within a few msec and then decays exponentially with the time constant of decay having a small negative voltage dependence (A = 0.0039 ± 0.0017, M ± SD, N = 15, 14-18°C). The peak EPSC amplitude increases with hyperpolarization with the I-V relationship approximately linear in the range -30 to -100 mV. In the presence of procaine (0.2-0.5 mM), peak EPSC amplitude was markedly decreased and the I-V relationship in this voltage range became flattened so that peak amplitude did not increase substantially with hyperpolarization. In some procaine treated cells, EPSC decay no longer appeared as a single exponential function but consisted of two exponential components; an initial fast component followed by a slow component. When present, the two components of the decay time course in procaine treated cells became more obvious with hyperpolarization. In many other cells the initial component appeared absent or was too small to analyze so that the decay appeared only slowed relative to the decay time course in control cells. These results suggest that procaine produces a voltage and concentration dependent postsynaptic blockade in sympathetic ganglion cells. Supported by NIH grant NS 14552.

2488 LEPTINOTARSIN: PRELIMINARY PURIFICATION AND CHARACTERIZATION OF A PRESYNAPTIC NEUROTOXIN. Richard D. Crosland*, T.H. Hsiao*, Joseph R. Stimers*, and W.O. McClure. (SPON: B.C. Abbott). Section of Cellular Biology, University of Southern California, Los Angeles, CA 90007, and Department of Biology, Utah State University, Logan, UT 84322.

Leptinotarsin is a presynaptic neurotoxin which occurs in the hemolymph of beetles of the genus Leptinotarsa (Hsiao, T.H. and Fraenkel, G., Toxcon 7: 119, 1969). It causes a massive, biphasic release of miniature end plate potentials (mepps) from a phrenic nerve-diaphragm preparation from the rat (Satin et al., Soc. Neurosci. Abstr. 4: 584, 1978). During the first one minute phase, about 10% of the total available store of mepps is released. A second phase immediately follows the first phase and results in the release of the remaining mepps within 10-15 minutes.

Preliminary purification of L. haldemani hemolymph on a Sephadex G-150 column results in two separate peaks of activity. Each peak contains material which releases radioactivity from rat brain synaptosomal preparations previously incubated with [^{3}H]choline. The summed activity of both peaks accounts for 62% of the activity applied to the column. The material in the first peak (Peak A) is purified 4.5 fold over the hemolymph and contains 65% of the recovered activity. The material in the second peak (Peak B) is purified 2.5 fold over the hemolymph and accounts for 35% of the recovered activity. The activities in both peaks are labile, but can be partially stabilized with dithiothreitol and bovine serum albumin. Propylene glycol and EDTA are ineffective as stabilizing agents.

When applied to a phrenic nerve-diaphragm preparation from the rat , the material in both peaks causes a biphasic release of mepps similar to that caused by unfractionated hemolymph. The second phase of release caused by the material in Peak B, however, is more prolonged than the second phase of release caused by the material in Peak A. Further purification and characterization of the active components of Peaks A and B should provide useful toxins with which to elucidate the mechanism of release of acetylcholine.

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2489 SIMULTANEOUS, INDEPENDENT CLAMPING OF VOLTAGE AND AGONIST-INDUCED CURRENT ACROSS THE ENDPLATE MEMBRANE. J. del Castillo, P. Specht* and A. Auerbach*.
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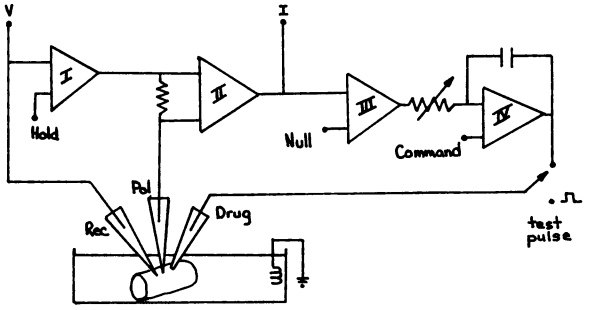
The circuit to clamp the potential across the endplate membrane by electrophoretic drug application recently described by del Castillo and Specht (J. Physiol. 284:95-96P, 1978) has been combined with a conventional two microelectrode voltage clamp system as shown below. This allows the simultaneous and separate control of both the current required to hold the membrane potential at a fixed level, and the agonist-induced current flowing through receptor-associated channels.



Amplifier I represents the voltage clamp, with a floating current monitor in amplifier II. The membrane potential (V) is controlled by the holding potential (Hold). A signal proportional to the injected current (1 mV/nA) is applied to buffer amplifier III, where any D.C. component is manually nulled to near zero. This output goes to the drug clamp amplifier (IV). A command signal of x mV, applied to amplifier IV, will generate the outward movement of agonist necessary to maintain a drug-induced current of x nA.

This system is useful for the analysis of agonist-induced current, as it makes it feasible to maintain a steady level of receptor activity for periods longer than those possible when the agonist application is controlled manually.

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2490 THE LIPIDS OF PURIFIED ELASMOBRANCH SYNAPTIC VESICLES: RELEVANCE TO THE MECHANISM OF EXOCYTOSIS. James W. Deutsch* & Regis B. Kelly. Dept Biochem. Biophys. Univ. Calif. San Francisco, CA 94143 (SPON: E. Mayeri).

A large body of evidence supports the hypothesis that the release of vesicle-contained transmitter occurs via fusion with the presynaptic membrane in the presence of Ca^{++}. Only recently has the availability of rigorously purified synaptic vesicles from Narcine brasiliensis electric organ (Carlson et al. (1978). Biochemistry 17, 1188) made it possible to address biochemically the question of how synaptic vesicles fuse. Studies of artificial liposomes reveal that Ca^{++}-mediated fusion requires negatively-charged lipid and a fluid membrane.

Purified synaptic vesicles were extracted and their lipid content analyzed. The major phospholipids are phosphatidylcholine (PC), 46 mol%; P-ethanolamine, 32%; sphingomyelin, 5%; and P-serine, which is negatively charged, 16%. The molar ratio of cholesterol to lipid phosphorus is 0.5, which is higher than that of whole electric organ (0.4) and lower than that of a non-vesicular membrane fraction which comigrates with synaptic vesicle in an early purification step (0.8). The fatty acids were analyzed by gas chromatography. The major fatty acids of synaptic vesicles are 16:0 (31 mol%), 22:6 (30%), 18:0 (13%), and 18:1 (11%). P-ethanolamine and P-serine contain high percentages of 22:6 (47% and 37%, respectively). Both whole organ and the non-vesicular membrane fraction contain significantly less 22:6 (9% and 16%, respectively).

The presence of a large amount of highly unsaturated fatty acid is consistent with the requirement from model studies that the membrane be fluid. The high concentration of cholesterol, which tends to decrease fluidity, is inconsistent, however. The amount of negatively charged lipid (P-serine) is not unusually high but it is necessary to know whether or not it is present in higher concentration on the protoplasmic surface of the vesicle membrane. This work was supported by NIH grant NS09878 (to RBK) and a NIH Postdoctoral Fellowship (to JWD).

2491 A STUDY OF SLOW POSTSYNAPTIC POTENTIALS IN SEVERAL MAMMALIAN SYMPATHETIC GANGLIA. N. J. Dun and A. G. Karczmar. Loyola Univ. Stritch School of Medicine, Maywood, Ill. 60153.

The slow postsynaptic potentials of the superior cervical ganglia (SCG) of the rabbit, rat and guinea pig were studied by means of the sucrose-gap technique. In the presence of hexamethonium (C_6,0.5 mM) which suppressed the initiation of ganglion compound action potentials, repetitive preganglionic stimulation (10-20 Hz, 1-2 sec) elicited in rabbit and rat SCG a biphasic synaptic response, an initial slow positive (P) potential followed by a late negative (LN) potential. The amplitude of P potential in the majority of these ganglia was much larger than LN potential which in several preparations was negligible. However, in guinea pig SCG superfused continuously with C_6, repetitive preganglionic stimulation evoked only a slow depolarizing response with no detectable hyperpolarization. Physostigmine (1μM) increased the amplitude of P and LN potentials in the majority of rabbit and rat SCG; in several preparations, the large increase in P masked the appearance of LN. In the SCG of guinea pigs, the amplitude of LN was first enhanced and then depressed by physostigmine, and after 15-20 min drug treatment, the LN was replaced by a hyperpolarizing potential. This effect of physostigmine was fully reversible. The effects on P and LN potentials of a number of adrenergic and dopaminergic antagonists were studied. Alpha-adrenergic blockers, phenoxybenzamine and phentolamine and beta-adrenergic blocker, propranolol, in concentrations up to 10 μM did not appreciably affect the amplitude of P and LN, whereas in higher concentrations, both P and LN were depressed by these agents. Dopaminergic blockers, haloperidol, chlorpromazine, metoclopramide and pimozide in concentrations up to 1 μM did not significantly depress the amplitude of P and LN, whereas, haloperidol (50μM) attenuated non-selectively the amplitude of P and LN in rabbit SCG. Atropine (1 μM) consistently and reversibly abolished the P and LN in all three species. These results demonstrate that muscarinic receptor is involved in the generation of P and LN potentials in mammalian sympathetic ganglia, whereas, the catecholaminergic nature of P is less certain. (Supported by NS 06455 and American Parkinson Disease Foundation).

2492 NON-VESICULAR RELEASE OF NEWLY SYNTHESIZED ACETYLCHOLINE FROM RAT CEREBRAL CORTICAL SYNAPTOSOMES. Gary E. Duncan* and Peter P. Rowell. Department of Pharmacology and Toxicology, University of Louisville School of Medicine, Louisville, KY 40232.

The release and subcellular distribution of ^{3}H-acetylcholine synthesized from ^{3}H-choline in synaptosomes were investigated in order to study the role of synaptic vesicles in acetylcholine release. Crude synaptosomal preparations were prepared from rat cerebral cortical homogenates and incubated for periods of 20 min to 2 hrs. ^{3}H-choline was shown to be transported into synaptosomes by a hemicholinium sensitive mechanism. Spontaneous release of the newly synthesized ^{3}H-acetylcholine was inhibited completely by 10mM magnesium. No detectable ^{3}H-acetylcholine was associated with particulate intrasynaptosomal contents as determined by preparative ultracentrifugation and sucrose gradient centrifugation. These results imply that synaptic vesicles are not required for acetylcholine release from synaptosomes.

2493 CORRELATIVE BIOCHEMICAL AND MORPHOLOGICAL INTEGRITY OF SYNAPTOSOMES: COMPARATIVE EFFECTS OF ISOLATION AND INCUBATION. Lynda H. Fleming*, Albertina E. Hodach*, and Norman C. Reynolds*. (SPON: D.A. Szech). Neurology Dept., Univ. of Wisconsin School of Medicine (Mt. Sinai Med. Cntr.) and Dept. of Pediatrics, Medical College of Wisconsin (Milwaukee Children's Hospital), Milwaukee, WI 53233.

Synaptosomes are often isolated in solutions with no regard to the role of pH in the production of viable nerve endings. However, isolation of viable synaptosomes which transport GABA and glutamic acid is critically dependent on the pH of the isolation solutions. (Hitzemann. J. Neurochem. 32: 669, 1979). Suboptimal isolation conditions yielding reduced numbers of metabolically active synaptosomes may also account for their relatively low levels of lactate dehydrogenase (LDH). Synaptosomes isolated in cold hypertonic sucrose solutions manifest the characteristic S-form when examined by electron microscopy. This morphology can be changed to one resembling in situ nerve endings by a brief incubation in iso-osmolar ion media. (Hajós and Csillag. Br. Res. 112: 207, 1976). Similar buffered salts solutions are often used in experiments designed to elucidate presynaptic events. Since these solutions approximate in vivo salt concentrations, it is assumed that the synaptosomes retain their integrity throughout the incubation period. However, by measuring LDH in Krebs-Ringer medium after incubation, it was shown that the addition of isotonic sucrose is essential for maintaining synaptosomal integrity. (Sperk and Baldessarini. J. Neurochem. 28: 1403, 1977).

The specific aim of this study was to provide an integrated approach to the measure of intactness of synaptosomes during isolation and incubation by using LDH activity and electron microscopy. Synaptosomes isolated on unbuffered sucrose gradients were compared with those isolated on sucrose gradients of known pH. After incubation in one of several different solutions, the synaptosomes were centrifuged and both supernant and pellet were assayed for LDH activity. In addition, effects of the incubation on synaptosomal morphology were also determined. By using this approach, we have outlined a basic technique for the optimum use of synaptosomes as a model system for the investigation of presynaptic events.

2494 APPLICATIONS FOR A MICROCOMPUTER SYSTEM IN CONVENTIONAL ELECTROPHYSIOLOGICAL EXPERIMENTS. G.H. Frederickson*, J.H. Fisher* and G.L. Ringham (SPON: H.M. Brown). Dept. of Physiology, University of Utah College of Medicine, Salt Lake City, UT 84108.

Experiments in this laboratory use conventional microelectrode stimulation and recording techniques to examine the characteristics of synaptic transmission at a synapse in the lamprey spinal cord. Recently, an inexpensive microcomputer system has been utilized in these experiments to help produce and evaluate the data. The system consists of a 4 MHz, Z80 microprocessor, multichannel digital-analog (D/A) and analog-digital (A/D) converters, dual disc drives, 32 K of memory, an external clock with a 10 MHz time base, and a teleprinter. In one application the microcomputer has been programmed to serve as a four channel, digital stimulator. In this capacity the computer delivers pulses, singly or in combination, of variable duration, delay, and frequency; it can also provide train-pulse combinations or direct voltage. The fixed amplitude pulse output of the computer itself is used to control variable voltage output amplifiers, which permit manual control of the intensity and polarity of the signal. This system is able to generate signals with an uncertainty of less than 5 μsec per cycle. A distinct advantage of this approach is that frequently used stimulation parameters can be pre-programmed into the computer and called up as needed during an experiment. Of course, any signal can be obtained when needed by simply entering the desired parameters. A second major use of the computer is for on-line analysis of the data. For example, a stimulus generated as described above can be used to produce a presynaptic action potential. This action potential and the resultant post-synaptic potential are fed to separate channels of the A/D converter of the computer, and the amplitude and time course of the potentials are measured. The computer then prints mean values for maximum amplitude and time to peak of the pre- and post-synaptic potentials produced during a given trial. Future programs will utilize the graphic capabilities of this system to produce graphs relating the pre- and post-synaptic potential changes. Experimental results obtained with this microcomputer system are presented elsewhere at these meetings.

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2495 DENDRITIC LOCALIZATION AND DENSITY OF ACETYLCHOLINE RECEPTORS IN SINGLE CELLS IN SLICES OF GOLDFISH OPTIC TECTUM. John A. Freeman. Dept. of Anat., Vanderbilt Univ., Nashville, TN 37232.

All three major classes of retinal ganglion cells terminating in the goldfish optic tectum appear to be nicotinic-cholinergic (Schmidt and Freeman, Brain Res., in press, 1979). In order to examine the localization and density of nicotinic acetylcholine receptors (nAChE) physiologically, tissue slices 100-300 μm thick were made parasagittally through the tectum, by cooling the fish to 4° C, rapidly removing the tectum and placing it on a Peltier-effect cooled movable stage, using a methacrylate adhesive. Slices, made by rapid vertical strokes of a razor attached to a rotary solenoid, were then transferred to a coverslip which formed the bottom of a perfusion chamber, and were viewed with an inverted microscope equipped with Nomarski optics and a Zeiss fluorescence epi-illuminator. Individual cell bodies and their axonal and dendritic processes could be clearly seen (mag. 500 X), as could the 3 layers of retinal afferents. Contrast was further enhanced by supravital staining with 0.5% methylene blue (20-30 sec). Slices perfused with oxygenated Ringer's maintained their viability for 8-10 hr; normal laminar field potentials could be elicited by stimulating the retinal fiber layers, and stable intracellular resting potentials (25-65 mV) were recorded from cell bodies in the superficial and deep gray layers (diameter 7-12 μm; Z_{in} = 70-120 MΩ). The dendritic surfaces of individual tectal neurons were mapped iontophoretically for their sensitivity to ACh. ACh sensitivity was sharply localized to 3 zones, corresponding to the regions of optic nerve terminal synapses. The reversal potentialsfor ACh were near 0 mV for all 3 zones, as determined by the use of double-barrelled intracellular microelectrodes. DTAF- and TRITC-conjugated α-Btx bound selectively to these same 3 layers and abolished ACh-induced depolarization, recorded intracellularly. Individual clusters of fluorochrome labelled receptors could be discerned both on the dendrites and on many cell bodies. Assuming an area of 0.13 μm^2 for the subsynaptic membrane, a receptor density of 1,500-3,500 nAChR molecules/μm^2 was estimated at retinotectal synapses. The dynamics of acetylcholine receptors during different phases of optic nerve degeneration and regeneration are currently being investigated.

(Supported by NIH Grant EY-01117-07).

2496 IONTOPHORESIS OF ACETYLCHOLINE EVOKES A SLOW MUSCARINIC DEPOLARIZATION IN NEURONS OF DISSOCIATED RAT SUPERIOR CERVICAL GANGLION. Joseph E. Freschi* and William G. Shain (SPON: D. E. Evans). Neurobiology Dept., Armed Forces Radiobiol. Res. Inst., Bethesda, MD 20014.

Synaptic transmission within the superior cervical ganglion (SCG) has been extensively studied because it is experimentally accessible and contains a diversity of types of synaptic transmission. Further experimental simplification has been achieved using tissue culture techniques, so that the biochemical basis of neural transmission and its correlation with electrophysiology have been usefully studied. It is puzzling, therefore, that the slow synaptic potentials seen in intact animals and isolated whole organ preparations have not been discovered in primary cultures of mammalian SCG (O'Lague, P. et al., Devel. Biol. 67: 424, 1978).

We have consistently found slow depolarizing potentials in response to iontophoresis of acetylcholine (ACh) onto neurons dissociated from neonatal rat SCG. With small pulses of ACh a fast depolarizing response associated with a fall in membrane resistance (Rm) is evoked. When the fast response reaches saturation with large pulses of ACh, a long-lasting depolarization of delayed onset becomes evident. The slow ACh response increases in amplitude and duration in a dose dependent fashion, a maximal response lasting 30 to 40 sec. The Rm during the slow response either remains unchanged or increases slightly. Curare eliminates the fast ACh potential without affecting the slow response. Atropine selectively blocks the slow potential. Excitability is increased during the muscarinic action of ACh. This effect is independent of ACh induced changes in membrane potential. In some cells hyperpolarizing the membrane with steady inward current reduces the amplitude of the ACh depolarization but does not reduce the increased excitability effected by ACh. The spike afterpotential is reduced following muscarinic ACh activation, and this effect, too, occurs separately from changes in membrane potential.

The requirement of a large ACh dose to evoke the slow depolarizing potential may suggest that both nicotinic and muscarinic ACh receptors of high affinity (Heilbronn, E. and T. Bartfai, Prog. in Neurobiol. 11: 171, 1978) must be saturated before lower affinity muscarinic receptors mediating the slow response can be evoked. Our findings offer the opportunity to more easily and unambiguously study the ionic and biochemical mechanisms of muscarinic ACh action.

2497 PURIFICATION OF AN α-BUNGAROTOXIN BINDING COMPONENT FROM *DROSOPHILA MELANOGASTER*. Janice I. Gepner and Linda M. Hall. Dept. of Biology, MIT, Cambridge, MA 02139.

An α-bungarotoxin binding component was solubilized from homogenates of *Drosophila* heads as an initial step in the purification of this component. Detergents with intermediate hydrophile-lipophile balance (HLB) numbers of 13 to 14.5 were the most effective in solubilizing toxin binding activity. (HLB number reflects the relative hydrophobicity of a detergent.) Optimal solubilization was achieved with 1% Triton X-100 in 1.0 M NaCl. Under these conditions, a maximum of 50% of the binding activity was solubilized.

The solubilized α-bungarotoxin binding component was purified by passage twice through an affinity column composed of Sepharose 4B covalently linked to the α-neurotoxin from the cobra *Naja naja siamensis*. The binding component which was adsorbed to the affinity column and eluted with carbamylcholine had a molecular weight of approximately 500,000 by agarose gel chromatography and 250,000 by sucrose gradient analysis. The specific activity of the final purified α-bungarotoxin binding component was 1 μmole α-bungarotoxin binding sites/g protein, representing a 700 to 1000-fold increase over that of the solubilized extract.

Cholinergic compounds were shown to inhibit [^{125}I]α-bungarotoxin binding to the purified extract. The concentrations required for 50% inhibition are as follows: dihydro-ß-erythroidine (0.026 μM), nicotine (0.35 μM), d-tubocurarine (0.66 μM), trimethaphan (1.3 μM), gallamine (1.6 μM), acetylcholine (3.0 μM), atropine (18 μM), carbamylcholine (23 μM), dexetimide (60 μM), decamethonium (63 μM) and pilocarpine (150 μM). The relative ability of cholinergic ligands to inhibit toxin binding to the purified component is very similar to that observed with crude *Drosophila* extracts and is consistent with the suggestion that the toxin binding component is a nicotinic acetylcholine receptor.

(Supported by grant 1126 from The Council for Tobacco Research-U.S.A., Inc., NSF grants BNS 75-22581 and BNS 78-24594 and NIH Training Grant GM 07287. L.M.H. is a McKnight Scholar in Neuroscience.)

2498 INHIBITORY POTENTIALS RECORDED FROM MAMMALIAN PARASYMPATHETIC GANGLIA. William H. Griffith, III, Joel P. Gallagher and Patricia Shinnick-Gallagher. Dept. of Pharmacol. and Toxicol. Univ. of Tex. Med. Br., Galveston, TX 77550.

The sucrose gap recording technique was used to study post-train hyperpolarizing potentials in the cat parasympathetic vesical pelvic ganglia (VPG). This is the first time this technique has been employed to record both action potentials and slow membrane potential changes from a parasympathetic ganglion.

Following nicotinic blockade of the ganglion with either hexamethonium (10^{-4}M), chlorisondamine (10^{-5}M) or nicotine (10^{-5}M) and orthodromic stimulation of 30 Hz for 1 sec, two types of hyperpolarizing potentials were observed. First, a slow inhibitory postsynaptic potential (slow i.p.s.p.) was observed with amplitude of 0.5-1.2 mV and duration of 3-9 sec. Second, a long lasting hyperpolarization that was small in amplitude (0.1 to 0.4 mV) but lasted up to 3 min. The long lasting hyperpolarization was more apparent after blockade of the slow i.p.s.p. No slow or late excitatory postsynaptic potentials were observed (N=25).

The slow i.p.s.p. could be blocked by atropine (10^{-7} - 10^{-6}M) but was not blocked by phentolamine (10^{-6}M) or propranolol (10^{-6}M). On the other hand, NE (10^{-4}M) added to the bath by superfusion depolarized the membrane; this depolarization blocked by phentolamine (10^{-6}M). The long lasting hyperpolarization was not blocked by atropine, phentolamine or propranolol. In addition, the long lasting hyperpolarization persisted in the presence of zero Ca^{++} - 1 mM EGTA. The long lasting hyperpolarization was probably not due to synaptic events, but rather resulted from the repetitive volley being conducted in axons or fibers traveling through the ganglion. The amplitude and duration of the long lasting hyperpolarization could be enhanced by increasing the frequency and duration of train stimulation.

These data suggest the presence of a slow inhibitory postsynaptic potential that is mediated via a muscarinic receptor. Hartzell et al. (1977)[1] found a similar muscarinic i.p.s.p. in parasympathetic ganglion cells that modulate heart beat of the amphibian mudpuppy.

[1] J. Physiol. 271: 817-846 (1977).

2499 LONG-TERM PLASTICITIES ARE DIFFERENT AT DIFFERENT TERMINALS OF THE SAME PRE-SYNAPTIC NEURON IN APLYSIA. Peter B. Guthrie, Werner T. Schlapfer and Samuel H. Barondes ,Dept. of Neurosciences and Psychiatry, UCSD, La Jolla, CA 92093, and Dept. of Psychiatry, VAH, San Diego, CA 92161.

The amplitude of the EPSP evocable at R15 upon stimulation of the right connective (called RC1-R15) has been shown to exhibit prominent changes (plasticities) as a function of both its very recent and more remote history of stimulation. The pre-synaptic neuron, referred to as RC1 and presumed to be in the right pleural ganglion, has been shown to be spontaneously active (Woodson et al. Neuroscience Abstracts III (1977) #1666). The bursting pattern of RC1 allows the plasticities at RC1-R15 to be expressed such that the amplitude of RC1-R15 can vary over a several-fold range. We have now found at least twenty different neurons in the abdominal ganglion that receive direct synaptic input from RC1. The amplitude variations due to the bursting pattern of RC1 are seen at all of these synapses, suggesting that the plasticities seen at RC1-R15 are expressed by these branches of RC1.

We have examined in detail synaptic depression, frequency facilitation and post-tetanic potentiation (PTP) at the RC1 synapse onto $L9_{G1,G2}$ (tentatively identified) for comparison with RC1-R15. Identical results were obtained by stimulating RC1's processes either in the right connective or in regions of the right pedal ganglion. Although the RC1-L9 EPSP amplitude is only 30% of RC1-R15, the short-term plasticities (synaptic depression and frequency facilitation) are quantitatively similar at the two synapses. The rising phase of PTP is, however, slower ($P<.05, n=8$), PTP-amplitude is larger ($P<.001$, n=19) and the PTP-decay rate is faster ($p<.001$, n=19) at RC1-L9 than at RC1-R15. We have evidence to suggest that these differences in PTP parameters are due to functional differences in the pre-synaptic terminals and are not due to heterosynaptic influences which can accelerate PTP decay.

Since the relationships between the various parameters of the plasticities studied hold for both RC1-R15 and for RC1-L9 (e.g. PTP slope is directly correlated with PTP amplitude), we suggest that both synapses utilize the same basic mechanisms. Both the mechanisms and the biological significance, if any, of the differences in plasticities of these branches of a single neuron remain to be determined.

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2500 PRE AND POST SYNAPTIC EFFECTS OF ELEVATED EXTRACELLULAR POTASSIUM UPON IN VITRO HIPPOCAMPAL SLICES. John J. Hablitz and Arvid Lundervold* Institute of Neurophysiology, University of Oslo, Oslo, Norway.

Accumulation of potassium in the extracellular space following repetitive neural activity has been repeatedly documented in the mammalian central nervous system. Subsequent changes in neuronal responsiveness during these periods have been observed. Although these changes in extracellular potassium (K+) have been shown to have particular spatial and temporal characteristics, it has not been possible to clearly delineate pre and post synaptic effects. The present study investigated the effect of manipulation of the ionic microenvironment upon extracellularly recorded indices of pre and post synaptic activity in the in vitro hippocampal slice.

Transverse slices of guinea pig hippocampus, 400-500 microns thick, were maintained in vitro. Slices were initially incubated in a medium containing 3.25 mm (K+) and KCl was subsequently added hyperosmotically to obtain (K+) levels of 6.25, 9.25, 12.25 and 15.25 mM. Low impedance glass micropipette recording electrodes were placed in stratum radiatum and the cell body layer of CA1 to record the pre synaptic fibre volley (PV), extracellular EPSP and population spike (PS) resulting from stimulation of the Schaffer collaterals. For a given constant input strength as measured by stimulus current or amplitude of PV, the population spike amplitude was directly correlated with (K+) in the range 3.25-12.25 mM. At high stimulus strengths multiple PS's were seen at (K+) concentrations of 6.25-12.25 mM and spontaneous bursts of PS's were observed in 9.25 and 12.25 mM (K+). PS's always extinguished rapidly in 15.25 mM and occasionally in 12.25 mM (K+). These effects on form and size of the PS were reversible. Elevation of (K+) also increased the size of the EPSP in response to a constant input strength in the range of (K+) from 3.25-9.25 mM. An occasional subsequent degression of EPSP amplitude was seen in 12.25 mM and again all activity was abolished in 15.25 mM.

These results suggest that raising (K+) increases excitability by effecting changes at both pre and post synaptic sites. Post synaptic changes can be attributed to a depolarizing action of (K+), while the pre synaptic EPSP changes may result from a specific effect of (K+) on the transmitter release system as described for the neuromuscular junction (Cooke & Quastel, 1973).

2501 AMINO ACIDS THAT EXCITE CEREBELLAR PURKINJE CELLS AND THEIR POTENTIAL ROLE AS CLIMBING FIBER AND PARALLEL FIBER NEUROTRANSMITTERS. J.T. Hackett, S.L. Cochran, and D.L. Brown. Dept. of Physiology, University of Virginia School of Medicine, Charlottesville, VA 22908

The climbing fiber-evoked, Purkinje cell (PC) EPSP is unitary, and its quite large amplitude easily distinguishes it from graded EPSPs evoked by parallel fiber stimulation. We have suggested that L-glutamic acid (Glu) may be the excitatory transmitter released from parallel fibers but not from climbing fibers, since the reversal potential for Glu-evoked postsynaptic potentials is similar to the reversal potential for parallel fiber EPSPs. We have examined this notion in an investigation of the Glu receptors with putative antagonists and with L-aspartic acid (Asp). Cerebella were quickly removed from anesthetized frogs and turtles. Sagittal sections (200-300 µm thick) of cerebellum were superfused with a Ringer solution from a system that permitted rapid exchange of test solutions containing known concentrations of agents. PC responses were recorded extracellularly and intracellularly with microelectrodes filled with 2 M K-citrate. We have found no distinguishing characteristics between the actions of Glu and Asp on PC responses. Thus both agents depolarize PC to the same extent, with the same time course, and over the same concentration range (0.1 to 1 mM). Several putative antagonists (methyl- and ethyl esters of glutamic acid, 2-amino-4-phosphonobutyric acid, and nuciferine) produced only excitation of PCs. These agents, at several concentrations tested (0.1 to 1 mM) did not block the PC depolarization evoked by Glu, parallel fiber, or climbing fiber stimulation. Similarly, the excitatory action of Asp was not affected by 0.2 mM D,L-α-aminoadipic acid, a putative Asp blocker--this agent also failed to block climbing fiber-PC transmission. In contrast, morphine, (in concentrations as high as 1 mM) did not cause a measureable change of PC activity evoked by stimulating parallel fibers or climbing fibers, but in the same experiments produce a 30-70% reduction in the PC excitation by Glu. Although some of these agents may have acted to depolarize presynaptic nerve terminals, Glu-evoked depolarizations were not reduced by a Ringer solution containing lowered Ca^{2+} and 2 mM Mg^{2+} which effectively blocked all synaptic transmission. In conclusion, the similar depolarizing effects of Glu, Asp and several putative antagonists suggest that they may be acting at the same receptor site. Based on a comparison of reversal potentials, the Glu site of action is more likely the subsynaptic receptors for the parallel fiber neurotransmitters than those for the climbing fiber. However, high concentrations of morphine may have acted to block nonsynaptic effects of Glu.

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2502 VOLTAGE DEPENDENCE OF SYNAPTIC CURRENTS AT A CNS SYNAPSE IN THE HATCHETFISH. William D. Huse, John W. Day and Michael V.L. Bennett. Dept. Neurosci., Albert Einstein College of Medicine, Bronx, NY 10461

The Mauthner fibers form axo-axonic chemical synapses on giant fibers just beneath the floor of the IVth ventricle. Two to six giant fibers on each side arise from their cell bodies in the lateral wall of the IVth ventricle, pass medially dorsal to the near Mauthner fiber from which they receive a single synapse and then bifurcate to form anteriorly and posteriorly running branches that receive several synapses from the far Mauthner fiber (Model et al., Brain Res., 45:288, 1972). The transmitter appears to be ACh (Spira et al., J. Cell Biol., 47:199a, 1970). The two Mauthner fiber PSPs in the giant fibers can be individually evoked by graded stimuli applied to the caudal spinal cord. Iontophoresis of the dye, Fast Green, into a giant fiber had no pronounced effect on cell excitability or on MPSP rise time, decay time or amplitude, and facilitated simultaneous penetration with current and voltage electrodes. Electrodes were positioned within 50µ of one another and within 100µ of the synapse from the near Mauthner fiber. The postsynaptic region was then voltage clamped at its resting potential and stepped to various levels 15 msec before antidromic activation of the presynaptic cells. At the resting potential (c.-85 mV) postsynaptic currents rise to a peak in less than 0.1 msec; after an initial more rapid phase, decline is exponential with a time constant of c.0.60 msec. Hyperpolarization augments the peak current and slows the decline (c.0.85 msec at -160 mV). Increasing inside positivity reduces and then inverts the synaptic currents. The currents are essentially absent at reversal potentials of c.-10 mV. Reduced and inverted currents decline more rapidly (c.0.28 msec at 90 mV). Peak amplitude is linearly related to voltage. These results are consistent with data from the frog neuromuscular junction (Magleby and Stevens, J. Physiol., 223:173, 1972) in which the decline in synaptic current is more rapid at more inside positive membrane potentials. In that case the decline is determined by the rate of closing of ACh activated channels.

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2503 PRIMARY AFFERENT DEPOLARIZATION IN THE FROG OLFACTORY BULB. C.E. Jahr* and R.A. Nicoll, Depts. of Pharmacology and Physiology, UCSF, San Francisco, CA 94143.

Primary afferent depolarization in the spinal cord and brain stem is believed to be causally linked to presynaptic inhibition (Schmidt, R.F., Ergeb. Physiol. 63:20, 1971). Using the isolated olfactory bulb of the bullfrog, we have recorded by the sucrose gap method a similar, but much longer lasting, depolarization of the olfactory primary afferents following stimulation of the same or adjacent olfactory nerve (ON) fibers. The ON depolarization lasted up to 40 sec., was blocked by Co^{++} and increased in amplitude and duration with increasing stimulus strength. These results indicate that intact synaptic transmission is necessary for the ON depolarization but that firing of the same fibers is not a prerequisite. The antidromic compound action potential evoked by stimulation of the primary afferent terminal area was increased by a preceding conditioning stimulus. This excitability increase paralleled in duration the ON depolarization and was blocked by Co^{++} suggesting that the ON terminals themselves were depolarized.

The n_1 wave of the orthodromic field potential recorded in the glomerular layer with a focal extracellular electrode is the sum of the currents produced by the synaptic excitation and subsequent action potentials set up in the mitral cells by the ON terminals. Upon paired stimulation, the n_1 to the second shock was blocked at short intervals. On analogy to the crayfish neuromuscular junction (Dudel, J. and Kuffler, S.W., J. Physiol. 155:543, 1961), one would not expect an inhibitory postsynaptic conductance increase to block the synaptic current. Blockade of the n_1 is, therefore, consistent with a presynaptic mechanism. A conditioning stimulus applied to the ON can inhibit the n_1 produced by a test stimulus to adjacent but distinct ON fibers, and thus rules out the possibility that depletion of readily releasable transmitter from the ON terminals is responsible for blockade of n_1.

While the mechanism underlying the ON depolarization has not been elucidated, a picrotoxin sensitive depolarization was produced by GABA. Picrotoxin, however, does not antagonize the electrically evoked ON depolarization. Potassium sensitive electrodes are being used to determine what role fluctuations in extracellular potassium play. Both glutamate and glycine also depolarized the ON and are, therefore, candidates.

2504 ROLE OF CATECHOL-O-METHYLTRANSFERASE (COMT) IN SLOW POSTSYNAPTIC RESPONSES TO PREGANGLIONIC IMPULSES, IN MAMMALIAN SYMPATHETIC GANGLION. B. Libet and John H. Ashe, Department of Physiology, School of Medicine, University of California, San Francisco, CA. 94143.

The COMT-inhibitor U-0521 (0.3mM) produces a reversible augmentation of both the dopamine (DA)-mediated s-IPSP and the ACh-mediated s-EPSP postsynaptic responses to preganglionic nerve impulses, in rabbit superior cervical ganglia. The relative increases in both slow PSP's were larger for the weaker test responses (to a single volley), but the overall potentiations were greater and more consistently apparent for the s-EPSP.

Augmentation of s-EPSP is explained as due to potentiation of the long-lasting DA-modulatory enhancement of the s-EPSP response to ACh (acting muscarinically) (Libet and Tosaka, Proc.Nat.Acad.Sci. 67: 667-673, 1970). This implies that DA spontaneously released by the dopaminergic interneurones ("SIF" cells) can be sufficient to induce a modulatory change, when this DA is protected from inactivation by COMT. This view is further supported by finding that bromocriptine (7μM), an antagonist of DA stimulation of adenyl cyclase in brain tissue, can antagonize the augmentation of s-EPSP responses by U-0521.

The present evidence also suggests that COMT can play a significant limiting role that is analogous to that of acetylcholinesterase, in at least some neurone-to-neurone synaptic functions involving catecholamine transmitters. For DA in this ganglion, COMT-access barriers would appear to be effective primarily against the postsynaptic actions. Access to presynaptic receptors is apparently not so limited by COMT, judging from presynaptic as opposed to postsynaptic effects obtainable with DA applied exogenously. A special ability of COMT-access barriers to limit the DA-modulatory action, which can be induced by the smallest amounts of DA moving from releasing sites to possibly more distant postsynaptic receptors, could be of significance in regulating potentially related modulatory actions of catecholamines in the brain. (Supported by U.S.P.H.S. grant NS-00884 from NINCDS.)

2505 SUBSYNAPTIC LOCALIZATION OF α-BUNGAROTOXIN BINDING WHICH BLOCKS NICOTINIC TRANSMISSION AT FROG SYMPATHETIC NEURONS. Lawrence M. Marshall* (SPON: R.M. Harris-Warrick). Department of Neurobiology, Harvard Medical School, Boston, MA 02115.

Sympathetic neurons receive primary synaptic input from cholinergic terminal boutons of preganglionic nerve fibers. The distribution of acetylcholine receptors at these synapses is not precisely known. This study shows that α-bungarotoxin, known to bind to nicotinic receptors on skeletal muscle, may also be useful for localizing nicotinic receptors on principal neurons of paravertebral sympathetic ganglia of the bullfrog.

α-Bungarotoxin (1-5 μM) produces a block of nicotinic (fast) excitatory postsynaptic potentials which is fully reversed after 5-8 hrs of washing. Nicotinic antagonists reduce the half-time of recovery from the toxin to one-third the control value, presumably by competing with the toxin for the same receptor sites. Furthermore, the response to applied carbachol is suppressed by the toxin, indicating that the block of synaptic transmission is due to reduced sensitivity of the postsynaptic membrane.

Peroxidase-labeled α-bungarotoxin is localized to small (0.2-0.5 μ diameter) patches beneath synaptic boutons. Each patch of peroxidase reaction product is restricted to those regions of the synaptic cleft just opposite the active zones of the presynaptic terminal. Additional experiments show that peroxidase-labeled antibodies against Torpedo acetylcholine receptor bind exclusively to these same subsynaptic regions; suggesting further that these patches represent the areas at which nicotinic receptors are concentrated on sympathetic neurons.

2506 RUTHENIUM RED INHIBITION OF THE CALCIUM DEPENDENT ^{3}H-GABA RELEASE DURING ONTOGENESIS IN THE MOUSE. Graciela Meza-Ruiz (SPON: Ricar do Tapia). Departamento de Neurociencias, Centro de Investigaciones en Fisiología Celular. UNAM. México.

Neuronal calcium-dependent release of GABA develops in a progressive fashion as shown in synaptosomes isolated from rat brain (Redburn et al, Brain Res. 152:511-519, 1978). On the other hand, we have shown that Ruthenium Red inhibits calcium-dependent GABA release from synaptosomes both in vitro and after intracranial injection of the dye (Brain Res. 126:160-166, 1977; Brain Res. 154:163-166, 1978).

In the present work, ^{3}H-GABA release was investigated during development in the mouse, both in the absence and in the presence of Ruthenium Red. Crude synaptosomal preparations were isolated from 5, 10, 15-day old and adult mice. They were loaded with ^{3}H-GABA (0.5 uM; 0.8 uCi) for 10 minutes at 37°C in Krebs-bicarbonate buffer, pH 7.4. ^{3}H-GABA release was performed in 4 parallel superfusion chambers at 37°C as previously described (Brain Res. 126:160,166, 1977). Calcium-dependent release was studied either by depolarization with 48 mM potassium in the presence of calcium or by calcium-stimulation in synaptosomes previously depolarized by 48 mM potassium in the absence of calcium. The effect of Ruthenium Red was studied only in the latter, in synaptosomes whose loading was performed in the presence of 40 uM Ruthenium Red in a calcium-free medium.

The release of ^{3}H-GABA was stimulated by high potassium 64, 89, 161 and 193 percent over the spontaneous baseline in 5, 10, 15-day old and adult mice, respectively. Spontaneous efflux was the same regardless of age. Stimulation by calcium was of 59, 110, 157 and 148 percent over the baseline for the same age periods. In the presence of Ruthenium Red, this stimulation was inhibited by 30, 55, 60 and 58 percent for 5, 10, 15-day and adult animals, respectively.

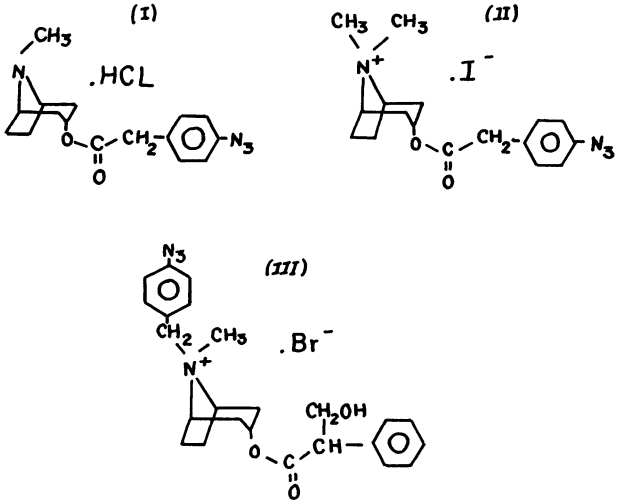
These results show that although spontaneous efflux of GABA does not change throughout development of the brain, the calcium-dependent, depolarization-induced release, sensitive to Ruthenium Red, seems to increase during ontogenesis.

The collaboration of Mrs. Consuelo Hernández and Miss Lilia Reynoso in some of the experiments is acknowledged.

2507 PHOTOAFFINITY LABELING OF SPECIFIC ^{3}H-QNB-BINDING SITES IN BRAIN MEMBRANES. Jose Moreno-Yanes* (SPON: Ruth S. Gurd). Chemistry Dept., Indiana University, Bloomington, IN 47405

The specific [^{3}H]-quinuclidinyl benzylate (QNB) binding sites of several membrane fractions from rat brain have been irreversibly inactivated by photoaffinity labeling with two azido-phenyl acetate esters of 3-tropanol (I and II) and N(p-azidobenzyl) methyl atropine (III). Without photolysis, the three compounds bind to the [^{3}H]-QNB binding sites with apparent dissociation constants of 3.7×10^{-6} M, 9×10^{-7} M and 4.2×10^{-8} M, respectively. Subsequent irradiation of these probes bound to these membrane sites converts their azido function to the highly reactive nitrene and results in an irreversible loss of the capability of the preparation to interact with [^{3}H]-QNB.

Atropine and oxotremorine, but not d-tubocurarine, afford protection against this irreversible photoinsertion of the probes. These findings suggest that the compounds may prove useful as specific photoaffinity labels for muscarinic receptors. (Supported by Research Grant NS 08309 from the NIH)



2508 A PROLONGED VOLTAGE-SENSITIVE INHIBITORY SYNAPTIC POTENTIAL IN MITRAL CELLS OF THE ISOLATED TURTLE OLFACTORY BULB. Kensaku Mori* and Gordon M. Shepherd. Section of Neuroanatomy, Yale Univ. Sch. Med., New Haven, CT 06510.

We have continued an intracellular analysis of synaptic potentials in the isolated turtle olfactory bulb preparation (Nowycky, Waldow and Shepherd, Neurosci. Absts. 4:583, 1978). Single olfactory nerve volleys induce a complex sequence of synaptic potentials in mitral cells. There is an early period which includes the EPSPs in the terminal tufts of mitral cell dendrites in the olfactory glomeruli, and a later period consisting of a slow hyperpolarizing IPSP. The IPSP can be divided into two phases, early and late. The early phase covers the first several hundred msec of the response, whereas the later phase lasts for very long periods; in some cases the hyperpolarization persists as long as 4 secs following a single volley. When the membrane potential was held at different levels by injected current, the early IPSP was found to have a reversal potential of -70 to -80 mV. In this respect it resembled IPSPs in motoneurons and other central neurons. Some degree of overlap with the initial EPSP may be present during this early phase. In contrast, the late IPSP did not reverse; it decreased in amplitude as the membrane was depolarized, and also when it was hyperpolarized. This is similar to the behavior of voltage-sensitive IPSPs previously demonstrated in piriform cortex neurons (Mori, Satou and Takagi, Proc. Jap. Acad. 54:484, 1978) and in parasympathetic ganglion cells (Hartzell, Kuffler, Stickgold and Yoshikami, J. Physiol. 271:817, 1978). Tests with brief injected current pulses indicated that membrane conductance was increased during the early phase. The results suggest at least two different types of synaptic receptors or receptor mechanisms for inhibition of mitral cells.

2509 TWO MORPHOLOGICALLY AND STRUCTURALLY DISTINCT POPULATIONS OF NEUROSECRETORY GRANULES PURIFIED FROM RAT NEUROHYPOPHYSIS. S.J. Morris and J.J. Nordmann*, Max Planck Inst. for Biophys. Chem., D-3400 Goettingen, FRG and INSERM U176, F-33077 Bordeaux Cedex, France.

The neurohormones vasopressin and oxytocin are synthesized and packaged as pro-hormones in cell bodies in the hypothalamus and transported down axons to nerve terminals, which together with axonal "swellings" and glia form the neurohypophysis, from which both hormones are released by exocytosis[1].

Subcellular fractionation of the granules on sucrose/Metrizamide gradients which were isoosmotic at 360 mOsm(=0.30 M sucrose)[2]produced 2 populations of granules:one with a density of ∿1.13 containing granules of 172 ±2(SEM) nm dia. and a second with a density of ∿1.11 and dia. of 197 ±9 nm. Both populations contained oxytocin, vasopressin and neurophysins. The denser granules were insensitive to osmotic change while the less dense particles behaved like perfect osmometers. When compared to the tissue homogenate, these fractions were purified 4-5 fold in relation to mitochondria and 7 fold compared to lysosomes. Chromatography of the gradient fractions on CPG 10-3000 porous glass produced further purification.

These structural differences do not reflect the cleavage of the precursor into neurophysin and hormone since virtually no precursor is found in the neural lobe[3]. Newly synthesized granules seem to move first into the nerve terminals and only then, if not released, into the "swellings"[4]. Our results suggest that the granules are transported as osmotically inert structures and that conversion of the pro-hormones to active oligopeptides and neurophysins would not greatly alter the osmotic activity of the core. We postulate that a subsequent aging process (probably exopeptidase activity) occurs in the neural lobe which renders the granule core osmotically active. Thus the osmotically inert granules fraction would represent those newly arrived while the granules sensitive to osmotic pressure would represent the older granules. In support of this, the osmotically sensitive granules have diameters similar to that of granules found in the swellings after fixation of the gland (188 ± 2 nm) whereas the non-osmotically active granules have diameters like those found in the nerve terminals (173 ± 3 nm). Thus the fractionation seems to produce granules from two anatomically different areas of the terminals of the neural lobe. The aging process might be of importance for the subsequent destruction and/or lysosomal digestion of the unused neurosecretory granules.

A complete account of this work is in press[5].

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2510 DIRECT ELECTRICAL SYNAPTIC CONNECTION IS MEDIATED BY AN INTERNEURON. Kenneth J. Muller and Sheryl A. Scott. Dept. of Embryology, Carnegie Institution of Washington, Baltimore, Md. 21210.

Touch sensory neurons (T-cells) in segmental ganglia of the medicinal leech make excitatory electrical synaptic connections upon the S-interneuron, whose axon is the "giant fiber" of each segment. Positive current but not negative current can flow from T-cells to S-cells, so that S-cells do not excite T-cells. Morphological and physiological measurements suggest that excitatory currents leave the T-cell at several points within the ganglion. For example, the amplitude of excitatory synaptic potentials recorded in the S-cell is diminished when T-cell impulses, propagating into the ganglia from anterior or posterior receptive fields, fail at central branch points and activate only a portion of the T-cell's presynaptic terminals. This branch-point failure occurs in response to natural stimuli, and by modulating synaptic transmission can affect integration within the ganglion.

Intracellular injection of the marker horseradish peroxidase (HRP) reveals that ventral and lateral T-cells do not make direct contact with the S-cell; therefore, another neuron must mediate the electrical connection between them. Intracellular injection of the S-cell with fluorescent dye of low molecular weight, such as Lucifer Yellow (Stewart, Cell 14: 741, 1978) or 6-carboxyfluorescein, which can cross between electrically coupled cells in the leech, stains with nearly uniform intensity the S-cell and two small interneurons in each ganglion, designated as Stewart cells. Positive currents can pass from Stewart cells to S-cells and vice versa. HRP injections show that Stewart cells provide the necessary link between T- and S-cells and can account for the spatially distributed connection between them. When Stewart cells are selectively eliminated from the ganglion with intracellular injection of protease, which does not measurably affect cells that are electrically coupled to the injected cell, the synaptic connection between T- and S-cells is selectively abolished. We conclude that Stewart cells mediate the distributed electrical connections between ventral and lateral T-cells and the S-cell in each ganglion, connections that by physiological criteria seem monosynaptic.

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2511 RELEASE OF β-ADRENERGIC AGONIST FROM SYMPATHETIC NEURONS IN CO-CULTURE WITH PINEAL CELLS. Andrew Parfitt* and William Shain (SPON: L. M. Masukawa). Dept. of Neurobiology, Armed Forces Radiobiology Research Institute, Bethesda, MD 20014.

Co-cultures of pineal cells and sympathetic neurons were prepared from dissociated pineal glands and superior cervical ganglia dissected from 2-day-old rats. Routinely 300,000 pineal cells were plated with 30,000 neurons. Cultures were maintained in Ham's F12 containing 10% fetal calf serum at 36.5°C in an atmosphere of 4% CO_2:96% air. Medium was changed every third day. Experiments were done 5 to 8 days post-culture preparation. Test compounds were incubated with the cultures for 6 hours. In order to monitor spontaneous release of β-agonist, co-cultures were incubated with compounds that block the reuptake of norepinephrine by nerve endings. Both desmethylimipramine (DMI) (10^{-5} M) and cocaine (10^{-6} to 10^{-4} M) caused an increase in acetyl CoA:serotonin N-acetyltransferase (NAT, EC 2.3.1.5) activity in the pineal cells. Neither DMI nor cocaine affected basal or isoproterenol(ISO)-stimulated NAT activity in pineal cells cultured alone. The DMI- or cocaine-stimulated increase in NAT activity in co-cultures was not affected by simultaneous treatment with tetrodotoxin (TTX) (10^{-7} M). This concentration of TTX was sufficient to block electrically initiated action potentials in the cultured neurons. The increase in NAT activity following treatment with DMI and cocaine varied between preparations, probably as a result of variations in the numbers of neurons in the cultures prepared on different days. This increase in NAT activity was never less than 50% and frequently approached 80% of the activity elicited by a maximally effective concentration of ISO (10^{-8} M). In order to monitor evoked release from neurons, co-cultures were incubated with batrachatoxin (BTX, 10^{-7} M), which is reported to stabilize voltage-dependent sodium channels in the open configuration, thus depolarizing the neurons. BTX treatment elicited an increase in NAT activity, which was blocked by simultaneous application of TTX, but was only slightly diminished by treatment with $4x10^{-2}$ M $MgCl_2$. Cobalt was tested but was found to kill both the neurons and the pineal cells. Neither TTX nor BTX had an effect on basal or ISO-stimulated NAT activity in pineal cells. Culture medium taken from the BTX- or DMI-treated co-cultures initiated an increase in NAT activity when added to cultures of pineal cells alone. This enabled us to bioassay β-adrenergic agonist released by the neurons in co-culture. Calculations on data from a representative experiment indicated that an amount of β-agonist equivalent to $6.7x10^{-12}$ moles of norepinephrine was released from each neuron during a 6-hour treatment with BTX.

2512 EFFICIENCY OF UTILIZATION OF QUANTAL ACETYLCHOLINE. P. Pennefather* and D. M. J. Quastel. Dept. of Pharmacology, The University of British Columbia, Vancouver, B. C., V6T 1W5, Canada.

Miniature end-plate currents (m.e.p.c.s) recorded at vertebrate neuromuscular junctions are produced by the release of quanta of acetylcholine (ACh); this gives rise to localized transient high concentrations of transmitter in the synaptic cleft. The m.e.p.c. height will be dependent on the relative likelihood of ACh interacting with receptor rather than it being lost through hydrolysis or diffusion. As a result, d-tubocurarine (d-TC) is less effective at reducing m.e.p.c. height after inhibition of acetylcholinesterase (AChE) and is more effective after partial receptor inactivation by myasthenic immunoglobulin G or α-bungarotoxin. With a few assumptions, a simple equation can be derived which relates the apparent potency of d-TC under varying conditions to the m.e.p.c. height in the absence of d-TC. This provides a basis whereby one can estimate the m.e.p.c. height that would have been achieved if all of the ACh in a quantum became bound to receptor, and the ratio of m.e.p.c. height to this maximum gives an indication of the fraction of ACh in a quantum that has become bound to receptor at the peak of the m.e.p.c. Although the equation ignores a number of complicating factors, it accurately describes the behavior of a variety of mathematical models of the synapse which include such complications. Experimental data from mouse diaphragm indicate that normally about 75% of ACh released as a quantum binds to receptor; after inhibition of AChE about 90% binds. Thus, m.e.p.c. height is little altered by inhibition of AChE. Katz and Miledi (J. Physiol. 231, 549, 1973) have pointed out that d-TC increases m.e.p.c. decay rate after inhibition of AChE, evidently by increasing the fraction of ACh that is not bound to receptor and is therefore free to diffuse from the cleft. This phenomenon can be used to estimate the fraction of ACh bound to receptor during the decay phase. We find that at e^{-1} of the peak of the m.e.p.c. only about 45% of ACh in the cleft is bound to receptor as opposed to 90% at the peak. This difference presumably arises from cooperativity in ACh binding. At e^{-1} of the peak, the concentration of ACh in the cleft has become relatively low and binding seldom proceeds beyond unstable intermediate forms. Further support for this interpretation comes from the observation that the m.e.p.c. decay rate is relatively slow early in its decay phase and progressively increases.

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2513 INTRACELLULAR RECORDING FROM NEURONS IN THE LOCUS COERULEUS AND MESENCEPHALIC NUCLEUS OF THE TRIGEMINAL NERVE (MNV) IN VITRO. Christine M. Pepper* and Graeme Henderson* (SPON: A. G. Karczmar) Department of Pharmacology, Loyola University Stritch School of Medicine, Maywood, Illinois 60153.

Intracellular recordings were made in vitro from slices (350μm thick) of guinea-pig pons. Locus coeruleus neurons had a resting membrane potential of -52.7 ±2.7 mV (mean ± SEM) (n=20), an input resistance of 58.0 ± 7.6 MΩ (n=21) and a membrane time constant of 7.3 ± 1.0 ms (n=21). Only 6 of 31 cells were spontaneously active. Focal stimulation either directly activated the cell soma to fire an overshooting action potential or elicited an excitatory post-synaptic potential (e.p.s.p.); e.p.s.ps of sufficient amplitude gave rise to action potentials. E.p.s.ps were graded with stimulus intensity and were abolished in calcium free solutions. action potentials recorded in locus coeruleus neurons were 1.38 ± 0.15 ms (n=21) in duration and were followed by an after-hyperpolarization. MNV neurons had a resting membrane potential of -51.9 ± 3.6 mV (n=13), an input resistance of 15.0 ± 1.8 MΩ (n=18) and a membrane time constant of 1.4 ± 0.2 ms (n=17). These cells showed time dependent anomalous rectification to injected hyperpolarizing current pulses and accommodation to depolarizing pulses. Focal stimulation gave rise to action potentials or to low amplitude (<10 mV) short duration (<5 ms) depolarizing potentials. The electrical properties of MNV cells are similar to those previously described for sensory ganglion cells.

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2514 THE EFFECTS OF VARYING Ca^{++} CONCENTRATION ON SYNAPTIC TRANSFER AT A SYNAPSE IN THE LAMPREY SPINAL CORD. G.L. Ringham. Dept. Physiology, University of Utah College of Medicine, Salt Lake City, Utah, 84108.

The synaptic transfer curve relating pre- and post-synaptic potential changes at a synapse between a Müller axon (the presynaptic element) and a large interneuron (the post-synaptic element) in the spinal cord of the lamprey has been described previously (Martin & Ringham, J. Physiol. 251, 409-426, 1975). In the tetrodotoxin-blocked preparation superfused with normal solution containing 3 mM Ca^{++}, the transfer curve saturates at presynaptic depolarizations on the order of 90-100 mV. Among the factors which could contribute to development of this plateau is a decreased inward driving force on Ca^{++} at these larger presynaptic depolarizations. This possibility can be tested directly by obtaining transfer curves at several different Ca^{++} concentrations. Those curves obtained at higher Ca^{++} concentrations should be shifted along the abscissa (toward lower levels of presynaptic depolarization) and have larger peak values.

Preliminary experiments have compared the synaptic transfer curves obtained in 3.0, 6.0 and 9.0 mM Ca^{++} solutions (prepared by isotonic substitution of $CaCl_2$ and NaCl). The curves obtained in the high Ca^{++} solutions are indeed shifted along the abscissa to the left, with a slight decrease in the apparent presynaptic "threshold" depolarization required for production of a detectable post-synaptic potential (PSP). The effects are somewhat greater in the 9.0 mM Ca^{++} solution than in 6.0 mM Ca^{++}. However, these curves often saturate at PSP values very similar to those observed in normal Ca^{++} solutions, and there is little difference in this effect between the 6.0 and 9.0 mM Ca^{++} solutions. Thus, it would appear that a decreased driving force on Ca^{++} is not the only factor determining the saturation level of these transfer curves. Perhaps this inconsistency in effect of the high Ca^{++} solutions on maximum PSP amplitude is related to transmitter depletion at those synapses with larger PSP's. Interestingly, the high Ca^{++} concentrations do not seem to depress the sensitivity of the post-synaptic membrane, because the amplitudes of spontaneously occurring miniature synaptic potentials (from numerous sources) recorded from the interneuron are essentially unchanged in these solutions.

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2515 MEMBRANE POTENTIAL OF OLFACTORY BULB SYNAPTOSOMES MONITORED WITH THE LIPOPHILIC CATION TETRAPHENYLPHOSPHONIUM. S. Rochel* and F. L. Margolis (SPON: R. Wurzburger). Dept. Phys. Chem. & Pharm., Roche Institute of Molecular Biology, Nutley, NJ 07110.

Measurement of changes in synaptosomal membrane potential associated with transmitter uptake and release cannot be performed by traditional electrophysiological techniques. Recently the utility of the lipophilic cation [^{3}H]tetraphenylphosphonium (TPP^+) to study neuronal membrane potential has been demonstrated (Lichtshtein, Kaback & Blume, PNAS 76:650-654 [1979]). Since [^{3}H]TPP^+ distributes across a membrane in proportion to the transmembrane potential it can be used to measure that potential according to the Nernst equation:

$$\Delta\Psi = \frac{-RT}{ZF} \ln [TPP^+]_{in}/[TPP^+]_{out}.$$

Two crude synaptosomal fractions were prepared from rat olfactory bulb: P_1-the pellet after centrifugation at 1000 g for 10 min containing multisynaptosomal particles and P_2-the pellet after centrifugation at 14,500 g for 20 min, which is a traditional mitochondrial-synaptosomal fraction. The internal volume of P_1 was 4.5 μl/mg protein and of P_2 was 6.5 μl/mg protein as measured by ^3H-H_2O and [^{14}C]inulin distribution. Using these volumes, the concentration of TPP^+ was calculated as nmoles TPP^+ per synaptosomal volume. [^{3}H]TPP^+ accumulation into P_1 and P_2 forms a concentration gradient which is four-fold higher at low external K^+ (4.5 mM) than at high external K^+ (122 mM), indicating the dependence of resting membrane potential on the potassium diffusion gradient (in→out). Assuming that the K^+ diffusion gradient is the major component of synaptosomal ΔΨ, a resting membrane potential of 100 ± 5 mV can be calculated. Veratridine, a neurotoxic alkaloid which causes depolarization of the synaptosomes by allowing electrogenic Na^+ influx, diminished [^{3}H]TPP^+ accumulation in high Na^+ medium. This effect of veratridine was not observed when Na^+ ions were omitted from the medium. The protonophore carbonylcyanide-m-chlorophenylhydrazone, which collapses membrane potential, abolished [^{3}H]TPP^+ accumulation in our synaptosomal preparations. Interestingly, the cardiac glycoside ouabain which inhibits the Na^+/K^+ ATPase caused a 30% decrease in [^{3}H]TPP^+ accumulation within 1 min and nearly abolished the accumulation at longer times, indicating the dependence of synaptosomal ΔΨ on pump activity. These results indicate that the accumulation of [^{3}H]TPP^+ can be used to monitor the transmembrane potential of olfactory bulb synaptosomal fractions. The influence of various neuroeffectors on this phenomenon is currently under investigation.

2516 USE OF DOPAMINE β-HYDROXYLASE ANTIBODIES IN THE STUDY OF VESICLE DYNAMICS. Robert A. Rush*, Thomas J. Millar*, Laurence B. Geffen*, Centre for Neuroscience, School of Medicine, Flinders University, Bedford Park, S.A. 5042, Australia. (SPON: R. Porter, Department of Physiology, Monash University, Clayton, Vic. 3168, Australia.)

Systemically administered antibodies to dopamine β-hydroxylase are taken up into noradrenergic nerve terminals by binding to membrane bound dopamine β-hydroxylase molecules which become exposed on the external surface of the neurone during exocytosis. In the presence of complement this antigen-antibody reaction leads to a lysis of the plasma membrane, resulting in the degeneration of the whole axon terminal. When lysis is prevented by the use of an Fab_2' fragment of the antibody or in the absence of complement, the binding of antibody to DBH results in the inhibition of enzyme activity. The inhibition of enzyme activity may be used to study the turnover of DBH in nerve terminals. With the use of an appropriate label, the bound antibody may also be visualised at both the light and electron microscope level. When DBH is coupled to horseradish peroxidase (HRP) and administered either systemically or locally into the anterior chamber of the eye, the DBH-HRP complex can be localised in membranous structures within axon terminals. In this way, the fate of vesicles which have participated in the release process can be studied.

Twenty-four hours after systemic injection, the sympathetic nerve terminals of the iris contain many densely labelled small vesicles. In addition, label was also clearly visible within large membranous organelles and cylindrical cisternae. Little staining was visible on the plasma membrane of axon terminals suggesting that, following exocytosis, the vesicle membrane is rapidly retrieved. This novel approach to the ultrastructural localization of DBH within neurones overcomes many of the difficulties associated with *in vitro* immunohistochemistry and moreover provides a method for studying the fate of synaptic vesicles after they have undergone exocytosis.

2517 SYNAPTIC VESICLE MORPHOLOGY IN THE RAT NEUROMUSCULAR JUNCTION FOLLOWING APPLICATION OF LEPTINOTARSIN. A.A. Sadun, A.K. Crews*, T.H. Hsiao*, J.R. Stimers*, W.O. McClure. Neuro. Res. Lab., Huntington Inst. of Appl. Med. Res., Pasadena, CA 91105; Dept. of Biological Sciences, Univ. of S. California, Los Angeles, CA 90007 and Dept. of Biology, Utah State Univ., Logan, UT 84322.

Satin et al (Soc. Neuros. Abs. 4, 1873,1978) described a biphasic response of miniature end plate potentials (mepps) following application of leptinotarsin (LPT), a toxic protein found in the hemolymph of the beetle Leptinotarsa haldemani. We confirmed that when LPT is added to a rat phrenic nerve-diaphragm preparation, intracellular recordings show a biphasic release of mepps. Shortly after application of LPT there is a burst of mepps reaching about 900 Hz and lasting about one minute. There follows, about six minutes later, a second cascade of activity peaking to about 400 Hz and lasting about 12 minutes before the frequency of mepps falls to zero.

In control preparations, normal ultrastructural features of the neuromuscular junction were noted. The axon terminals are uniformly filled with small round vesicles and the junctional folds demonstrate a repeating precise architecture.

Preparations to which LPT was applied were then examined. Tissue which was fixed immediately after the first burst of mepps activity does not markedly differ in ultrastructure from control preparations. The terminals are filled with spherical vesicles. Mitochondria and junctional folds appear nearly normal.

In a third preparation, the tissue was quickly fixed after the second response, at the time the frequency of mepps fell to zero. Numerous morphological distinctions were noted. Significantly, the terminals contain vesicles many of which are collapsed. Mitochondria in the terminals contain swollen cisternae while mitochondria in the muscle cells look normal. The post-synaptic junctional folds show morphological variation.

Suszkiw (Soc. Neuros. Abs. 4, 1876,1978) has shown that a discrepancy may exist between the vesicular content of ACh and the number of vesicles seen. This fact, the present study, and other recent investigations suggest that vesicles may be conserved in the face of massive neurotransmitter release. These experiments provide evidence for ultrastructural changes in axon terminals secondary to the pulsed release of neurotransmitter. Some of the remaining vesicles have a distinctive morphology which may provide a clue to alternate modalities for neurotransmitter release or vesicle formation.

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2518 RELEASE OF ENDOGENOUS CATECHOLAMINES IN THE CAUDATE NUCLEUS AND PREOPTIC AREA OF THE ANTERIOR HYPOTHALAMUS OF FREELY MOVING RATS. Steven K. Salzman*, and Matej Stepita-Klauco (SPON: T. Stanton). Dept. Biobehavioral Sci., Univ. of Connecticut, Storrs, Ct. 06268

The release of endogenous catecholamines (CA), norepinephrine, dopamine and epinephrine, was studied in vivo, using a push-pull perfusion technique followed by high pressure liquid chromatography with electrochemical detection (Bioanalytical Systems). Push-pull cannulae were constructed from stainless steel hubs (David Kopf) and tubing (24g for pull, 32g for push, Small Parts) and machine screws (Small Parts). Cannulae were implanted stereotaxically, under chloral hydrate anesthesia (350mg/kg), in either the caudate nucleus or the preoptic area of the anterior hypothalamus of rats. Both the push and pull ends of the cannulae were connected, via silastic tubing (Sil-Med) to the output and input ends respectively of miniature electrically pulsed pumps (Valcor), mounted directly on a mercury commutator, thus allowing the rat free movement during the perfusion session. The pumps were pulsed remotely through the commutator by a timer with variable settings (Harvard Apparatus). Artificial cerebrospinal fluid, 0.1 micron filtered and warmed to 37°C, was perfused at a constant rate of 30µl/min. Samples were collected at 5-50 min. intervals into vials containing 50-100pg of an internal standard (dihydroxybenzylamine, Aldrich) in 75-750µl of ultrapure perchloric acid (Baker). After extracting the CA on alumina, 20µl aliquots of the alumina eluates were valve loaded (Rheodyne) onto the column (Vydac, strong cation exchange) and separated under 400 psi pressure. The mobile phase was a degassed phosphate-citrate buffer. The column was maintained at 28°C by means of a water jacket. The glassy carbon working electrode in the thin layer electrochemical cell was preset at a potential of +720 mV against a Ag/AgCl reference electrode. The current resulting from the oxidation of the CA was monitored on a pen recorder (Houston Inst.). A release profile was determined under basal conditions during waking and sleep (behavioral criteria). The optimal perfusion time for the sensitivity of the assay was also determined. Specificity of release was determined by an increase in CA output after an i.p. injection of amphetamine (5mg/kg) at the end of the session. Results are discussed in terms of the validity of the in vivo approach and its possible utility for studying transmitter release during different behavioral states. Preliminary results indicate that the concentrations of DA in the perfusate from the caudate and NE in the perfusate from the hypothalamus are in the nanomolar range.

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2519 HABITUATION: CELLULAR MECHANISMS IN MAMMALIAN HIPPOCAMPUS. Linda K. Simmons and R. J. W. Mansfield. Psychology Department, Harvard University, Cambridge, Mass. 02138.

Habituation, the response decrement due to repetitive stimulation, is a simple yet ubiquitous form of non-associative learning that is observed across a wide phylogenetic range. We have utilized the in vitro rat hippocampal slice, a reduced preparation that produces evoked responses manisfesting 8 of the parametric criteria characterizing behavioral habituation (T. J. Teyler and B. E. Alger, Brain Res., 115, 413, 1976), to study the cellular mechanisms underlying this form of synaptic plasticity.

After surgery the 400 micra hippocampal slices were maintained in perfusion chambers that enabled the quick (within 30 seconds) and complete transfer of various perfusates. Stimulating electrodes were placed in the perforant pathway and recording electrodes were placed in the granule cell or molecular layer to obtain monosynaptic population spikes or EPSP's, respectively, from the dentate gyrus region of the hippocampal formation.

Studies with other preparations such as the Aplysia suggested that certain cyclic nucleotides can modulate synaptic plasticity by manipulating the voltage-dependent Ca^{2+} influx which controls levels of transmitter release (M. Klein and E. R. Kandel, P.N.A.S. (USA), 75, 3512, 1978). In the hippocampal slice both theophylline, a phosphodiesterase inhibitor, and N^6,O^2-dibutyryl adenosine 3'-5' cyclic monophosphate (Bu_2cAMP), a more membrane-permeable form of the nucleotide, significantly and reversibly attenuated the relative habituation of the evoked population spikes and EPSP's when compared to controls. Preliminary studies indicated that N^2,O^2'-dibutyryl guanosine 3'-5' cyclic monophosphate (Bu_2cGMP) has no effect upon the habituation observed at this group of synapses. This depression of relative habituation was invariably accompanied by an increase in the absolute amplitudes of the evoked responses. Since habituation is inversely related to the stimulus intensity, the decreased relative habituation displayed in the drugged solutions may be caused by the observed increase in synaptic efficacy. Control studies performed to determine the locus of the drug-induced synaptic change indicated that the effect of the experimental solutions is not due to either a change in the afferent volley size or the gross postsynaptic membrane impedance. The above results suggest that cAMP levels indirectly decrease relative habituation by enhancing transmitter release (presumably by increasing the voltage-dependent Ca^{2+} influx) for a given stimulus intensity. Whether cAMP levels initiate habituation by manipulating the Ca^{2+} current has to be determined.

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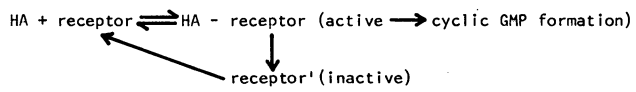
2520 HISTAMINE H_1 RECEPTORS ON NEUROBLASTOMA CELLS: SHORT- AND LONG-TERM REGULATION OF SENSITIVITY. J.E. Taylor* and E. Richelson (SPON: R.M. Weinshilboum) Depts. of Psychiatry and Pharmacology, Mayo Foundation, Rochester, MN 55901

Mouse neuroblastoma cells (clone N1E-115) responded to histamine H_1 receptor-agonists with a large and transient increase in the intracellular levels of guanosine 3',5'-cyclic phosphate (cyclic GMP). Short-term ($\leq$ 30 min) pre-incubation of intact cells with histamine (HA) resulted in a receptor-specific, time- and concentration-dependent decrease in responsiveness to HA (desensitization). The rate at which desensitization developed was dependent on the concentration of HA used to desensitize (at 50 µM, $t_{1/2}$ = 5 min; at 100 µM, $t_{1/2}$ = 2 min). When the cells were washed free of HA, normal sensitivity to HA rapidly returned and the rate of resensitization was independent of the concentration of histamine used to desensitize (half-time ≈ 8 min). Data from the dose-response curves using cells partially desensitized with HA suggested a loss of agonist binding sites. However, binding studies with the H_1 receptor antagonist, [^{3}H]pyrilamine, to whole cells showed that there was no significant change in the number of antagonist binding sites associated with short-term desensitization. These data for short-term desensitization fit the following cyclic model as described by Gosselin[1]:



The nature of the inactive receptor for short-term desensitization is not certain, but could result from a reversible change in receptor conformation which only affects agonist binding. Long-term exposure (> 4 hrs) to HA resulted in a desensitization that was only slowly and partially reversible. Furthermore, this long-term desensitization was accompanied by a marked decrease in the number of specific [^{3}H]pyrilamine binding sites on intact cells. These results suggest that different mechanisms are involved in the short-term and in the long-term desensitization of histamine H_1 receptors. (Supported by Mayo Foundation and USPHS Grants MH 27692, MH 07925 and DA 1490.)

[1]Gosselin, R.E., in Kinetics of Drug Action (J.M. Van Rossum, ed) Springer-Verlag, Berlin, pp. 323-356 (1977).

2521 BIPHASIC RESPONSE OF PYRAMIDAL NEURONS TO GABA IONTOPHORESIS IN HIPPOCAMPAL SLICES. R. H. Thalmann*, E. J. Peck and G. F. Ayala. Dept. Neur. and Cell Bio., Baylor Coll. Med., Houston, TX 77030.

Changes of membrane potential during iontophoresis of GABA were studied in CA1 pyramidal cells of hippocampal slices maintained at 34-35°C and bathed in synthetic CSF with a K^+ concentration of 3.5 mM. As others have reported, iontophoresis of GABA in the pyramidal cell layer produced a suppression of action potentials when recorded extracellularly. However, intracellular recording revealed that the changes in membrane potential during GABA iontophoresis near the soma may be biphasic: an early hyperpolarization followed by a depolarization. The hyperpolarization had properties similar to those of the IPSP; an increased membrane conductance, the same reversal potential as the IPSP, and sensitivity to picrotoxin. The depolarizing phase was accompanied by an increase of membrane conductance, it was reversed by transmembrane injection of outward currents, and it was abolished by picrotoxin. Topical application of a low Cl^- solution reversably decreased the size of the hyperpolarization and shifted the equilibrium potential of the depolarizing phase toward a more positive level. Application of Co^{++} at a concentration sufficient to block synaptic transmission did not change the amplitude of the biphasic response to GABA iontophoresis near the soma. The response was different if the GABA iontophoresis was near the apical dendrites. A depolarizing phase was observed which was not preceded by a hyperpolarization as in the case of somatic application. These results were obtained either by monopolar or bipolar iontophoresis and with K-acetate and K_2SO_4 microelectrodes (50-80 Mohms). Our present working hypothesis is that both components of the biphasic response are chloride dependent. Elsewhere in the meeting, we report that when the conductances during the IPSP are enhanced by sodium pentobarbital, a biphasic response to endogenous transmitter release can be evoked which has similar properties to the response elicited by iontophoresis of GABA.

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2522 METHYLTRANSFERASES ARE INVOLVED IN NEUROTRANSMISSION. Jeffrey M. Thompson, Peter K. Chiang,* Robert R. Ruffolo, Jr.*, Giulio L. Cantoni* and Marshall Nirenberg. Lab of Neurosciences, Natl Inst on Aging, Baltimore, Md. 21224; Lab of Gen and Comparative Biochem, NIMH and Lab of Biochem Genetics, Natl Heart, Lung, and Blood Inst, NIH, Bethesda, Md. 20205.

Methyltransferases can be inhibited by S-adenosyl homocysteine (AdoHcy) or by analogues of this compound. Analogues which either increase levels of AdoHcy or inhibit methyltransferases directly include 3-deazaadenosine(DZA), adenosine-2', 3'-diazido-5'-carboxamide (744-99), 5'-deoxy-5'-isobutylthioadenosine(SIBA) and 5'-deoxy-5'-isobutylthio-3-deazaadenosine(DZ-SIBA). (Chiang, Richards and Cantoni, 1977, Mol. Pharmacol. 13:939-947; Chiang et al., 1978, Biochem. Biophys. Res. Commun. 82:417-423.) Neurons from 8-day chick embryo retina grown in culture with rat striated muscle cells for 24 hr formed synapses. Synapses were detected by recording spontaneous depolarizing synaptic muscle responses with intracellular microelectrodes (Puro and Nirenberg, 1976, Proc. Natl. Acad. Sci., USA, 73:3544-3548; Ruffolo et al., 1978, Proc. Natl. Acad. Sci. USA, 75:2281-2285). These synapses were investigated with the above probes to discover whether methylation reactions occur during synapse formation or neurotransmission. After treating the cultures for 2 hr with one of the AdoHcy analogues, the number of muscle responses/min in these synapses was inhibited up to 95% by DZ-SIBA (ED_{50} = 1. 5 x 10^{-6}M), SIBA (ED_{50} = 3 x 10^{-5}M), DZA (ED_{50} = 1.5 x 10^{-5}M) and 744-99 (ED_{50} = 1 x 10^{-4}M). Homocysteine thiolactone, 5'-deoxyadenosine and tubercidin, which do not increase levels of AdoHcy or inhibit methyltransferases, had no effect. DZ-SIBA inhibited the muscle responses with a half-time of 3.5 min. No inhibition of the number of synapses formed was seen. Inhibition of the number of muscle responses/min by DZA was reversed within 2 hr after removal of the compound. Further, homocysteine thiolactone potentiated the inhibition by DZA by 6-fold when the two compounds were added together. These results strongly suggest that a methyltransferase mediated reaction occurs during neurotransmission.

2523 PROPERTIES OF STORAGE OF DOPAMINE AND ACETYLCHOLINE IN GRANULES OF PC12, A CLONAL PHEOCHROMOCYTOMA CELL LINE. Lawrence Toll*, William P. Melega*, Robert V. Rebois*, Elwood E. Reynolds* and Bruce D. Howard. Biol. Chem. Dept., Sch. Med., UCLA, Los Angeles, CA 90024

PC12, a clonal line of rat pheochromocytoma, synthesizes acetylcholine and catecholamines, stores each in different granules, and exhibits a spontaneous and depolarization-induced, Ca^{2+}-sensitive secretion of each. These properties allow us to compare the mechanism of transmitter storage in two different types of storage granules produced by the same cell. The mechanism of storage of dopamine in PC12 granules is similar to that for catecholamines in storage granules from adrenal medulla and brain. Transport of catecholamines into these granules is driven by a transmembrane pH gradient (inside acidic), which also causes the retention of catecholamines within the granules. The pH gradient is established by the activity of a proton-translocating Mg^{2+}-ATPase present in the membrane of the catecholamine-containing storage granules. Transport of dopamine into PC12 granules is stimulated by ATP and inhibited either by proton ionophores that dissipate transmembrane pH gradients or by agents that inhibit the associated Mg^{2+}-ATPase. The proton ionophores cause dopamine, but not acetylcholine, to efflux from the granules. These effects can be demonstrated with isolated PC12 granules or with granules in intact cells. Other results suggest that the system involved in the loading of newly synthesized acetylcholine into granules operates relatively slowly and can be saturated. PC12 cells take up dopamine, tyrosine and choline and quickly convert the choline to acetylcholine and the tyrosine to dopamine. The newly accumulated or synthesized dopamine enters storage granules more readily than does newly synthesized acetylcholine. Incubation with the cholinesterase inhibitor eserine causes an increase in the cytosol level of newly synthesized acetylcholine but does not cause a corresponding increase in the amount of newly synthesized acetylcholine that is loaded into granules or that is released upon stimulation of the cells. Our studies indicate that PC12 will have much utility for examining the metabolism of dopamine and acetylcholine in secretory cells.

2524 STIMULATION-INDUCED RELEASE OF ^{3}H-NOREPINEPHRINE FROM EXPLANTS OF THE RAT SUPERIOR CERVICAL GANGLION: EFFECT OF ALPHA-ADRENERGIC AGENTS. N.D. Vu* and N.B. Thoa* (SPON: R.W. Colburn). Howard Univ., Washington DC 20059 and NIMH, Bethesda, MD 20205.

Alpha-adrenergic receptors have been identified in the rat superior cervical ganglion (SCG) (Kafka and Thoa, Biochem. Pharmacol. In Press). In culture, SCG cells were found to develop axonal sprouts with physiological and pharmacological properties of adrenergic nerve endings (Silberstein: in Neurosci.Res. 5:1-34, 1973). A calcium-dependent release of ^{3}H-Norepinephrine (^{3}H-NE) could be elicited in the cultured SCG with electrical field stimulation (Vu, Thoa and West, The Pharmacologist, 1979). In this study, the effect of alpha-adrenergic agents on release of ^{3}H-NE elicited by field stimulation (stimulated release) was examined. Rat SCG were cultured for 48 hours and labeled with 0.2μM of dl-^{3}H-NE in presence of 30μM corticosterone (CX) to inhibit uptake of catecholamines into extraneuronal structures. The explants were then perfused with a modified Krebs solution. After 60 min., electrical field stimulation (3Hz, 20 volts, 5 msec. duration) was applied for 1 min., and 4 min. samples were collected prior to and immediately following stimulation. ^{3}H-NE and its deaminated metabolites ^{3}H-DOMA and ^{3}H-DOPEG were assayed. Perfusates collected in the pre-stimulation period contain mostly ^{3}H-DOPEG (51%) and smaller amounts of ^{3}H-NE (26%) and ^{3}H-DOMA (23%). Field stimulation primarily increases ^{3}H-NE release (200%) and induced smaller increases of both ^{3}H-DOMA (100%) and ^{3}H-DOPEG (50%). Cocaine ($7x10^{-5}$M) blocks ^{3}H-NE uptake by 85% but does not affect spontaneous release of either ^{3}H-NE or of the two metabolites. It however potentiates stimulated release of ^{3}H-NE and completely blocks stimulated release of ^{3}H-DOPEG. The alpha-adrenergic antagonists phenoxybenzamine, phentolamine and piperoxane all increase spontaneous release of ^{3}H-NE in a dose-dependent manner with the following order of potency: phenoxybenzamine > phentolamine > piperoxane. In cocaine-treated preparations, stimulated release of ^{3}H-NE is inhibited by alpha-adrenergic agonists. This effect is stereospecific, l-epinephrine (l-EPI) being more potent than d-epinephrine. The order of potency of alpha-agonists is l-EPI > alpha methyl NE ≫ l-NE and resembled that observed at presynaptic alpha2 receptors sites (Berthelsen and Pettinger, Life sci. 21:595-606, 1977). Clonidine and dopamine are as potent as l-NE at lower doses but the former increases stimulated release of ^{3}H-NE at higher doses and the effect of the latter never reaches levels comparable with those of l-EPI or l-NE. Thus there seems to be a presynaptic feed back control of NE release (Langer, Biochem. Pharmac. 23:1793-1800, 1974) at newly formed sympathetic terminals of the rat SCG when this organ is maintained in culture.
(N.D.Vu supported by NIH Grant #5-T02-GMO-5000-02-MARC)

2525 TRANSMITTER RELEASE STUDIES WITH DIAMIDE AT FROG NEUROMUSCULAR JUNCTION AND IN BRAIN SLICES. Patricia D. Wade*, Lawrence C. Fritz* and P. Siekevitz. Rockefeller University, New York, N. Y. 10021

Diamide, a sulfhydryl oxidizing agent, is known to dramatically increase the frequency of miniature endplate potentials at the frog neuromuscular junction in a Ca^{++}-independent manner (Werman, R. et al., Nature New Biology 233:120, 1971) similarly to a toxin from the black widow spider (α-latrotoxin) (Frontali, N. et al., J. Cell Biol.58:462,1972). Diamide also parallels α-latrotoxin in that following the waning of a maximal response further attempts to activate the terminal are unsuccessful. For example, upon electrical stimulation the endplate potential is blocked. Since toxin-treated terminals are empty of vesicles (Clark, A. W. et al., J. Cell Biol. 52:1, 1972), it is of interest to know how diamide-treated ones appear in the EM. Although not always entirely normal in appearance, diamide-treated terminals typically do contain vesicles, even though the terminal cannot be activated electrically nor even by the addition of α-latrotoxin. It might be that in the presence of diamide α-latrotoxin cannot interact with the terminal. However, we find that the toxin is still capable of resulting in a large decrease in the number of vesicles in the terminal as seen in EM.

Since α-latrotoxin releases every kind of transmitter from vertebrate neurons where the phenomenon has been studied (cf., Tzeng et al., PNAS 75:4016, 1978), and since the possibility exists that diamide may act on a general release mechanism as well, the effect of diamide on γ-aminobutyric acid (GABA) release from slices of rat cerebral cortex was studied. Diamide does not cause GABA release under conditions where high K^+ or α-latrotoxin does. The specificity or generality of the release mechanism of different transmitters as demonstrated by diamide action is being studied for acetylcholine and norepinephrine in brain.

2526 SPIKE AFTER HYPERPOLARIZATION AND TRANSMITTER RELEASE IN INTERNEURON L_{10} OF APLYSIA. Rafiq Waziri. Dept. of Psychiatry, Univ. of Iowa College of Medicine, Iowa City, IA 52242.

In Aplysia neurons, spike configuration in the soma may not always be representative of spikes occurring near the terminal where transmitter is released. Transmitter release by interneuron L_{10} can be modulated by the polarization of another neuron (L_{20}) which is electrically coupled to L_{10} (Waziri, Science, 195:790-792, 1977). The biphasic electrotonic potentials produced in L_{20} under experimental conditions may provide a "window" on some aspects of spike configuration that are important in the release of transmitter. Hyperpolarization of L_{10} terminal branches, by passing hyperpolarizing currents into L_{20}, diminishes transmitter release as evidenced by changes in the chemical postsynaptic potentials observed in other cells. The electrotonic potentials in L_{20}, produced by action potentials of L_{10}, show that the hyperpolarizing phase of electrotonic potentials is diminished or abolished depending on the level of induced hyperpolarization. Hyperpolarization of L_{10} which diminish transmitter release, also diminish the spike after hyperpolarization in L_{10} and the hyperpolarizing phase of the electrotonic potential. Tetraethyl ammonium ions which increase spike duration and transmitter release, also increase the amplitude of the spike after hyperpolarization. Lowering of extracellular calcium concentration does not decrease the amplitude of spike after hyperpolarization. These observations indicate that in this cell inward calcium movement which is essential for transmitter release is enhanced by outward movement of K^+ during the spike after hyperpolarization.

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2527 ARE PRESYNAPTIC ALPHA-RECEPTORS INHIBITORY? G.J. Whiting*, D.A. McAfee, J.P. Horn, and N. Wenger*. Div. of Neurosciences, City of Hope National Medical Center, Duarte, CA 91010.

Synaptic transmission is dependent upon Ca^{++} in most preparations. Recently, Horn & McAfee (Science, in press) demonstrated that activation of postsynaptic α-receptors inhibits Ca^{++} current in postganglionic neurons. Activation of presynaptic α-receptors can inhibit synaptic transmission at low frequencies of preganglionic stimulation (Christ & Nishi, J. Physiol. 213, 107, 1971). We have tested the hypothesis that presynaptic α-receptors act to control presynaptic Ca^{++} influx by studying synaptic depression. Synaptic depression is thought to be due to depletion of readily releasable transmitter. We propose that activation of presynaptic α-receptors reduces Ca^{++} influx, slightly inhibiting low-frequency transmission, but profoundly facilitating high-frequency transmission.

Superior cervical sympathetic ganglia were isolated from mature rats and maintained in vitro at 24° C. Suction electrodes were used to apply stimulus trains (5-40 Hz for 1 sec.) to the preganglionic (cervical sympathetic) nerve and to record compound action potentials from the postganglionic internal carotid nerve. At frequencies above 10 Hz, the amplitude of the compound action potential progressively decreased, reaching a plateau about 1 sec. after the start of the train. At 17 Hz, the action potential was reproducibly depressed to 50% of control (EF_{50} = 17 Hz). Depression was directly proportional to extracellular Ca^{++}. Increasing extracellular Ca^{++} from a control level (2.2 mM) to 8.0 mM reduced the EF_{50} for depression about 40%, and decreasing extracellular Ca^{++} to 1.0 mM increased the EF_{50} by 40% over control values. Catecholamines had similar effects to low Ca^{++}. They reduced depression during trains at concentrations which had little effect on single responses. In the presence of norepinephrine (30 μM), the EF_{50} for depression was 25 Hz. At low frequencies (10 Hz) of stimulation, α-agonists actually facilitated synaptic responses. The order of potency was epinephrine (EC_{50} = 10^{-7} M), norepinephrine, dopamine, and isoproterenol. The effect of epinephrine and norepinephrine on depression was antagonized by the α-adrenergic blocker, phentolamine (10 μM), but not by the β-antagonists, propranolol (1 μM) or MJ-1999 (5 μM).

Our results support the above hypothesis inasmuch as the effect of low Ca^{++} was mimicked by α-agonists. We demonstrated that the α-agonists can actually promote synaptic responsiveness during repetitive activity. Intracellular studies are in progress to determine the relative contributions of postsynaptic mechanisms to synaptic depression. (Supported by NS-05820)

2528 RELATIONSHIP OF HIGH ENERGY PHOSPHATES AND pHi TO SYNAPTIC TRANSMISSION FAILURE DURING HYPOXIA IN THE IN VITRO HIPPOCAMPAL SLICE. Tim S. Whittingham* and Peter Lipton. Dept. Physiology, Sch. Med., Univ. of Wisconsin, Madison, Wis. 53706.

In vivo brain function fails rapidly during hypoxia. Transverse slices (.5mm) of guinea pig hippocampus are incubated in bicarbonate buffer containing 4mM glucose and aerated with O_2:CO_2 or N_2:CO_2 to study hypoxic transmission failure in vitro. Field potentials are recorded from the dentate granule cell region following stimulation of the perforant path. 75" after changing from O_2:CO_2 to N_2:CO_2 there is approximately a 25% decrease in the evoked response, while complete transmission failure occurs in ∿ 3'.

Increased extracellular potassium ($[K^+]_e$) and other depolarizing conditions all accelerate the rate of transmission failure during hypoxia, indicating that depolarization of neurons is leading to the hypoxic transmission failure.

Two means by which this hypoxic depolarization could occur are: 1) inhibition of the Na-K pump, and/or 2) an increase in cell membrane permeability. In either case it is important to establish whether decreased levels of ATP are responsible for the alteration in function.

Thus, experiments were done to determine if the cellular [ATP] falls rapidly enough during hypoxia to account for the decay in the evoked response. The slices were dissected such that the portion of tissue analyzed for ATP and phosphocreatine (PCr) was predominantly composed of the dentate gyral region in which transmission failure was monitored. In normal 4.4mM K^+ superfusate, the first noticeable decrease of the post-synaptic response occurred in ∿ 2'15". At this time [PCr] had fallen ∿ 37% (from 42± 3 (SEM) to 27±2 µmoles/mg protein), while ATP had decreased ∿ 15% (from 19±1 to 16±1µmoles/mg protein.) When these same experiments are done at 13.4 mM K^+, the first noticeable decrease occurs at ∿30". At this time the decrease in PCr is only 13% (from 40±2to ∿35 µmoles/mg protein) and the [ATP] remains unchanged (16± 1 µmoles/mg protein compared to ∿15.5 µmoles/mg protein in control slices). The failure of [ATP] to decrease prior to the first decrement in the post-synaptic response in 13.4 mM K^+ superfusate indicates that a decrease in [ATP] may well not be leading to the decay in the evoked response during hypoxia.

Intracellular pH has been observed to decrease during hypoxia. This fall could result in Na pump inhibition or changes in membrane permeability, resulting in neuronal depolarization. Experiments correlating pHi with the evoked response during hypoxia are currently being done and will be described.

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2529 SLOW SYNAPTIC MODULATION OF EXCITABILITY MEDIATED BY INACTIVATION OF CALCIUM-DEPENDENT POTASSIUM CONDUCTANCE IN MYENTERIC NEURONS OF GUINEA-PIG SMALL INTESTINE. J.D. Wood, P. Grafe* and C.J. Mayer*. Physiol. Dept., Univ. Kansas Med. Ctr., Kansas City, KS 66103 and Physiologisches Institut der Universität München, 8 Munich 2, GFR

5-Hydroxytryptamine (5-HT) is the putative neurotransmitter for slow synaptic modulation of a particular kind of neuron (tonic-type) within the myenteric plexus (J. Neurophysiol. 42:582, 1979). Low excitability within these neurons is associated with a relatively low input resistance of the cell soma and long lasting hyperpolarizing after-potentials, both of which appear to be reflections of increased gK. Electrical stimulation of presynaptic fibers or iontophoresis of 5-HT produce a prolonged increase in excitability (slow EPSP) which is characterized by membrane depolarization, increased input resistance, repetitive spike discharge and reduction of postspike hyperpolarizing potentials in these neurons. We now report that the calcium antagonists Mg^{++} and Mn^{++} mimic all of the characteristics of the slow EPSP. When the neurons were superfused with solutions containing either 16 mM $MgCl_2$ and 1 mM $CaCl_2$ or 2 mM $MnCl_2$ and 2.5 mM $CaCl_2$, the input resistance increased, the membrane depolarized, hyperpolarizing after-potentials were abolished and repetitive spike discharge occurred. The organic calcium antagonists verapamil and D-600 did not produce these effects. The effects of elevated Mg^{++} and Mn^{++} were reversible, and were observed only in tonic-type neurons. We also studied the effects of elevated Mg^{++} and Mn^{++} on the relationship between external potassium concentration and the resting membrane potential in these cells. The results, when plotted on a logarithmic scale, showed a close fit to the Goldman equation and the slopes of the plots were significantly reduced when either 16 mM Mg^{++} or 1 mM Mn^{++} was present. These effects did not occur in other types of myenteric neurons and putative glial cells. We used intracellular current injection to control membrane potential, and estimated the reversal potential for the slow EPSP to be -73 mV. The results are consistent with the hypothesis that low resting excitability in these neurons is related to two different calcium-dependent gK systems within the membranes. One calcium-dependent increase in gK is triggered by calcium entry during the spike and results in prolonged hyperpolarizing after-potentials. The second calcium-dependent gK is associated with a high resting gK that is reflected by high membrane potential, low input resistance and low excitability. The calcium antagonists and 5-HT appear to produce augmented excitability by suppressing the two calcium-dependent gK systems. Therefore, our results suggest that the slow synaptic modulation of excitability in the tonic-type myenteric neurons involves a mechanism in which the primary action of the neurotransmitter is to interfer with the availability of calcium for the calcium-dependent gK systems.

2530 A SECOND NOVEL PRESYNAPTIC TOXIN PRESENT IN THE GENUS LEPTINOTARSA. J.E. Yoshino*, D. Baxter*, T.H. Hsiao*, and W.O. McClure. Section of Cellular Biology, University of Southern California, Los Angeles, CA 90007, and Department of Biology, Utah State University, Logan, UT 84322.

Previously we reported the ability of leptinotarsin, a neurotoxin found in the hemolymph of Leptinotarsa haldemani (LPT-H), to stimulate the release of acetylcholine (ACh) from synaptosomes and to increase spontaneously the frequency of miniature end plate potentials (mepps) at the rat neuromuscular junction (nmj) (Satin et al, Soc. for Neuroscience Abs., Vol. 4, p. 584, 1978; Yoshino et al, loc. cit., p. 586). In this study we have examined the neurochemical properties of leptinotarsin from a related species, L. decemlineata (LPT-D). LPT-D stimulated the preferential release of [^{3}H]ACh from rat brain synaptosomal preparations previously incubated with [^{3}H]choline. The releasing activity present in LPT-D was also partially dependent upon the presence of Ca^{++} in the perfusing medium and was heat labile, similar to those results found with LPT-H. Other preliminary studies with the partially purified toxin suggest, however, that LPT-D and LPT-H are not identical. The curve which relates releasing activity to increasing concentration of LPT-D is sigmoid, with a Hill coefficient equal to 1.8. In contrast, the corresponding curve for LPT-H does not exhibit any sigmoid character. Whereas the release of radioactivity by LPT-H can be well characterized by first order kinetics, the activity of LPT-D is more complex. Since the ability to stimulate release of ACh was similar to that of black widow gland extracts (BWGE), we examined the immunological similarity of LPT-D and LPT-H to BWGE by incubating the two toxins with commercial antivenin to BWGE (Lyovac). The ability of LPT-D to stimulate release was reduced 35% ($p \leq 0.05$) by treatment with 300 units of antibody/ml. Under similar conditions, the activity of LPT-H was not impaired.

We have begun the purification of LPT-D from the lyophilized hemolymph. After passage over Sephadex G-100 and gradient elution from DEAE Sephadex A50 we find an increase in the specific activity of the toxin of about 30 fold. These procedures partially separate two proteins, each of which appears to stimulate the release of radioactivity from synaptosomes. Preliminary observations indicate that the hemolymph of LPT-D stimulates an increase in the frequency of mepps at the rat nmj. When LPT-D and LPT-H are purified to homogeneity, they may be useful as mechanistic probes in examining the processes underlying the release of neurotransmitters.

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2531 MORPHOLOGICAL AND ELECTROPHYSIOLOGICAL CHARACTERIZATION OF A DISCRETE CLASS OF STATOACOUSTICAL INTERNEURONS IN A VERTEBRATE BRAIN. Steven J. Zottoli and Donald S. Faber. Div. of Neurobiology, Dept. of Physiol., SUNY at Buffalo, Buffalo, NY 14214 and NYS Research Institute on Alcoholism, 1021 Main Street, Buffalo, NY 14203.

Interneurons of the goldfish medulla which are inhibitory to the Mauthner cell (M-cell) are electrophysiologically identifiable by the presence of a passive hyperpolarizing potential (PHP) coincident with the M-cell's antidromic impulse (Korn and Faber, J. Neurophysiol., 38:452, 1975). We have studied the physiological and morphological properties of one class of these neurons which are excited by eighth nerve inputs.

The majority (>90%) exhibited graded short latency depolarizations (SLDs) to subthreshold stimulation of the ipsilateral eighth nerve. SLDs were occasionally followed by mono- or polysynaptically mediated PSPs. The short latency of the SLD (430 ± 100 µsec; mean ± SD, n=92) and its amplitude independence of membrane potential suggest these PSPs are electrotonically mediated. As stimulus strength was increased, SLDs could bring the PHP neurons to threshold. Stronger stimuli evoked a shorter latency impulse (340 ± 60 µsec; n=84); collision tests and membrane hyperpolarizations did not reveal an underlying EPSP. These findings and the latency difference between this spike and the SLDs (103 ± 80 µsec; n=77) suggested the former were antidromically activated and that the neurons might be efferents (Zottoli and Faber, Soc. Neurosci., 2:1063, 1976). However no efferent process could be identified after intracellular iontophoresis of either Procion yellow or cobalt acetate (n=77). Their morphology rather suggests that they are interneurons and that the short latency spike may be generated by an electrotonic input at a more distant locus than that which mediates the SLD.

The somata of the statoacoustic interneurons were clustered dorsolateral and posterior to the M-cell soma. The apparent axon could be traced to a contralateral tract that projects caudally and lies lateral to the VIIth cranial nerve. Its ramifications project bilaterally to the vicinity of other statoacoustical neurons, large and small reticular neurons and the M-cells. Their widespread medullary projections (see also Triller and Korn, C.R. Acad. Sci., D, (Paris), 286:89, 1978) suggest that these commissural interneurons not only mediate afferent inhibition of both M-cells but of many other brainstem areas as well. (Supported by PHS Grant No. NS 12132 to D. Faber and Postdoctoral Fellowship F 32 NS5282 to S. Zottoli.)

TISSUE CULTURE

2532 NEURITE PROMOTION IN CILIARY GANGLIONIC CULTURES. Ruben Adler and Silvio Varon. Dept. Biol., Sch. Med., UCSD, La Jolla, CA 92093.

We have previously reported that chick embryo ciliary ganglionic (CG) neurons will survive in monolayer cultures if supplied with either chick embryo extracts or medium previously conditioned over chick heart cell cultures (HCM), in addition to serum. The two supplements, however, differed in their neurite-promoting effects, as embryo extracts elicited neurite growth only on collagen and HCM did so only on polyornithine (PORN) substrata (Varon et al, Brain Res., 1979, in press). We report here that HCM contains two distinct and separable agents. One is a trophic agent (HCM-CNTF), which supports the survival of CG neurons on either substratum. This agent does not seem to adsorb to either substratum, and may act directly on the cells. Its relationship to the CNTF which we have obtained from intraocular target tissues (Adler et al, Science, 1979, in press; Manthorpe et al, Trans. Am. Soc. Neurochem. 10: 76, 1979) has not yet been investigated. The CG culture assay previously developed for the eye-CNTF indicates that our HCM contains 10-20 trophic units/ml. The second agent (HCM-NPF), first described by Collins (Proc. Natl. Acad. Sci. USA 75: 5210-5213, 1978), is presumably an acidic material which adsorbs to PORN but not to collagen surfaces, and confers to the substratum a neurite-promoting activity for CG neurons that are supported by CNTF (from either HCM or embryonic extracts). Pre-exposure of PORN-coated dishes to serially diluted HCM, followed by seeding of CG neurons in the presence of HCM-CNTF, allows to assign to the original HCM an NPF activity of 3-6 unit/ml.

Neurite-promoting activity can be imparted to a PORN surface also by other means. Ciliary ganglia, explanted either on untreated PORN or on PORN precoated with HCM-NPF, display no neuritic outgrowth unless CNTF is also provided. On the pretreated PORN, CNTF-supported neuritic outgrowth is radial and unrestricted. On untreated PORN, CNTF-supported neuritic outgrowth also occurs, but it extends only within the visible confines of some substratum-attached material (SAM), which originates within the explant and coats the surrounding PORN in widening circles. Thus, it appears that both the tissue of origin (ganglion) and potential target tissue (muscle) are capable of providing supportive terrains for the trophic-driven elongation of a neurite. (Supported by NINCDS grant NS-07606).

2533 COLD-INSOLUBLE GLOBULIN ENHANCES NEURITE OUTGROWTH FROM CULTURED NEURAL RETINA AGGREGATES. Rebecca M. Akers, Deane F. Mosher* and Jack E. Lilien*. Depts. of Zoology and Medicine, University of Wisconsin, Madison, WI 53706.

Aggregates of embryonic chick neural retina extend few neurites on plastic tissue culture dishes. However, pre-incubation of culture dishes with human plasma cold-insoluble globulin (CIG) dramatically increases both the number and length of outgrowing neurites. Within 24 hours of plating on CIG-treated dishes, retinal aggregates elaborate straight, sparsely-branched processes which extend radially over the culture surface for several hundred microns. Enhancement of neurite outgrowth is evident on culture dishes treated with as little as 2.5 µg CIG (in 1 ml of medium); maximal effects are observed after pre-incubation of dishes with 25 µg CIG. Treatment of dishes with equivalent amounts of other serum proteins (BSA or fetuin) has no apparent effect on neurite outgrowth. CIG-treated dishes incubated with affinity-purified anti-CIG antibodies no longer support neurite outgrowth; aggregates plated on such dishes resemble those cultured on untreated plastic surfaces.

Neurites growing on CIG-treated substrata have a different morphology than neurites grown on poly-lysine (PLYS)-treated dishes. Like CIG, PLYS enhances neurite outgrowth from retinal aggregates. However, these neurites do not extend radially from the aggregate. Rather, they grow concentrically around the aggregate, enveloping it in a network of fine, multiply-branched processes. In addition, neurites adhere differentially to CIG- and PLYS-treated dishes. Neurites growing on PLYS adhere to the substratum along their entire length, whereas on CIG adhesion of the neurite occurs solely at the growth cone. Although CIG and PLYS both provide favorable substrata for retinal neurite outgrowth, the interactions of neurites with these two substrata appear to be different.

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2534 HORMONAL REGULATION OF GLIAL RELEASED PROTEIN IN CELL CULTURE. Alaric T. Arenander* and Jean de Vellis. Department of Anatomy and the Laboratory of Nuclear Medicine and Radiation Biology, UCLA, Los Angeles, CA 90024.

Glial cells synthesize and release a variety of macromolecular factors including glycosaminoglycans, proteins (e.g., Nerve Growth Factor), glycoproteins and polypeptides, some of which are capable of influencing the morphological and/or biochemical differentiation of various neuronal populations. There has been little analysis of the range of potential protein factors or of the control mechanisms which modulate their production and release.

We report here that monolayers of rat clonal and primary glial cells release into the culture medium a reproducible, broad spectrum of soluble macromolecules. Furthermore, a number of these glial-released proteins (GRPs) are shown to be regulated in a specific manner by a variety of hormonal agents. Conditioned medium was collected from C6 glioma cell monolayers after being incubated for 24 hr in serum-free medium and labelled during the last 6 hr with either [^{3}H] or [^{14}C]leucine. SDS-polyacrylamide slab gel electrophoresis of soluble GRPs revealed 20 major peaks of labelled protein distributed over a molecular weight (MW) range of 10,000 to 300,000. Gel radio-labelled patterns were reproducible both within and between experiments based on visual examination of plots, $^{3}H/^{14}C$ isotope ratios and label reversal. Exposure of the cells to hydrocortisone (HC, 1-2 µM) for 24 hr altered the pattern of GRP in a selective and specific manner. Six major GRP peaks were consistently observed to either increase or decrease based on plots of isotope ratios. The effect could be observed at 0.02 µg/ml (5.4×10^{-8} M) but not at 0.002 µg/ml. Treatment of cells with 17-β-estradiol (2 µM) gave no observable change in pattern of GRP. Dibutyryl cyclic AMP (1 mM) and isoproterenol (10 µM) were also capable of selectively influencing the GRP patterns but in a manner different from HC. A number of the GRP peaks are glycoproteins based on metabolic labelling with [^{14}C]glucosamine. Pure primary cultures of astroglia and oligodendroglia obtained from newborn rats generated a spectrum of GRP closely resembling that of C6, differing mainly in the high MW region. HC treatment also influenced the GRP pattern from mixed glial cultures. Thus, both clonal and primary glial cultures released a wide MW range of proteins (GRP) that were under specific hormonal control. The origin and possible role in neuronal-glial coupling of GRPs is being investigated. Supported by DOE and USPHS.

2535 RECIPROCAL REGULATION OF ACETYLCHOLINE RECEPTOR AND MYOSIN IN CHICK MYOTUBES BY A PHOSPHODIESTERASE INHIBITOR AND CYCLIC NUCLEOTIDE ANALOGUES. James C. Blosser and Stanley H. Appel. Dept. Neurology, Baylor College of Med., Houston, TX 77030.

Acetylcholine receptor (AChR) and myosin are reciprocally regulated under a number of conditions. Denervation increases AChR and decreases myosin. In primary cultured muscle cells tetrodotoxin also increases AChR and decreases myosin while electrical stimulation decreases AChR synthesis and increases myosin synthesis. We have observed a similar effect with a phosphodiesterase inhibitor. A number of phosphodiesterase inhibitors were found to elevate AChR levels in primary cultures of chick 11 day embryonic muscle. Using ^{125}I-α-bungarotoxin binding to surface receptors as an index of AChR content, caffeine and theophilline (10^{-3} M) were found to cause moderate increases in AChR (25-30%). Papaverine (3×10^{-5}M) increased levels by 50% while Ro20-1724 (3×10^{-4}M) elevated receptor by over two fold. The effect of Ro20-1724 appeared to be largely due to an increased rate of receptor accumulation. The apparent rate constant of accumulation (k_{acc}) increased from 32 cpm/h (^{125}I-toxin binding sites) to 55 cpm/h while half life, as measured by loss of ^{125}I-α-bungarotoxin from prevously labeled cells, was unchanged. Under these conditions myosin heavy chains were extracted and quantitated by densitometry on acrylamide gels. After 48 hours of treatment myosin content was 11.2 ± 0.9 µg/plate in control and 5.6 ± 0.9 µg/plate in the Ro20-1724 treated cells. Pulse chase experiments with ^{35}S-methionine indicated that increased degradation is primarily responsible for the decreased myosin content (control $t_{\frac{1}{2}}$ = 72 h, Ro-1724 $t_{\frac{1}{2}}$ = 22 h). No measurable differences in DNA content were observed. To gain a better understanding of the mechanism of action of Ro20-1724, several cyclicnucleotides were tested. Dibutyryl cAMP (10^{-3}M) was found to increase AChR levels by 80% while dbcGMP (10^{-3}M) had little effect. Both dbcAMP and 8BrcAMP increased k_{acc} by 50% with little if any affect on half life. In contrast dbcGMP decreased heavy chain myosin levels by over 40% after 48 h of treatment. Similar treatment with dbcAMP had little effect on myosin content. These results suggest that cAMP and cGMP each play a role in the reciprocal regulation of AChR and myosin but by different mechanisms - cAMP affecting AChR at a synthetic or insertion level and cGMP influencing myosin degradation.

2536 ACTIONS OF GLUTAMIC ACID AND KAINIC ACID ON MUSCARINIC RECEPTOR BINDING IN SPINAL CORD CELL CULTURES. Neville Brookes and David R. Burt. Dept. Pharmacol. & Exptl. Therap., Univ. of Maryland Sch. Med., Baltimore, MD 21201.

The specific binding of [^{3}H]quinuclidinyl benzilate ([^{3}H]QNB) in surface cultures of dissociated murine spinal cord (SC) cultures was previously reported to increase with maturation of the cultures in vitro (Brookes, Burt and Goldberg, Neurosci. Abstr. 4:590, 1978). To establish that this muscarinic receptor binding is neuronal, we have treated SC cultures with selectively neurotoxic amino acids and have assayed for the disappearance of [^{3}H]QNB binding. The cultures were prepared by mechanical dissociation of spinal cords (including dorsal root ganglia and meninges) from 13-day-old mouse embryos. Cell proliferation was inhibited by application of 5-fluoro-2'-deoxyuridine after about 5 days of incubation. SC cultures at 2–7 weeks of incubation were exposed to monosodium glutamate (10^{-3} M) for 3 hr. The fraction of control [^{3}H]QNB binding present immediately after exposure was 0.88 + 0.12 (mean + SEM, N=3). The fractions remaining 1, 2 and 4 days after exposure were 0.47 + 0.05 (N=6), 0.40 + 0.05 (N=7) and 0.21 + 0.02 (N=6), respectively. The mean control values of [^{3}H]QNB binding were 125–175 fmole per mg protein. The non-collagen protein content (Wallace and Partlow, Analyt. Biochem. 87:1, 1978) of the exposed cultures was 0.86 + 0.10 (N=6) of the control value (189 µg protein per culture) 4 days after exposure. The loss of binding was not increased by prolonging the glutamate exposure to 24 hr and was detectable with exposures as short as 10 min. When [^{14}C]glutamate tracer was included with cold glutamate (10^{-3} M) in the culture medium, sample counts did not change after 24 hr and the radioactivity still ran as a single peak on thin layer chromatograms using two solvent systems. The concentration-effect relationship for the action of glutamate was very steep. The mean fraction of control [^{3}H]QNB binding two days after a 3 hr exposure to glutamate (10^{-4} M) was 0.91 + 0.14 (N=5). However, the loss of binding observed at 10^{-3} M was not increased by further raising the concentration of glutamate. The fraction of [^{3}H]QNB binding remaining 4 days after a 3 hr exposure to glutamate (10^{-3} M) was dependent on the maturity of the cultures at the time of exposure. Cultures exposed at 8, 12 and 19 days of incubation yielded 59%, 21% and 18%, respectively, of control binding. Kainic acid was only moderately more potent than monosodium glutamate. When SC cultures at 2–7 weeks of incubation were assayed 2 days after a 3 hr exposure to kainic acid (10^{-4} M and 10^{-3} M) the remaining fractions of control [^{3}H]QNB binding were 0.65 + 0.09 (N=3) and 0.83 + 0.09 (N=3), respectively. The actions of glutamic and kainic acids were not influenced by the presence of 10% heat-inactivated horse serum in the culture medium. In conclusion, at least 80% of muscarinic receptor binding in mature SC cultures is associated with a sub-population of cells, presumably neurons, which is destroyed by the neurotoxic amino acids. The time course of disappearance of the receptor binding is measured in days. (Supported, in part, by USPHS Grant MH 29011.)

2537 QUANTITATIVELY DEFINED CELL CULTURES OF THE CEREBELLUM D. Neil Currie* and Gary R. Dutton. Brain Research Group, The Open University, Milton Keynes MK7 6AA, U.K.

Primary cell cultures have great potential as models for the study, particularly by biochemical methods, of many aspects of neuronal development which are currently rendered inaccessible by the complexity and heterogeneity of the intact brain. However interpretation of biochemical results must rest on clear knowledge of cellular composition in cultures based on reliable methods of cell identification.

In order to define and quantify the composition of our monolayer cultures of rodent cerebellum, we have used three types of marker: (a) Autoradiography of ^{3}H-GABA uptake using specific GABA analogues to distinguish glial uptake from uptake into putative GABAergic neurons. (b) Established immunological markers for neurons, astrocytes, oligodendrocytes, and fibroblasts. (c) New monoclonal antibodies to cell populations in culture.

Cerebellar cultures have been found to contain at 6 days in vitro (DIV): granule neurons(84% of total cells), inhibitory GABA neurons(4%, probably stellate and basket cells), astrocytes(11%), oligodendrocytes(0.5%), and fibroblasts(0.5%). Cerebellar glial cultures have also been prepared from the same starting cell suspension using a different medium: these contain at 6 DIV over 85% astrocytes and small proportions of the other cell types.

Once cell composition can be defined in this way, it is possible to adjust conditions to favour particular cell types, such as the inhibitory neurons. Cultures of greater than 95% granule neurons can now be obtained, being the purest cultures of a single neuronal type yet available.

The availability of a range of culture systems of differing and known composition provides a sound basis for biochemical studies of cellular development.

2538 IN VITRO, GROWTH, GLUTAMINE AND GLUCOSAMINE TOXICITY STUDIES OF HUNTINGTON'S CHOREA FIBROBLASTS. I.DeMartini*, J.Archer*, S. Kiel* and E.Mancall. Departments of Neurology and Pediatrics, Hahnemann Medical College, Philadelphia, PA 19102.

Tissue culture studies were performed on skin fibroblasts from 6 patients with Huntington's Disease (HD), 6 age-matched normal controls (NC) and 6 "at risk" subjects. For growth studies, cells were plated at 1x10^5 cells/60mm plate in Waymouth/McCoy medium containing 20% fetal calf serum (FCS). Three plates were counted each day for 5 days in 3 separate experiments. HD fibroblasts grew to a significantly higher maximal cell density than NC fibroblasts ($P < 0.05$ on the 5th day) as previously demonstrated (Leonardi,A., DeMartini,I., et al. N.Eng.J.Med. 298:632,1978 inter alia). One of the 6 "at risk" subjects' fibroblasts grew to a maximal cell density in the HD range (2 standard deviations (SD) above the mean of the NC cells). The other 5 "at risk" subjects could not be clearly identified in this manner; in 3 cases the maximal cell density was within 1 SD of the mean of the HD cells, and in 2 cases within 1 SD of the mean of the NC cells.

Glutamine and Glucosamine toxicity studies were then performed with the aim of obtaining a more significant and clearly-defined difference in behavior between HD, NC, and "at risk" cells in culture. When HD and NC fibroblasts were grown for 3 days in medium containing 5% FCS (partially depleted medium) growth rates for the two groups of cells were similar (P-not significant). However, the addition of glutamine (2mM) after 24 hours growth in partially depleted medium, caused a significant reduction in the growth of HD cells but not of NC cells. (day 3 vs. day 1: Δ %=-31% for HD. $P < 0.01$, Δ %=1% for NC. P not significant). When glucosamine (1mM) was added under the same conditions, the growth of both HD and NC cells was significantly reduced $P < 0.05$. Studies with "at risk" cells are in progress, as are toxicity studies using glucosamine and glutamine in a concentration range 0.5-10mM, with plating efficiency (mean colony formation) as the end point.

These preliminary findings suggest a greater toxicity of glutamine in HD cells as compared to NC cells, perhaps as an expression of partial impairment of one or more of the metabolic pathways for glutamine and glutamic acid. This formulation would be consistent with reported findings of reduced levels of GABA and glutamic acid decarboxylase in the brain of HD subjects.

2539 EXPLANT CULTURES OF ADULT RAT SUPERIOR CERVICAL GANGLIA (SCG) MAINTAINED IN CHEMICALLY DEFINED MEDIA. Anthony Dombrowski* and Frederick C. Kauffman, Dept. Pharm. Exptl. Ther., Univ. MD, Sch. Med., Balto., MD 21201.

Organotypic cultures of adult rat SCG explants have been maintained in chemically defined media for periods of at least two weeks. Desheathed ganglia from rats weighing 125–150 g were cut transversely into 300 µm sections and placed in culture on either a collagen or Millipore filter (THWOP2500) surface. Virtually no growth was obtained in a serum-free medium (Eagle's MEM, 10 mM glucose, 100 units/ml nerve growth factor and antibiotics) supplemented with 1% bovine serum albumin (BSA). Significant outgrowth occurred when the medium was further supplemented with at least one or combinations of the following: insulin (1 µg/ml), l-thyroxine (4 µg/ml), and hydrocortisone (.05 µg/ml). On the collagen surface growth observed in the presence of BSA and all three hormones was comparable to that observed with a 10% Fetal Calf Serum (FCS) supplement. Increasing the FCS concentration to 20% or adding all three hormones plus 10% FCS did not alter this.

The explant outgrowths are long neuronal-like processes containing catecholamines but no detectable DNA. After freeze-drying the culture assembly the entire explant body can be removed from either the collagen or Millipore filter leaving the outgrowth attached to the surface.

Outgrowth may be quantitated by measuring $NADP^+$-dependent dehydrogenases localized in the growing processes. Glucose-6-phosphate dehydrogenase (G6PDH) was the most active of the four major NADPH generating enzymes in SCG explants. Activities of G6PDH, isocitrate dehydrogenase, 6-phosphogluconate dehydrogenase (6PGDH), and malic enzyme were 150 + 10, 70.8 + 11.8, 34.6 + 7.7, 8.6 + 1.5 pmole NADPH/µg protein/min, respectively in FCS media at 37°. This pattern differs from that in situ where isocitrate dehydrogenase is the most active $NADP^+$-dependent enzyme. Activities of the dehydrogenases, except 6PGDH, were higher in the presence of FCS than in explants cultured with BSA and hormone supplemented media. Both G6PDH and 6PGDH showed a trend of decreasing activity in the serum-free media. Highest activity was noted in the presence of all three hormones and decreased progressively in media supplemented solely with insulin, thyroxine or hydrocortisone.

The SCG explant maintained in chemically defined media promises to be a useful system to quantitate growth and growth promoting substances in neural tissue. (Supported by USPHS Grant NS-14728.)

2540 DEVELOPMENT OF RESTING POTENTIAL IN TISSUE CULTURED SKELETAL MUSCLE: A REQUIREMENT FOR LOW [K^+] IN BATHING SOLUTIONS. J.K. Engelhardt, K. Ishikawa*, and D.K. Katase*. Dept. of Neurology, USC School of Medicine, Los Angeles, CA 90033.

The developmental increase in skeletal muscle resting potential has been a common observation in embryonic tissue (Boethius & Knutsson, J. Exp. Zool., 174: 281, 1970; Kano, J. Cell. Physiol., 86: 503, 1975) and in primary cultures (Fischbach et al., J. Cell. Physiol., 78: 289, 1971; Ritchie & Fambrough, J. gen. Physiol., 66: 327, 1975). The experiments reported here were specifically designed to test the effect of low [K^+] in the bathing solution on the ability to observe a developmental increase in resting potential.

Our tissue culture and electrophysiological methods have been described elsewhere (Engelhardt et al., Brain Research, 110: 170, 1976). Myotube resting potentials were measured using conventional glass micropipette electrodes filled with 3 M KCl. Our 2.7 mM K^+ solution had the following composition in mM: NaCl 140, KCl 2.7, $CaCl_2$ 1.8, Glucose 11.7, HEPES 16.7, pH adjusted to 7.4 with NaOH. 5.4 and 10.8 mM K^+ solutions were prepared by substituting the appropriate amount of KCl for NaCl. The experimental protocol consisted of removing a culture dish from the incubator and discarding the media. The dish was rinsed with the appropriate experimental solution, refilled and allowed to equilibrate for 10 minutes before resting potential measurements were attempted. Experiments were conducted at room temperature (20-24°C). The statistical significance of differences in average resting potential was tested using analysis of variance.

Resting potentials measured in 2.7 mM K^+ increased from 42.1 ± 8.6 mV (mean ± SD, n = 10) on day 4 to 86.1 ± 4.2 mV on day 8, while resting potentials measured in 5.4 mM K^+ did not show a statistically significant change during the same period of time (67.8 ± 4.2 vs 71.5 ± 4.0). The change in average resting potential measured in 10.8 mM K^+ was statistically significant (51.7 ± 2.4 vs 56.5 ± 2.4), but the increase was less than 5 mV during the same period of time that resting potentials measured in 2.7 mM K^+ increased an average of 44 mV.

Our tentative explanation for these observations is that low [K^+] leads to K-channel inactivation and an increased dependence of the resting potential on other ions in the system, ions that exhibit changes in their ability to penetrate developing muscle membrane.

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2541 PULSATILE MOVEMENTS OF SCHWANN CELLS IN VITRO STUDIED BY TIME-LAPSE CINEMICROGRAPHY. David S. Forman, William G. Shain, Jr., David A. Fuchs* and Clarence H. Braddock*. Naval Medical Research Institute and Armed Forces Radiobiology Research Institute, NNMC, Bethesda, Maryland 20014.

Schwann cells (Pomerat, Science 130:1759 [1959]) and oligodendroglia in tissue culture display slow pulsatile movements which may be unique to these myelin-forming cells. This behavior has been reported by several laboratories but has rarely been analyzed quantitatively. We have used cinemicrography to study the movements of Schwann cells in dissociated cell cultures of sciatic nerves, vagus nerves and superior cervical ganglia from 3-day old rats. The cells were grown on glass coverslips in F12 medium containing 10% foetal calf serum and 5 µg/ml gentamycin. In most of our cultures the proliferation of fibroblasts was suppressed either by X-irradiation (1000 rad), treatment with cytosine arabinoside, or culture in N2 medium (Bottenstein and Sato, Proc. Nat. Acad Sci. USA 76:514 [1979]). The cells were observed with phase microscopy in a continually perfused Dvorak-Stottler chamber at 36°C and photographed at 6 frames/minute. Most of the Schwann cells were elongated and bipolar with small, highly refractile cell bodies. However, they often extend more than two processes, and can flatten against the substrate. The cultures were usually filmed for an hour, during which an average of 56% of the Schwann cells displayed intermittent pulsatile activity. The duration of these active periods varied from 1 to 7 minutes, with an average of 2.7 minutes. The quiescent interval between pulsatile periods also varied widely; intervals ranging from 1 to 17 minutes were measured between pulsations (average = 4.3 minutes), but there were many longer pauses which extended beyond the ends of the filming sessions. The average cell had 4.0 periods of pulsatile activity per hour. Two types of pulsatile movement were seen: single strong contractions of the cell body or a series of low amplitude contractions or side-to-side movements of the cell body. The distributions of the duration of the active periods and of the intervals between activity were closely similar for these two types of movement, suggesting that they are two forms of the same behavior. Pulsatile motility depended upon continual perfusion of the chamber; it stopped about an hour after perfusion was discontinued and began again when perfusion was restored. Pharmacological, ionic, and developmental factors which control the pulsatile behavior are being studied. Preliminary results are that serotonin (10^{-4}M) and dibutyryl cyclic AMP (10^{-3}M) reduce the frequency of the pulsations. Raising the K^+ in the medium to 20 or 40 mM had no obvious effect upon the pulsation. The function of Schwann pulsations is unknown, but one hypothesis is that it forms a mechanism for the peristaltic pumping of materials through the long, attenuated cytoplasmic processes in myelin.

2542 ANALYSIS OF THE MECHANISM OF NEURITE TRANSECTION IN CULTURE WITH PULSED UV LASER MICROBEAM IRRADIATION. Guenter W. Gross*, M. Louise Higgins*, Marilyn N. Smith*. Dept. of Biology, Texas Woman's University, Denton, TX 76204. (SPON: J.F. Hines)

To develop a reliable and convenient method for the micromanipulation of cultured neurons and their processes in closed chambers we are investigating the effects of UV laser microbeam cell surgery on mouse neuroblastoma cells with light, scanning electron and transmission electron microscopy. It has been determined that highly localized transections can be achieved with three basic techniques: (a) direct transection due to vaporization of tissue in the laser focus; (b) indirect transection due to shock waves caused by vaporization of minute quantities of substrate below the neurite; and (c) slow coagulation of cytoplasm due to irradiation with power densities below the threshold for immediate physical damage (approx 10^9 W/cm^2). Contrary to impressions gained from preliminary data (Rieske, Gross and Kreutzberg, Laser und Elektro-Optik 2,44, 1977; Rieske and Kreutzberg, Brain Res. 148, 478, 1978; Gross, Higgins and Smith, Neurosci. Abs. 4, 590, 1978), transections are usually not direct but result from shock waves and associated physical phenomena due to vaporizing substrate. These transections are not as accurate as previously believed and often show neurite separation at sites 3 to 5 µm distal or proximal from the laser impact area along the process. Scanning electron microscopy also reveals occasional residual processes below the resolution of LM at laser impact sites. Although accuracy and reproducibility are best with technique (a) it has the disadvantages of higher levels of scattered UV and a higher risk of widespread damage if the laser focus is accidentally located in the substrate. Techniques (a) and (b) must be carried out with single shots because structural alterations in substrate cause higher absorption with successive shots and larger shock waves of unpredictable magnitudes. Despite these problems laser cell surgery allows manipulations with unprecedented accuracy and ease. Remarkably little cell damage can be demonstrated on the ultrastructural level. (Supported by NIH grant NS15167-01 and Texas Woman's University Institutional Grant 0958).

The laser microbeam system was constructed by BTG Biotechnic, Munich and consists of a nitrogen laser coupled to a Leitz microscope; max. power densities 10^{11} W/cm^2, min. focus 0.8 µm; pulse length 10 nanoseconds; frequency 337 nanometers. A HeNe laser is used to mark the target and aid in focusing the UV laser.

2543 ACETYLCHOLINE SENSITIVITY IN XENOPUS NERVE-MUSCLE CELL CULTURES DURING SYNAPTOGENESIS. Raphael Gruener and Yoshiaki Kidokoro. Dept. Physiol., Univ. Arizona Col. Med., Tucson, AZ 85724 and Salk Institute, La Jolla, CA. 92112.

We recently showed (1) that in combined cultures of Xenopus nerve and muscle cells, miniature endplate potential (mepp) amplitude and frequency increase in parallel with the nerve-induced accumulation of acetylcholine receptors (2) at the area of contact between the neurite and muscle cell. These findings are consistent with the well-described changes in acetylcholine receptor (AChR) redistribution which occurs during in-situ synaptogenesis of the neuromuscular junction.

We examined the sensitivity of muscle cells to iontophoretic application of ACh to ascertain if ACh sensitivity, at the developing contacts, may be correlated with mepp properties and to determine the sensitivity of non-contact areas on the muscle cells during development of functional interactions between nerve and muscle cells in-culture. ACh sensitivity was measured by the slope method (3) on muscle cells grown alone or with nerve-cord cells.

Application of ACh anywhere on the surface of cells grown alone produced depolarizations. In addition, small spots were randomly encountered with much higher sensitivity to ACh ("hot spots"). Muscle cells grown with neurons but without visible neurite contacts were similarly sensitive to ACh. In cells with neurite contacts and from which mepps could be recorded, more than four-fold increase in ACh sensitivity at the site of contact with the nerve correlated well with the incremental mean mepp amplitudes. The ACh sensitivity outside the contact areas, however, was not significantly different from that found in non-innervated cells.

We conclude that the increase in mepp amplitude during synaptogenesis, in this system, may be accounted for solely by the post-synaptic accumulation of receptors at the site of nerve-muscle contact.

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2544 BIOSYNTHETIC REQUIREMENTS FOR NEURITE OUTGROWTH IN GOLDFISH RETINA EXPLANTS. Anne M. Heacock. Neuroscience Laboratory, University of Michigan, Ann Arbor, MI 48109

Our previous studies of goldfish retina explants have documented the dependence of the neuritic outgrowth on prior optic nerve crush and have demonstrated that the retinal ganglion cells are the cells of origin of these neurites. This preparation then serves as an in vitro model for investigations of many aspects of optic nerve regeneration and nerve growth and maintenance in general. In the experiments described here, we examined the biosynthetic requirements for neurite outgrowth, making use of inhibitors which are known to interfere with specific metabolic events. Those agents include an inhibitor of protein synthesis (cycloheximide, CXM), of cholesterol synthesis (diazacholesterol, DAC) and of glycoprotein biosynthesis (tunicamycin). Neurite outgrowth was quantitated using a morphological nerve growth index.

As might be expected, prevention of protein synthesis by addition of CXM (100 μg/ml), either at the time of explantation or at subsequent times, completely inhibits neurite outgrowth. However, maintenance of existing neuritic outgrowth is not so drastically affected by the continued presence of CXM. The neurites remain intact and appear normal for several days following inhibition of protein synthesis, then gradually deteriorate. This result implies that newly synthesized protein is not necessary for the maintenance of the axon over this interval and is in contrast to the relatively rapid degeneration within 24 h of a neurite fascicle which has been cut off from its parent explant.

Another treatment which was found to cause intact neurites to degenerate within 24 h was incubation with the cholesterol synthesis inhibitor DAC (10^{-4}M). A lower concentration of DAC (10^{-5}M) did not cause degeneration of intact neurites but did inhibit neurite outgrowth by 70-80%. This inhibition was not overcome either by the presence in the medium of fetal calf serum (which contains cholesterol) or by supplementation with added cholesterol (2.6×10^{-5}M). The DAC inhibition was however reversed by the addition of mevalonic acid (1 mg/ml), the product of a rate-limiting enzyme in cholesterol biosynthesis, HMGCoA reductase. The reversal by mevalonic acid and not by cholesterol suggests that DAC may have, either directly or indirectly, an inhibitory effect on HMGCoA reductase.

Mevalonate is the precursor not only of cholesterol but of all terpenoids, including the dolichols, long-chain isoprenoid alcohols which function as glycosyl carriers in the biosynthesis of glycoproteins. A role for the polyprenol-linked sugars in goldfish retina explant glycoprotein synthesis was investigated by examining the effect on neurite outgrowth of an inhibitor of this pathway, tunicamycin. The latter, when present continuously in the culture medium (at 5 μg/ml), resulted in a 70% inhibition of outgrowth after 5 days in vitro and greater than 90% inhibition at 12 days. These results indicate that the functioning of the dolichol sugar pathway is necessary for neurite outgrowth.

2545 EXPLANT CULTURES OF FETAL RAT BRAIN: CHARACTERIZATION BY IMMUNOFLUORESCENCE AND NEUROTRANSMITTER CONTENT. Robert J. Milner*, Quentin J. Pittman, William J. Shoemaker, Alejandro Bayon and Floyd E. Bloom. Salk Inst., La Jolla, CA 92037.

Explants of fetal rat cerebellum (CB), locus coeruleus (LC), substantia nigra (SN), caudate nucleus (CN) and arcuate nucleus including median eminence (A-ME) can be maintained in culture for several weeks. To characterize the cell types obtained in these cultures we have used immunofluorescence techniques that distinguish neurons, astrocytes and oligodendrocytes (oligos). Neurons were further characterized by assay of peptide or catecholamine neurotransmitters. Specific regions were dissected out of the brain of ED19 rats, teased into small pieces and cultured for several weeks in DMEM containing 10% horse serum. Neurons were labelled by sequential treatment with tetanus toxin and horse anti-toxin; oligos were labelled with rabbit anti-galactocerebroside; and astrocytes were labelled with rabbit anti-glial fibrillary acidic protein. The cells were visualized by reaction with rhodamine or fluorescein labelled goat anti-rabbit or horse IgG. Enkephalins and β-endorphin were measured by specific RIA and catecholamines by a radio-enzyme assay. The staining techniques were shown by double labelling to be specific for each cell type and could account for the majority of cells present in cultures of all brain regions. Neurons and astrocytes were the predominant cell types while oligos were comparatively infrequent. In cultures of CB the majority of neurons were small, with interconnecting networks of neurites. Some larger cells, possibly Purkinje cells, were also found. Cultures of LC and SN contained large neurons with extensive processes; some cells also exhibited positive catecholamine fluorescence. Both large and small neurons were found in CN cultures. Oligos were seen most frequently in cultures of CB, LC, and SN and rarely in CN. This cell type was usually found singly, although oligos were seen associated with nerve fascicles in LC and SN cultures. In all cultures large flat astrocytes formed a monolayer over the dish surface but in explant outgrowth regions astrocytes formed long fibrous processes. Only LC and SN cultures possessed measurable amounts of norepinephrine and dopamine. Enkephalins and β-endorphin were found only in A-ME cultures. Although enkephalins were undetectable by RIA in CN cultures, some CN neurons stained for enkephalin using an immunoperoxidase technique. Characterization of neuronal and non-neuronal cell types in these cultures is essential for monitoring their development under different culture conditions and for electrophysiological recording.

2546 ULTRASTRUCTURAL AND CYTOCHEMICAL CHARACTERIZATION OF SCHWANN CELLS CULTURED FROM RAT PERIPHERAL NERVE. Ward F. Odenwald*, Valerie Askanas, W. King Engel, Linda S. Carter*, Jane V. Lawrence,* NIH, Bethesda, MD 20205

Schwann cells were cultured from 3 day old rat sciatic nerves with techniques established in this laboratory for human nerve cultures. Six 1 mm^3 nerve explants were placed on collagen-coated 35 mm petri dishes, and subsequent reexplantations were performed until almost all non-schwann cells were eliminated. Cultures were fed with media as described (Neurology 25:58-67, 1975). After satisfactory growth was obtained the cultures were processed for cytochemical reactions or routine electron-microscopy (EM). EM studies were performed on areas of cultured schwann cells (CSC) preselected by light microscopy as described (Stain Technology 52: 249-254, 1977). CSC of peripheral nerve possessed similar ultrastructural characteristics to schwann cells of adult rat sciatic nerve (ASC); those were: large ovoid nucleus, numerous free and rosetted form ribosomes, narrow RER, mitochondria, golgi apparatus, ~7nm microfilaments, and ~18nm microtubules. CSC were surrounded by a well-defined plasma membrane (PM) ~9nm thick. The majority of CSC had characteristic membrane surface invaginations, ~25nm deep. Major differences between CSC and ASC were lack of a basement membrane and the presence of polarly orientated bundles of helical filaments in single and double helix configuration (helix periodicity ~56nm) in the cytoplasm of the CSC.

Positive peroxidase-post-coupled DAB staining of concanavalin A (100 μg/ml) binding revealed the presence of α-D-glucoside and/or α-D-mannoside groups on the PM of CSC. Staining with a low concentration (10μg/ml) of ConA revealed possibly stronger affinity of the invaginations toward ConA, suggesting an increased density of receptors there. Omitting the ConA from the incubating medium or pretreatment with α-methyl-glucoside resulted in lack of characteristic staining.

Horseradish peroxidase (HRP), employed as a cytochemical tracer, was added to the medium of the living cultures. After two hours, high HRP activity was observed within lysosomal-like (LL) structures in the CSC cytoplasm. HRP activity was also located at distinct sites along the PM. Uptake of HRP was much more prominent in the CSC than in the non-schwann cells. When cultures were examined for endogenous peroxidase activity, almost no activity was observed with the exception of a few grains of oxidized DAB within a small number of the LL structures.

The knowledge of ultrastructural and cytochemical characteristics of normal CSC is needed to study the influence on them of different substances suspected to cause dyschwannian neuropathies in humans.

2547 ACETYLCHOLINESTERASE, OTHER MUSCLE PROTEINS, AND DNA OF FIBROBLAST-FREE IRRADIATED MUSCLE CULTURES. W.R. Randall*, P.A. Nieberg*, and B.W. Wilson. Dept. Avian Sci., UCD, Davis, CA 95616.

Experiments that compare properties of muscle cultures from different genetic backgrounds and involve treatments that may alter distributions of cells are complicated by the presence of fibroblasts and other cell types in mixed cultures. Chemical treatments are often used to suppress dividing cells. X-ray irradiation has also been reported to destroy fibroblasts without affecting fusion of myoblasts into myotubes (Friedlander et al.; Dev. Biol., 66, 457, 1978). The experiments presented here examine the differentiation of some muscle-specific properties in such fibroblast-free cultures.

Pectoral muscle cultures from 11 day embryos were irradiated one day after plating and examined for up to 2 weeks in vitro. There was maximum elimination of fibroblasts and minimum loss of myoblasts at approximately 4000 rads. Fusion and gross development proceeded normally; 5 day old cultures contracted spontaneously and 8 day old cultures appeared morphologically normal with the electron microscope.

A sample experiment is shown below. Accumulation of ACHE (cell and medium) for cultures 10-13 days in vitro was little changed by irradiation of New Hampshire (N.H.) or White Leghorn (W.L.) lines. However, ACHE per DNA and per protein increased greatly due to loss of fibroblasts.

	ACHE	ACHE/DNA	ACHE/Protein
N.H.	5.98±0.43	0.27±0.04	7.47± 1.22
N.H. X-ray	5.34±0.86	2.35±0.93	36.1 ±13.7
W.L.	9.69±1.89	0.50±0.15	12.4 ± 2.6
W.L. X-ray	11.6 ±1.18	2.06±0.46	32.7 ± 4.3

ACHE in umoles substrate hydrolyzed/min/dish; DNA in ug/dish; protein in mg/dish. Means ± SD (N=4)

DNA content of irradiated cultures was reduced 3 fold (W.L.) and 9 fold (N.H.). Non-collagen protein was 3 fold less (both lines) than in non-irradiated controls. Similar results were obtained when activities were expressed on a protein basis. Reinoculation of the cultures with fibroblasts did not alter the properties of the myotubes. Determinations of acetylcholine receptor, myosin, creatine kinase and lactic dehydrogenase and studies of normal and dystrophic chick lines are in progress.

The results show that differentiation of physiological, biochemical and histological properties of multinucleated myotubes proceed normally after irradiation, producing cultures virtually free of fibroblasts for periods up to two weeks in vitro. (Supported by the MDA and NIH ES00202)

2548 AN α-NEUROTOXIN THAT INDUCES THE RAPID INTERNALIZATION OF FLUORESCENT α-BUNGAROTOXIN BOUND TO AUTONOMIC NEURONS. Peter Ravdin*, Ralph Nitkin, and Darwin Berg. Dept. of Biology, UCSD, La Jolla, CA 92093.

Chick ciliary ganglion neurons and sympathetic neurons both have a high affinity binding site for α-bungarotoxin (Bgt 2.2), but the toxin does not block acetylcholine (ACh) sensitivity on the neurons. We have previously shown that another α-toxin (Bgt 3.1) from the same snake venom does block ACh sensitivity on the neurons. We now report that Bgt 3.1 causes the rapid internalization of fluorescent Bgt 2.2 bound to the cells. Blockade of ACh sensitivity by Bgt 3.1, however, does not appear to depend on the internalization revealed with fluorescent Bgt 2.2.

Dissociated neurons prepared from 8-12 day embryonic chick ciliary and sympathetic ganglia were grown in cell culture for 1-3 weeks with skeletal myotubes. High affinity Bgt 2.2 binding sites on the neurons were fluorescently labeled by incubating the cultures in 4 x 10^{-8} M tetramethyl rhodamine-conjugated Bgt 2.2 (R-2.2) for 1 hr at 37°. Subsequent incubation with 10^{-7} M Bgt 3.1 at 37° led to nearly complete loss of surface fluorescence within 0.5 hr with the concomitant appearance of numerous small foci of intense fluorescence throughout the interior of the neurons. This apparent internalization of bound R-2.2 was blocked by low temperature (4°) or by preincubation of the labeled neurons with 0.1 mg/ml concanavalin A. It was not blocked by 50 μM colchicine or 200 μM cytochalasin B; it did not occur spontaneously and was not induced by Bgt 2.2.

Two observations suggest that the induced internalization may be confined to Bgt 2.2 sites. First, neurons remained electrically excitable after Bgt 3.1 treatment, indicating that they retained membrane components necessary for action potentials. Second, sympathetic neurons bound fluorescent nerve growth factor (R-NGF) in addition to R-2.2, but Bgt 3.1 did not induce rapid internalization of the bound R-NGF.

Blockade of neuronal ACh sensitivity by Bgt 3.1 can be separated from the internalization revealed with R-2.2. When R-2.2 labeled neurons were incubated with 10^{-7} M Bgt 2.2 or D. viridis 4.7.3. toxin before and during challenge with Bgt 3.1, internalization of the bound R-2.2 was blocked. Under these conditions Bgt 3.1 still produced complete inhibition of iontophoretically measured ACh sensitivity.

These data indicate that Bgt 3.1 causes two separate events: inhibition of neuronal ACh receptors and internalization of bound R-2.2. The Bgt 3.1 binding site that produces receptor inhibition apparently is not blocked by Bgt 2.2 or D. viridis toxin. (Supported by USPHS grant #12601, The Muscular Dystrophy Association, & the American Heart Association.)

2549 HUMAN SKIN FIBROBLASTS EXHIBIT HIGH-AFFINITY CHOLINE ACCUMULATION IN CELL CULTURE. Donald Kay Riker, Robert H. Roth and Xandra O. Breakefield. Depts. Pharmacology, Psychiatry, & Human Genetics, Yale Univ. Sch. Med., New Haven, Ct. 06510.

^{3}H-choline can be transported across cell membranes by high-affinity (K_T < 4μM) and low-affinity (K_T>> 4μM) systems. High-affinity choline accumulation (HACA) has been demonstrated in synaptosomes made from cholinergic brain regions, such as the hippocampus and caudate-putamen; thus, regional choline accumulation is coextensive with afferent cholinergic nerve terminals. HACA has been shown to be a function of impulse flow in central cholinergic neurons and may be the rate-limiting step in acetylcholine synthesis in the septal-hippocampal tract. In cell culture HACA has been demonstrated in avian telencephalon and spinal cord, glia, neuroblastoma, and glioma cells. Recently, Barald & Berg (Dev. Biol. 65:90, 1978) reported HACA in fibroblasts cultured from avian muscle. HACA in these fibroblasts had a K_T and V_{max} similar to that observed in dissociated spinal cord cells in culture.

We examined ^{3}H-choline accumulation in normal human fibroblasts cultured from skin biopsy. ^{3}H-choline accumulation was temperature dependent and linear with incubation time up to 6 min at 0.125 μM choline. Isoosmotic replacement of Na^+ with Li^+ (137mM) or sucrose (274 mM) severely reduced ^{3}H-choline accumulation (by 70-90%). Preincubation with ouabain (10μM) or 2,4-dinitrophenol (10μM), or replacement of Ca^{++} with Mg^{++} had little or no effect on subsequent ^{3}H-choline accumulation. ^{3}H-choline accumulation was hemicholinium-3 (HC-3) sensitive with pre-incubation in HC-3 at 37°C for 10 min. The IC_{50} at 0.125 μM choline was 3-4 μM. The K_T for ^{3}H-choline was about 4 μM, which compares favorably to that observed in brain synaptosomes and avian fibroblasts. The HC-3 sensitivity, Na^+-dependence, and low K_T suggest that human skin fibroblasts have a high-affinity transport system for choline. Presently, we are investigating HACA in fibroblasts of patients with inherited neurologic disorders which may have a cholinergic etiology. (Supported by grants to D.K.R., R.H.R. & X.O.B. from the Dystonia Medical Research Foundation).

2550 ANTINEURONAL IN VITRO ACTIVITY OF SERA FROM PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS. F. J. Roisen*, H. Bartfeld*, H. Donnenfeld* and J. Baxter*, (Spon: A. Hess), Dept. of Anatomy, CMDNJ-Rutgers Medical School, Piscataway, NJ 08854, and the Amyotrophic Lateral Sclerosis Research Center of St. Vincent's Hospital and Medical Center, NY, NY 10011.

Amyotrophic lateral sclerosis (ALS) is a fatal, adult-onset, neurological disorder of unknown etiology which is characterized pathologically by degeneration of the anterior horn motor neurons as well as neurons in the motor nuclei of the lower cranial nerves. Wolfgram and Myers reported a high proportion of sera from patients with ALS was toxic to cultured anterior horn neurons (1, 2). Conflicting reports have been published (3, 4).

Blood samples from 18 patients were randomly selected from a total pool of 73 ALS patients. Age and sex matched sera were also obtained from 27 patients with neurological diseases (cerebral infarction, Parkinsonism, senile dementia, CNS trauma and tumors), as well as 5 from normal controls. The sera were incorporated into the media, applied to long-term monolayer cultures and assayed for a neuron-specific cytotoxic effect on developing neonatal anterior horn segments. The criteria for cytotoxicity included a reduction in neuritic processes, increased cytoplasmic granularity and vacuolarization, loss of characteristic neuronal shape, and eventually the extrusion of the nuclei.

These blind studies between our laboratories have shown that ALS sera when incorporated into the culture media have a significantly greater degree of antineuronal toxicity ($p<0.01$) than sera from patients with other neurological diseases or normal controls. Dialysis of previously tested positive sera against balanced salt solution did not reduce the antineuronal activity. The cytotoxicity appears highly specific since known positive sera did not affect embryonic sensory or sympathetic ganglia, S20 neuroblastoma, RN22 schwannoma, L-929 fibroblasts or primary dissociated embryonic muscle cultures. At present the relationship between the factor and the disease remains unclear since studies to date have shown no correlation between the course or severity of the disease and the degree of cytotoxic activity.

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2551 CELL SURFACE ACETYLCHOLINESTERASE ON CULTURED CHICK EMBRYO MUSCLE CELLS. Richard L. Rotundo and Douglas M. Fambrough. Carnegie Institution of Washington, Dept. of Embryology, Baltimore, Maryland, 21210.

Chick embryo muscle cells grown in tissue culture contain two major molecular forms of acetylcholinesterase (AChE) having sedimentation coefficients of 11.5S and 7.1S. In order to study the cellular distribution of AChE forms in culture we have modified the Johnson and Russel AChE assay so that it can distinguish AChE molecules located on the surface membrane from those located inside the cell (to be described). We can demonstrate that approximately 30-40% of the total cells' contents of AChE is located external to the plasma membrane. This cell surface AChE is not removed by treatment with 1M NaCl or by competition with various sugars; however, this enzyme is easily solubilized using the detergent Triton X-100.

To determine which molecular forms of AChE are located on the cell surface, cultures were pre-treated with BW284c51, a reversible water soluble inhibitor which in this experiment regime blocks only the surface enzyme, followed by treatment with DFP, an irreversible esterase inhibitor which does penetrate the cells to inactivate the intracellular enzyme. Analysis by velocity sedimentation of the forms protected from DFP by BW284c51 indicates that both the 11.5S and 7.1S forms of AChE are located on the muscle cell surface.

Using DFP to irreversibly inhibit all AChE at different stages of muscle cell development in culture we can demonstrate that: 1) the cells synthesize the surface AChE forms in vitro, 2) the rate of surface AChE deposition increases during the time of cell differentiation in culture, 3) following treatment with DFP there is a partial recovery of the cell surface AChE in mature cultures, and 4) the surface AChE molecules have a half-life which is several times longer than that of another well-defined muscle cell surface protein, the acetylcholine receptor. During recovery from DFP treatment muscle cells in culture synthesize both the 11.5S and 7.1S AChE forms which are initially located inside the cells, and later give rise to the surface membrane fraction as well as to the secreted forms. In these studies we have not observed any apparent precursor/product relationship between the 7.1S and 11.5S molecular forms. A model depicting the synthesis, transport, and turnover of these membrane bound AChE forms will be presented.

This research has been supported by the Muscular Dystrophy Association of America.

2552 DEXAMETHASONE INDUCES BIOCHEMICAL DIFFERENTIATION IN CULTURED MURINE NEUROBLASTOMA. Dean Sandquist*, Larry Williams*, Asa C. Black, Jr., Shail Sahu*, and Terence H. Williams. Dept. of Anatomy, University of Iowa, Iowa City, Iowa 52242.

It has been observed previously that dexamethasone has a dose-dependent effect on morphological differentiation and growth inhibition of murine neuroblastoma (clone NBP_2) in vitro. We now report that dexamethasone also induces biochemical differentiation (as determined by increased dopamine content and tyrosine hydroxylase activity), and morphological differentiation (as determined by glyoxylic acid-induced histofluorescence microscopy). Dopamine content was determined by the method of Schmidt and Bhatnagar (Brain Res., in press), while tyrosine hydroxylase was assayed according to Waymire et al. (Proc. Natl. Acad. Sci., 69:2241).

Cells treated for seven days with 25 µM dexamethasone in ethanol have more intense and uniform greenish-yellow catecholamine fluorescence than solvent-treated or untreated controls. Dopamine content increased from 3.7 ± 0.6 µg. per mg. protein (untreated controls) or 5.1 ± 0.5 (solvent-treated controls) to 11.2 ± 1.2 µg. per mg. protein after dexamethasone treatment. Tyrosine hydroxylase activity increased from 0.015 ± 0.003 nanomoles $^{14}CO_2$ per hour per mg. protein (untreated controls) or 0.10 ± 0.02 (solvent-treated controls) to 0.30 ± 0.01 nanomoles $^{14}CO_2$ per hour per mg. protein. Tyrosine hydroxylase activity rose to a maximum of 0.82 ± 0.10 nanomoles $^{14}CO_2$ per hour per mg. protein after five days of treatment with 125 µM dexamethasone. 200 µg/ml Ro 20-1724 (an inhibitor of cyclic AMP phosphodiesterase) increased tyrosine hydroxylase activity to a similar extent. The increases produced by 25 µM dexamethasone in tyrosine hydroxylase activity and dopamine content were significantly different from both untreated and solvent-treated controls ($P<0.001$; Student's "t" test).

Thus dexamethasone appears to induce both morphological and biochemical differentiation in cultured murine neuroblastoma. Since agents which increase cyclic AMP levels (such as Ro 20-1724) are known to produce the same effects on differentiation as dexamethasone, and since dexamethasone does not elevate cyclic AMP levels in these cells (as measured by radioimmunoassay) we are currently determining whether dexamethasone and cyclic AMP-increasing agents have interacting or synergistic mechanisms for producing differentiation. We are also determining the characteristics of the glucocorticoid receptor in murine neuroblastoma. (Supported by NIH grants CA 24241-02 to DS and NS 11650 to THW).

2553 EFFECT OF CULTURE DURATION ON THE ELECTRIC MEMBRANE PROPERTIES OF ADULT MOUSE NEURONS. B.S. Scott and B.A.V. Edwards*. Surrey Place Centre, 2 Surrey Place, Toronto, Canada M5S 2C2.

Dorsal root ganglia (DRG) were dissected from adult mice (6 to 9 months), softened with collagenase, dissociated and plated on collagen-coated coverslips using 10% fetal calf serum in CMRL-1415 as medium (Scott, J. Neurobiol. 8: 417-427, 1977). After a various number of days in culture (DIV) the following electric membrane properties were determined 1. resting membrane potential (Vm) 2. specific membrane resistance (Rm) 3. membrane time constant (τ) 4. specific membrane capacitance (Cm) 5. duration of the action potential (DT) 6. absolute refractory period (ARP) 7. rheobasic threshold depolarization (ΔV).

Neurons had action potentials either with a simple monophasic falling phase (M type) or a more complex biphasic or triphasic falling phase (B type). M and B types had small but significant differences in their EMP and relative frequency (RF) of occurrence. Since the EMP of both types varied similarly with DIV, the data was pooled and mean values were obtained by analysis of variance with cell size and type as covariates.

	Days in Culture							
	0	1	2	4	6	12	24	40
RF	81.3	79.1	59.6	49.2	50.8	39.7	44.3	20.8
Vm	60.2	59.4	58.4	59.1	60.0	57.4	53.9	49.7
Rm	399	354	380	475	463	557	636	832
τ	1.15	1.17	1.32	1.37	1.63	1.61	2.20	2.80
Cm	3.22	4.63	3.68	3.15	3.83	3.19	3.99	3.91
DT	1.87	1.92	1.53	2.00	2.22	2.27	2.45	2.65
ARP	1.75	1.75	1.88	2.23	2.26	2.18	2.30	2.67
ΔV	24.4	21.0	21.5	24.6	19.5	14.8	9.50	7.74

Regression analysis indicated that all variables except Cm varied linearly with days in culture ($p<.001$). Since neuron survival was relatively constant for the first 6 days and even after 40 days was 60%, it seems unlikely that significant selection of neuronal types occurred. Thus, the observed changes in EMP reflect modifications in membrane functions of individual neurons. Since similar changes in EMP (decreased Vm and ΔV, and increased Rm) have been observed for denervated muscle in situ (Nicholls, J. Physiol. 131: 1-12, 1956), in vitro (Engelhardt et al., Brain Res. 126: 172-175, 1977) and embryonic chick DRG in culture (Handa, Tohoku J. exp. Med. 121: 13-25, 1977), it seems likely that these modifications are due to the isolation in culture of the DRG neurons from their usual central and peripheral connections.

2554 CYTOSINE ARABINOSIDE EFFECTS ON DEVELOPING CEREBELLUM IN TISSUE CULTURE. Fredrick J. Seil, Arnold L. Leiman*, Nathan K. Blank*, and William R. Woodward. Research Service, V.A. Med. Ctr. and Dept. Neurology, Univ. Oregon Health Sci. Ctr., Portland, OR 97201 and Dept. Psychology, Univ. California, Berkeley, CA.

Cerebellar explants prepared from newborn mice were exposed to 1-10 µg/cc cytosine arabinoside (Ara-C), an inhibitor of DNA synthesis, for the first 5-9 days in vitro, after which they were maintained in normal nutrient medium. Observations are reported from such cultures after 14-23 days in vitro. The cortices of explants exposed to Ara-C contained numerous closely packed large neurons with a paucity of intervening elements. Normal cortical lamination including layers of granule cells was not evident. In Golgi-Cox preparations, the surviving large neurons appeared to be Golgi II neurons and Purkinje cells, the latter with persistent dendritic spines. A marked increase in density of intracortical and subcortical neurites was apparent in silver stains, and proved to be Purkinje cell axons and axon collaterals by fiber tracing. In spite of the relative increase in axonal elements, myelin did not form in Ara-C treated cultures. Ultrastructural examination of cortical regions revealed an increase of Purkinje cell recurrent axon collateral terminals forming numerous synapses on Purkinje cell somata and dendrites. Both spontaneous electrical activity and stimulus elicited responses were recorded electrophysiologically. An increase in cortical discharges was induced by addition of picrotoxin, a GABA antagonist. Stimulation of cortex and subcortical fibers evoked both excitatory and inhibitory responses. These responses were interpreted as consistent with a cortical reorganization in which Purkinje cell axon collaterals were the dominant inhibitory elements.

2555 SERUM-FREE CULTURE MEDIUM MAINTAINS DIFFERENTIATED PROPERTIES OF NEURONS IN FETAL RAT BRAIN EXPLANTS. Wm.J.Shoemaker, J.E.Bottenstein, R.J.Milner*, B.R.Clark* and F.E.Bloom. The Salk Inst. and Dept. Biology, UCSD, La Jolla, CA 92037.

Explant cultures from fetal rat brain can be maintained for several weeks using a completely defined medium (N_1) without the use of serum. The following substances were added to DME medium: insulin 5µg/ml, transferrin 5µg/ml, progestrone 20nM, putrescine 100µM, and selenium 30nM; (P.N.A.S.76:514,1979). Explants of different brain regions were cultured in order to assess the effect of serum-free conditions on a variety of cell types and neurons: locus coeruleus (LC), substantia nigra (SN), cerebellum, caudate nucleus, and hypothalamus. Although attachment of the explants to either collagen or poly-D-lysine coated dishes was better in medium containing medium than in N_1 medium, addition of fibronectin aided attachment of explants under serum-free conditions. Attachment could also be enhanced by incubating in serum for the first 4-5 days in vitro and then switching to N_1 medium. The outgrowth pattern and morphological appearance of cultures maintained in N_1 for up to 8 weeks differed in several respects from identical brain regions grown in serum-supplemented medium. Regardless of the initial attachment conditions, cultures grown in N_1 had fewer background cells and less flattening of the explant. The morphology of the neurons and their processes in phase optics appeared healthy, similar to neurons grown in serum. To distinguish different cell types neurons were labelled with tetanus toxin and horse anti-toxin, oligodendrocytes were labelled with rabbit anti-galactocerebroside, and astrocytes with rabbit anti-glial fibrillary acidic protein. Comparison of cultures grown in the presence of serum or in N_1 medium revealed that in N_1 the neurons appeared to have shorter processes and that cells having a similar morphological appearance by phase optics did not all stain positively with tetanus toxin. Although there is a reduced number of non-neuronal cells in N_1 medium, large numbers of oligodendrocytes were observed. Astrocytes, however, were somewhat fewer in number in N_1 media and displayed very few processes. A major criterion for completely defined medium would be the ability of neurons cultured in its presence to maintain neurotransmitter synthesis. We assessed the ability of LC and SN cultures to synthesize and store catecholamine transmitters by a sensitive radio-enzyme assay and by fluoresence microscopy. Both assessments indicated that the number of catecholamine-containing neurons and the amine content did not differ in the two media. The later findings imply that catecholamine-containing neurons of the CNS do not require NGF or other serum factors in agreement with our previous results.

2556 CHRONOTROPIC RESPONSES MEDIATED BY ALPHA- AND BETA-ADRENERGIC RECEPTORS ON CULTURED CARDIAC MUSCLE CELLS. Christopher Nelson Sinback, Jr. Laboratory of Cell Biology, National Cancer Institute, NIH, Bethesda, MD. 20014.

Action potential rate, duration, and amplitude, maximum diastolic potential, etc, were recorded with intracellular microelectrodes from embryonic hamster cardiac muscle cells in primary cell culture. Noradrenaline, isoproterenol, and acetylcholine were delivered by iontophoresis.

Noradrenaline, and not isoproterenol, elicited positive chronotropic responses (increased action potential rate and hence increased beat rate) by activation of alpha-adrenergic receptors. Alpha-adrenergic responses were due to a fast, latency less than 500 msec, brief, 5-10 sec, depolarization which was abolished when external sodium was replaced with choline. Alpha-adrenergic responses desensitized rapidly.

Isproterenol, and not noradrenaline, elicited positive chronotropic responses by activation of beta-adrenergic receptors. Beta-adrenergic responses were characterized by a slow, latency longer than 3 sec, sustained, 20 sec - 2 min, interval of increased action potential frequency due to increased slope of the pacemaker potential. Beta-adrenergic responses did not desensitize. Beta-adrenergic responses were abolished by propranolol. During beta-adrenergic responses maximum diastolic potential and action potential overshoot generally decreased, but in some cells maximum diastolic potential increased.

2557 Intracellular Transport of Acetylcholinesterase and Acetylcholine Receptor. H. Smilowitz. Univ. Conn. Health Center, Farmington, Connecticut 06032

Most of the acetylcholinesterase (ACHE) that is synthesized by cultured chick embryo pectoral muscle cells is secreted into the culture medium by a process that is rapidly and reversibly inhibited by the monovalent ionophores (Smilowitz, H. Molecular Pharmacol. July 1979). The secretion of other continuously released glycoproteins such as collagen and fibronectin are similarly inhibited by the monovalent ionophores (Uchida, N., H. Smilowitz & M. Tanzer P.N.A.S. 76: 1868 (1979).

IN contrast, we have shown that the rate of appearance of new acetylcholine receptors (ACHR) at α-bungarotoxin (α-BGT) accessible sites, the turnover of ACHR, the total number of ACHR (total α-BGT accessible sites) and the size of the intracellular pool are nearly the same in both control and monovalent ionophore treated cells. Since the monovalent ionophores reportedly block the flow of membranes out of the golgi (Tartakoff, A. & Vassalli, P. J. Cell Biol. 79:694 (1978)) our data suggests that there are at least two types of membrane vesicles or carriers derived from the golgi- one whose formation is inhibited by the monovalent ionophores(normally transporting secretory proteins such as ACHE) and one whose formation is unaffected by the ionophores (normally transporting integral membrane proteins such as the ACHR).

Detailed histochemical and electron microscopic analysis of the ACHE secretory process show that in control myotubes, ACHE is found in the golgi, the nuclear membrane and in the sarcotubular system. After cycloheximide treatment (100 ug/ml for 2-3 hours) ACHE reaction product can no longer be found in the golgi; after monovalent ionphore treatment, two classes of ACHE containnng membrane vesicles appear. One class is perinuclear and is not accessible to externally applied ferritin; one class is cytoplasmic and is accessible to externally applied ferritin. The perinuclear vesicles derive from the golgi within two hours after the addition of 4×10^{-8} M nigericin. The cytoplasmic vesicles appear to be ampulae of the t-system. Upon removing nigericin, the golgi derived vesicles break up into smaller vesicles which resemble the t-system in appearance. We propose that the ACHE resides in the golgi following synthesis and is then transported by vesicle to cytoplasmic channels or the t-system of muscle. Once in the t-system, the enzyme is in communication with the external bathing medium. Experiments are now in progress to visualize the route of ACHR transport. (Supported by NS 13860)

2558 IMMUNOCHEMICAL CHARACTERIZATION OF ISOLATED OLIGODENDROCYTES MAINTAINED IN LONG TERM CULTURE. Kari Stefansson*, Raymond P. Roos*, Robert L. Wollmann*, Barry G. W. Arnason, Sara Szuchet*, Dept. Neur., The Pritzker Sch. of Med., Univ. of Chicago, Chicago IL 60637.

Oligodendrocytes have been isolated from lamb brains employing the procedure developed in our laboratory (Szuchet, et al., Biophys. J., 21, 51, 1978). Immediately after isolation cells are suspended in Dulbecco's modified Eagle's medium supplemented with 10% horse serum, plated at a density of 2.5×10^5 cells/cm^2 and kept in an incubator with 5% CO_2 and humidity at saturation. Freshly isolated cells exhibit surface staining, as viewed by indirect immunofluorescence, with anti-galactocerebroside (a generous gift of Dr. M. Rapport) and anti-sheep myelin basic protein antisera (a gift from Dr. A. Noronha). The pattern of staining with both antisera is patchy but the patches appear larger and more bulky with antimyelin basic protein than with antigalactocerebroside. The positive staining for myelin basic protein is consistent with the notion that components of myelin remained attached to the cells during the process of isolation. Galactocerebroside, on the other hand, is a normal constituent of the oligodendrocytic membrane (Raff, et al., Nature, 274, 813, 1978). Cell kept in culture for 21 days possess morphological characteristics which have been ascribed to oligodendrocytes (Wolfgram and Rose, J. Neuropath. and Experim. Neurol., 16, 514 (1957). Cells stick to the surface and emit processes. The majority of cells are bipolar, but multipolar cells and cells with aborted processes are also seen. Bead-like swellings which stain dark blue with Giemsa are often present either along processes or close to the cell body, these are reminiscent of "gliosomos". Some cells do not emit processes but surround themselves with extensive membranous sheets. Cells in culture exhibit positive surface staining with anti-galactocerebroside but not with antimyelin basic protein. The cells also stain with anti-oligodendrocytes antiserum obtained by immunization of guinea pigs with freshly isolated cells. Thus, cells kept in culture for an extended period retain similar surface characteristics as freshly isolated cells. This fact attest to their oligodendrocytic character. These results support our contention that oligodendrocytes can be maintained in culture for an extended period and retain their characteristic properties.

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2559 IMMUNOCYTOCHEMICAL LOCALIZATION OF GLIAL FIBRILLARY ACIDIC PROTEIN AND FIBRONECTIN IN PRIMARY ASTROGLIAL CULTURES. P.E. Stieg*, H.K. Kimelberg*, J.E. Mazurkiewicz*, and G.A. Banker*, Albany Medical College, Albany, N.Y. (Spon: A.J. Popp).

The development of primary astroglial cell cultures promises to provide a valuable means for studying their specific properties under a variety of conditions. We have studied the types of cells present in such cultures using immunocytochemical methods employing antisera prepared against glial fibrillary acidic protein (GFAP), a specific marker for astrocytes, and against rat plasma cold-insoluble globulin (CIg). The latter antiserum cross-reacts with cell surface fibronectin. Meninges were removed from the brains of 1-3 day old rats and dissociated-cell cultures were prepared from the cerebral hemispheres essentially by the method of Booher and Sensenbrenner (Neurobiol. 2:97,.1972).

Two major types of cells were observed in cultures which had been maintained for 4 to 28 days. Cells of the first type, which are predominant, stain positively with antiserum against GFAP but do not stain for fibronectin when both antigens are localized simultaneously. GFAP-positive cells comprise 70-90% of the total number of cells found in such cultures. Such cells are typically broad, flat, and polygonal in shape and in confluent cultures are mainly present in large patches. Some of these cells undergo a morphologic transformation when exposed to 0.1mM norepinephrine, developing numerous radially-oriented processes and closely resemble astrocytes as seen in vivo; 95% of such processing-forming cells stain intensely for GFAP. However, 75% of the cells which show no morphologic response to norepinephrine also stain for GFAP.

Cells of the second type do not stain for GFAP but show surface staining for fibronectin. In confluent cultures these cells are typically spindle-shaped and are present in densely-packed "colonies" which appear as "sheaves of wheat". Cultures prepared from meninges, or cultured neurons prepared from the rat hippocampus, showed no staining for GFAP. Cells derived from the meninges do show intense surface staining for fibronectin.

These results indicate that astrocytes, as identified by the presence of GFAP, are the predominant cell type in these cultures and that such cells do not contain detectable fibronectin. A smaller number of other cells which do not contain detectable GFAP but which do contain fibronectin are also present. Techniques to reduce or eliminate the fibronectin-positive cell population are currently under study. Supported by NINCDS grant NS13042, NIH SO7-RR05394 and by a grant from the Sinsheimer Fund.

2560 ELECTRICAL PROPERTIES OF ANEURALLY CULTURED ADULT HUMAN MUSCLE
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Histochemical, ultrastructural and biochemical studies of aneurally cultured diseased human muscle have proved useful in elucidating the pathogenesis of neuromuscular disorders. Electrophysiological study of cultured diseased human muscle may also prove useful for investigating neuromuscular disorders characterized by electrical abnormalities. In order to define the electrical properties of the cultured adult muscle fibers and to compare their properties to normal innervated adult human muscle, six biopsies from five patients were cultured according to our established techniques (Neurol. 25:58-67, 1975). The individual cultures were examined daily by phase contrast microscopy. When the muscle fibers became well-differentiated and cross-striated, after 21-28 days of growth, they were selected for study. Spontaneous, slow, tonic-like contractions were visible in only one culture.

For electrophysiological studies, the cultures were placed in a perspex chamber mounted on the stage of an inverted phase contrast microscope. They were maintained at 37°C in a water-saturated 95% air 5% CO_2 atmosphere. Recording and stimulating electrodes were inserted intracellularly. The grouped values (Mean ± S.D.) for 34 fibers from all 6 cultures were: a) Resting membrane potential (Vm) of 52.4 ± 6.6 mV. This was approximately 30 mV lower than the mean reported from several studies of adult innervated human skeletal muscle. b) Input resistance of 5.5 ± 3.4 MΩ. This value was much higher than those reported for adult muscle fibers. c) Only two of 34 fibers were electrically excitable at Vm. These fibers were present in the only culture in which spontaneous contractions were visible. d) When the fibers were hyperpolarized to 80 mV, an action potential could always be elicited. The threshold for excitation was 22.6 ± 8.7 mV and the action potential amplitude was 83.4 ± 28.9 mV.

These data indicate that aneurally cultured adult human muscle fibers are electrically excitable. The membrane characteristics are different from those of innervated adult human muscle in vivo. These studies demonstrate that the electrophysiological investigation of cultured adult human muscle may prove useful in analyzing the influence of neural factors on the electrical characteristics of normal muscle as well as the electrical characteristics of diseased muscle.

2561 EFFECT OF NERVE GROWTH FACTOR ON UPTAKE OF ^{3}H-DOPAMINE AND SENSITIVITY TO 6-HYDROXYDOPAMINE TOXICITY IN A HUMAN NEUROBLASTOMA CELL LINE.
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The dopamine (DA) analogue 6-hydroxydopamine (6OHDA) is selectively toxic to cells of sympathetic neuronal origin as well as to certain non-neuronal cell lines. Cytotoxicity has been linked to higher uptake of catecholamines by sensitive cells than by resistant cells. Nerve growth factor (NGF) protects cells from 6OHDA toxicity both in vivo and in vitro by an unknown mechanism. There appear to be two mechanisms by which cells accumulate and bind DA: a low capacity system requiring DA concentrations of 10^{-8}M; and a high capacity system requiring 10^{-4}M DA. The high capacity system involves non-specific diffusion and binding of DA oxidation products within the cell. Since 6OHDA exerts its cytotoxic effect only at high concentrations, it is presumed that most of its incorporation occurs via the high capacity mechanism. However, cocaine, which blocks dopamine uptake, confers a small degree of protection from toxicity on human neuroblastoma cells in culture. The mechanism blocked probably corresponds to the low capacity system. We tested the hypothesis that NGF, which also protects the cells to a slight extent, acts in part by inhibiting the low capacity uptake system.

We studied the neuroblastoma clonal cell line SK-N-SH-SY5Y (SY5Y) which is very sensitive to 6OHDA toxicity, and which becomes less sensitive when treated with NGF. Uptake of ^{3}H-dopamine (^{3}H-DA) in untreated SY5Y cells was compared to that of cells treated with NGF or cocaine, as well as to ^{3}H-DA accumulation by a fibroblast-like cell line A_1B_1, which is more resistant to 6OHDA. Line A_1B_1 was cloned from the human glioma line U-251-MG. Uptake was measured by liquid scintillation counting of solubilized cells. ^{3}H-DA was added to cultures in serum-free F-12 medium, containing 20µg/ml ascorbic acid, at a concentration of 1µCi/ml (4 x 10^{-8}M), of which only 0.2 - 0.4% was taken up by the cells. The level of uptake plateaued within 30 minutes. Cocaine (9 x 10^{-6}M) blocked 23% of the total uptake in SY5Y cells indicating that most of the uptake is non-specific. Furthermore, A_1B_1 cells accumulated twice as much ^{3}H-DA as SY5Y cells. NGF was observed to increase the level of ^{3}H-DA uptake in SY5Y cells by 30%. We conclude that low capacity dopamine uptake is not a factor in the sensitivity of SY5Y cells to 6OHDA toxicity.

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2562 MYELINATION IN ROTATION-MEDIATED AGGREGATING CELL CULTURES.
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Myelination was studied in aggregating cell cultures of mechanically dissociated 15-16-day fetal rat brain utilizing ultrastructural, biochemical and immunocytochemical methods. Myelination began at or shortly before 15 days in vitro. As in vivo, the first evidence of myelination was the appearance of myelin basic protein (MBP) in oligodendroglia. At 15 days in vitro, MBP antiserum stained oligodendrocyte cytoplasm and a few cells extended stained processes which occasionally surrounded axons. Oligodendrocytes and axons surrounded by few myelin lamellae were identified ultrastructurally in 15-day aggregates. As the cells within the aggregates continued to differentiate the number of oligodendrocytes stained by MBP antiserum increased. By 25 days in vitro oligodendrocytes had extended numerous stained processes which were continuous with stained myelin sheaths. Ultrastructurally mature oligodendrocytes and myelinated axons were present. The periodicity of the myelin lamellae was similar to that found in vivo. Myelin can be isolated in sufficient quantities for biochemical analysis. The yield of myelin protein from 30-day aggregates was 0.9% of the protein in the initial homogenate. The specific activity of the myelin associated enzyme, CNP, was 1,095 in the 30-day aggregate myelin. Myelin proteins from 30-day aggregates were compared to those from normal rat brain on 12.5% SDS slab gels. All the major myelin proteins were present in aggregate myelin. Although the amount of myelin synthesized in aggregating cell cultures was less than that found in vivo, the biochemical composition of this myelin (thus far analyzed) was similar to its in vivo counterpart. In summary, aggregating cell cultures provide a useful model system for multidisciplinary investigation of myelination in vitro.

2563 TISSUE CULTURE OF NORMAL ASTROCYTES FROM RAT OPTIC NERVE.
P.A. Trimmer*, P.J. Reier and T.H. Oh. Dept. of Anat., Sch. Med., Univ. of Maryland, Balto.,MD 21201

Previous studies of gliogenesis in the rat optic nerve have shown that most astrocytes are generated within the first week. Since it has also been reported that a preferential production of this cell can be induced by denervation during this time, the present study was undertaken to determine whether immature optic nerves could provide a favorable source of cells for the establishment of homotypic populations of astrocytes in tissue culture. Optic nerves were excised from 5-day-old, Sprague-Dawley rats. Following removal of the meningeal sheath, the nerves were incubated in collagenase. Subsequent mechanical dispersion yielded a low-density suspension of cells (1.0-4.0 X 10^5 cells/ml) which was plated on collagen-coated culture dishes. The cells were maintained in a solution of Dulbecco's Modified Eagle's Medium, horse serum and embryo extract in a humidified CO_2/air environment. During the first 5 days in vitro (DIV) two types of cells could be distinguished by phase contrast microscopy: (1) a population of small cells having a few attenuated processes emerging from their perikarya and (2) many flattened, polygonal cells with a light cytoplasmic matrix. Between 7-14 DIV two basic cellular profiles could be identified in addition to the flattened cells which were still present. The first (Type I) was characterized by a pyramidal or bipolar perikaryon from which two or more processes emerged and extended radially for considerable distances. The second (Type II) cell exhibited a small, round perikaryon with numerous processes which terminated near the cell soma. By 20-40 DIV Type I cells represented 60%-80% of the culture population, and the processes of this cell formed a complex meshwork over the collagen surface. Examination of these cultures with the electron microscope showed that the Type I cell and its processes contained numerous fibrillar bundles, characteristic of astrocytes. A few, mature oligodendrocytes (probably represented by the Type II cell) were also distributed randomly within the culture. As seen in vivo, several gap junctions were established between overlapping astrocytic processes and glial growth cone profiles were also apparent. It has thus been possible to establish from immature optic nerves an enriched population of well-developed, viable astrocytes which can be maintained as a primary culture for extensive periods. Since astrocytes represent a major reactive component following axonal injury within the CNS, development of a suitable culture system can permit more direct study of the influence of this cell on neuritic elongation.

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2564 PURIFIED NEURONAL CULTURES FROM GANGLIONIC AND CENTRAL NERVOUS TISSUES OF SEVERAL SPECIES BY USE OF A DEFINED, SERUM-FREE MEDIUM. Silvio Varon, Stephen D. Skaper*, Ruben Adler, Jane Bottenstein and Gordon Sato*. Dept. Biol., Sch. Med., UCSD, La Jolla, CA 92093.

Cultivation of neural cells in synthetic media routinely requires serum supplementation and, sometimes, trophic factors. The addition of serum results in several problems, not the least of which is proliferation of nonneuronal cells. The eventual formation of a confluent monolayer of nonneurons in these cultures limits their usefulness in neurobiological studies. Also, simplification of medium composition to recognizably essential elements would eliminate the complex and relatively undefined nature of serum. A mixture of defined ingredients (insulin, transferrin, progesterone, putrescine and selenium) has recently been formulated which supports proliferative growth of rat neuroblastoma clonal cells in serum-free medium (J.E. Bottenstein and G. Sato, Proc. Natl. Acad. Sci. 76: 514, 1979).

In the present studies, dissociated embryonic chick dorsal root ganglionic cells were plated on collagen-coated culture dishes in medium containing 10% fetal calf serum. After allowing 48 hr for adequate cell attachment, the cultures received fresh medium supplemented with 10% serum or serum-free defined medium (N_1), which comprised insulin, transferrin, progesterone, putrescine and selenium. Nerve Growth Factor was also required in both media. The N_1 medium selectively maintained the neurons and did not support proliferation or even survival of nearly all nonneuronal elements. Survival of neurons in N_1 was initially as good and eventually better than in serum-containing medium. After 6 days in N_1 the cultures consisted almost entirely of neurons (>95%).

The N_1 medium was also used to culture cells from a variety of chick embryo central nervous system tissues (optic lobe, neural retina, spinal cord, telencephalon), and fetal or neonatal rodent. As with the chick sensory neurons, N_1 supported the survival of fiber-bearing cells (features typical of cultured neurons), while proliferation and in large part survival of flat cells were always suppressed. This, then, may provide a general way to obtain purified subpopulations of process-bearing, neuronal-like cells. (Supported by NINCDS grants NS-07606 and NS-12893).

2565 A FACTOR, DISTINCT FROM NERVE GROWTH FACTOR, THAT, LIKE NERVE GROWTH FACTOR, INDUCES INCREASED ADHESIVENESS AND MORPHOLOGICAL DIFFERENTIATION IN ANAPLASTIC GLIOMA AND PHEOCHROMOCYTOMA CELLS. Stanley A. Vinores* and Gordon Guroff. NIH, Bethesda, MD. 20205.

Nerve growth factor (NGF) induced increased cell-to-plastic and cell-to-cell adhesivesness in F98 rat anaplastic glioma cells. Increased adhesiveness could be observed at NGF concentrations as low as 1 ng/ml. Since malignancy is often associated with a decrease in cellular adhesiveness, this could explain, at least in part, the decreased tumor growth rate observed in anaplastic glioma-bearing rats treated with NGF (S. Vinores and A. Koestner, J. Neuropath. Exp. Neurol., 37: 704, 1978; Fed. Proc. 38: 1269, 1979). Conditioned medium from the NGF-secreting tumor cells, C-6 rat glioma and S-180 mouse sarcoma, also increased the adhesiveness of these cells, but this response was unaffected by anti-NGF IgG. Conditioned medium also induced adhesiveness in PC12 rat pheochromocytoma cells, and this was reduced by anti-NGF IgG. Conditioned medium collected from C-6 cells that were pretreated with 17β-estradiol, which stimulates increased NGF secretion, induced the highest degree of adhesiveness observed in both F98 and PC12 cells and this was unaffected by anti-NGF IgG. Insulin, epidermal growth factor, anti-NGF IgG, cytochrome-C, bovine serum albumin, dibutyryl-cAMP, and conditioned media from PC12 and IMR-32 human neuroblastoma cells did not increase adhesiveness. The factor inducing these effects is non-dialyzable, heat sensitive, and ammonium sulfate precipitable, and its secretion appears to be stimulated by 17β-estradiol. Supplementing conditioned medium with control medium produced an even greater increase in adhesiveness than did 100% conditioned medium, suggesting that factors depleted from control medium by growing cells also are necessary for maximum adhesiveness. As little as 1% conditioned medium had some effect on adhesiveness.

The processes induced by NGF in PC12 cells in vitro were longer and more filamentous than those induced by conditioned media. PC12 cells treated with conditioned medium also appeared flatter than NGF-treated cells. In F98 cells, the processes induced by NGF take longer to appear than those induced by conditioned media. None of the in vitro morphological responses to conditioned media in PC12 or F98 cells were neutralized by anti-NGF IgG. The data indicate that both NGF and certain conditioned media increase adhesiveness and induce morphological differentiation in F98 anaplastic glioma and PC12 pheochromocytoma, and that factors in addition to and other than NGF are active in the conditioned media.

2566 ION DEPENDENCY OF NERVE GROWTH FACTOR ACTION ON DORSAL ROOT GANGLIA. Norman R. West and Robert W. Stach. Depts. Anat. and Biochem., SUNY Upstate Med. Ctr., Syracuse, NY 13210.

Embryonic sensory neurons from 8 to 10 day chick embryos have an absolute requirement for the nerve growth factor (NGF) to survive and grow fibers in culture. We have shown that the inorganic cations magnesium and potassium are also necessary for the survival of these cells. On the other hand, calcium is not needed for the survival of these neurons, but is needed for these neurons to grow fibers. Our culture medium is simply Gey's balanced salts solution (GBS), (Amer. J. Cancer 27, 45-76, 1936) with various concentrations of NGF, 0.1 to 300 ng/ml, added. Therefore, it is an easy matter to remove a particular cation from the medium and determine what effect that cation has on the biological activity of NGF. When ganglia are cultured in potassium free GBS with various concentrations of NGF for 24 hours, there is no fiber outgrowth and 57% of the ganglia are floating. If normal GBS, containing the same concentrations of NGF, is now readded to the cultures and incubation is continued for an additional 48 hours, there is still no fiber growth and 71% of the ganglia are floating. Using the same paradigm, after 24 hours of incubation in magnesium free GBS containing various concentrations of NGF, there is no fiber growth and 49% of the ganglia are floating. With readdition of normal GBS and an additional 48 hours of incubation, there is no fiber growth and 67% of the ganglia are floating. Using floating ganglia and no fiber outgrowth as criteria for dead cells, our results show that potassium and magnesium are necessary for sensory neurons to survive. When calcium free GBS (CFGBS) is used as the culture medium, there is no fiber growth after 24 hours of incubation with various concentrations of NGF. However, only 5% of the ganglia are floating which is equivalent to control GBS cultures. Also, upon readdition of GBS, containing various concentrations of NGF, there is a normal fiber outgrowth at the normal concentration of NGF and again only control numbers (12%) of ganglia are floating. In GBS the biological activity of NGF is 6.9 ± 3.6 ng/ml. Upon readdition of GBS to the CFGBS cultures and an additional 48 hours of incubation, the biological activity of NGF is 4.6 ± 1.4 ng/ml. These results show that calcium ions are not needed for sensory neurons to survive but are needed for the neurons to grow fibers. The results obtained for the CFGBS can not be explained by low levels of calcium being present since the concentration of free calcium ions is below 5×10^{-11} M.

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TROPHIC FUNCTION

2567 LONG-TERM ALTERATIONS OF ELECTROTONIC SYNAPSES, Anderson, T.E., Bittner, G.D. and Ballinger, M.L.*, Dept. of Zoology, University of Texas, Austin, TX 78712.

Adjacent single lateral giant axon segments (SLGA) in the crayfish (Procambarus clarkii) CNS are coupled by septate electrotonic junctions in the abdominal ganlia. Following injury to an SLGA (cutting the segment in the connective rostral to the ganglion) the resistance across the septum in the posterior ganglion increased more than seven-fold, while input resistances for both intact and lesioned axonal segments remained at normal levels (Asada & Bennett, J. Cell Biol. 49:159). Ultrastructural studies revealed a complete absence of previously abundant gap junctions in these septa (Pappas, Asada & Bennett, J.Cell Biol. 49:173). However, these studies examined only short-term (1-6 hr) changes in lesioned segments in contact with their original cell body.

The distal portion of an SLGA is capable of surviving for up to one year, with no discernible hypertrophy of surrounding glia (Bittner et al, J. Exp. Zool. 180:13). However, if an SLGA is double cut, the segment survives less than 7 days, again with no glial hypertrophy (Meyer & Bittner, Br. Research 143:195). It thus appears that septal connections are important for long-term survival of axonal segments of SLGA's separated from their cell bodies, raising the possibility that the initial separation and loss of gap junctions might reverse over time to allow direct passage of trophic substances through pore-like structures found at the junctions. We have found that gap junctions made with the distal SLGA segment, anterior to the cut, disappear shortly after cutting the axon, and further, that they are essentially absent even 100 days post-operatively. Clear vesicles 400-800 A^0 in diameter remain abundant however. Associated with ultrastructural absence of gap junctions there is a maintained increase in the septal resistance, although the input resistance for both intact and severed SLGA are within normal limits. Resistance changes are observed at septa both anterior and posterior to the cut initially, but resistance posterior returns to normal within 10 days. Following nerve cord ligation, increased resistance is found only at the anterior septum. Thus, in spite of apparent transfer of trophic substances from intact to isolated SLGA, electrotonic connections are not re-established. The most likely hypothesis for long-term survival of severed SLGA segments is an exocytotic-pinocytotic transfer involving the clear vesicles.
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2568 ACCELERATED DEGRADATION OF JUNCTIONAL α-BUNGAROTOXIN-ACETYLCHOLINE RECEPTOR COMPLEXES IN DENERVATED RAT DIAPHRAGM. R.S. Brett and S.G. Younkin (SPON: L.H. Younkin). Dept. of Pharmacol., Case Western Reserve Univ. Sch. of Med., Cleveland, Ohio 44106.

The AChR in innervated and 5-day denervated left hemidiaphragms were labelled in vivo by injecting 1.0 μg of ^{125}I-αBT into the thoracic cavity of 250-350 g male Wister rats and maintaining them in a vertical position overnight. Virtually all animals survived this procedure which labelled about 35% of the AChR in the left hemidiaphragm. Toxin binding was measured 5 days after labelling. Because of the rapid degradation of extrajunctional toxin-receptor complexes essentially all of the toxin remaining bound to muscle 5 days after labelling is associated with the more slowly degraded junctional AChR. Denervation had a dramatic effect on junctional toxin-receptor complexes; the toxin bound to denervated hemidiaphragms was only 21% of that bound to innervated hemidiaphragms ($p < 0.0005$ by Student's t test). The result of this experiment could be partially or completely due to a denervation-induced increase in the rate of degradation of junctional AChR. We therefore measured toxin-receptor complexes in innervated and 5-day denervated left hemidiaphragms 1, 3, 5 and 8 days after the labelling procedure (i.e., 6, 8, 10 and 13 days after denervation). The loss of toxin from the endplate-containing area of innervated hemidiaphragms occurred with an apparent half-time of 11 days. The loss of toxin from the endplate-containing area of 5-day-denervated hemidiaphragms was much more rapid, occurring with an apparent half-time of 2 days ($p < 0.005$ by Student's t test). This result indicates that the normal metabolic stability of junctional toxin-receptor complexes is reduced during the period between 6 and 13 days after denervation. Because of the uncertainty inherent in the estimation of receptor half-life in vivo we cannot establish whether the increased rate of degradation accounts entirely for the decrease in toxin binding seen 5 days after labelling. The influence of nerve stump length on the denervation-induced change in junctional toxin-receptor complexes was examined. The toxin-binding to areas of muscle near the point of nerve entry (short stump) and areas far from the point of nerve entry (long stump) were compared in innervated and 2-, 5- and 8-day denervated hemidiaphragms. Animals were sacrificed 5 days after the labelling procedure (i.e., at 7, 10 and 13 days after denervation) when virtually all of the extrajunctional toxin-receptor complexes had been degraded. The decrease in ^{125}I-αBT bound to denervated hemidiaphragms occurred earlier in proximal (short stump) segments of the muscle. The fact that the denervation-induced alteration of junctional toxin-receptor complexes is influenced by nerve-stump length indicates that it is at least partly independent of the loss of muscle activity associated with denervation.

2569 PROPERTIES OF SOLEUS MUSCLE AND OF INDIVIDUAL SOLEUS MUSCLE UNITS AFTER CROSS-INNERVATION BY FDL MOTONEURONS. R. E. Burke, R. P. Dum, M. J. O'Donovan*, J. Toop*, and P. Tsairis. Lab. of Neural Control, NINCDS, NIH, Bethesda, MD 20205 and Dept. of Neurology, Cornell U. Med. Sch., New York, NY 10021.

We studied the histochemical and physiological properties of homogeneous "slow" soleus (SOL) muscles in adult female cats 9 - 10 months after re-innervation by heterogeneous, mainly "fast" flexor digitorum longus (FDL) motoneurons. Individual motor units were isolated with intracellular methods, enabling precise identification of motoneurons as originally FDL. Data from cross-innervated (XIN) muscles/motor units were compared with results from self-innervated (SI) SOL and from size-matched normal cats (NL SOL; Burke et al. J. Physiol. 238:503, 1974). XIN SOL muscles were close to normal in size, weight, force output and gross appearance when the FDL to SOL nerve anastomosis was mechanically intact, in contrast to XIN FDL (see Dum et al., this meeting). In all XIN SOL, over 90% of fibers had histochemical profiles identical to NL SOL fibers (type I); the minority fiber type was IIA (NL SOL can contain up to 4-5% type IIA fibers). However, the whole XIN SOL twitch times ranged from 55 to 65 msec (mean 61 msec; N = 4), versus 106 - 150 msec (mean 119; N = 4) for SI SOL and 99 - 186 msec (mean 140 msec; N = 14) for NL SOL. In one case, both SOL and FDL axons reinnervated a SOL muscle, giving a twitch time of 61 msec when FDL axons were stimulated versus 108 msec with SOL axons (tension deficit with simultaneous tetanization was only 18%, suggesting little dual fiber innervation). There was no detectable histochemical mosaic in ATPase staining at any pH, although the FDL-innervated fibers appeared to have lower neutral fat content (inferred since 1 unit glycogen-depleted from an FDL motoneuron had low fat in Oil Red O stain). We sampled a total of 17 SOL muscle units innervated by identified FDL motoneurons (4 were also studied histochemically by glycogen depletion). All 17 were type S and resembled NL SOL muscle units except for faster twitch contraction times. Range and mean (paren.) values for relevant properties of NL SOL muscle units and NL FDL motoneurons are given below (PTD = post-tetanic depression of twitch). The heterogeneous FDL motoneurons appear to reinnervate SOL muscle readily to produce typical type S muscle units with faster contraction times and less PTD than normal SOL units.

MOTOR UNITS IN:	NL SOL (S)	XIN SOL (S)	NL FDL (all)
Tw time (msec)	64 - 131 (97)	37 - 80 (53)	
Tw/ Tet.	0.16 - 0.41 (0.26)	0.05 - 0.44 (0.18)	
PTD incidence	10/26	1/16	
C. V. (m/sec)		64 - 111 (83)	72 - 111 (97)
AHP (msec)		42 - 71 (58)	32 - 68 (45)

2570 NERVE DEGENERATION CONTRIBUTES WITH INACTIVITY TO THE CHANGES OCCURRING IN MUSCLE AFTER DENERVATION. A. Cangiano and L. Lutzemberger*. Istituto di Fisiologia, Università di Pisa, Italy.

Overwhelming evidence indicates that factors different from nerve impulses are involved, together with inactivity, in the development of the membrane modifications occurring in muscle after denervation. One line of evidence shows, for instance, that pure muscle inactivity is less effective than denervation in producing spike resistance to tetrodotoxin and fibrillation or extrajunctional acetylcholine receptors. Lack of a hypothetical "neurotrophic" factor could be involved, although an alternative possibility is represented by the release of products of nerve degeneration between the muscle fibres. We have now obtained data in support of the latter interpretation with experiments of partial denervation of rat EDL and soleus muscles whose nerves were in addition subjected to impulse conduction block with tetrodotoxin containing cuffs for 2-3 days. We found that junctional and extrajunctional spike resistance to tetrodotoxin (TTX) as well as extrajunctional sensitivity to acetylcholine (ACh) developed virtually to the same extent in the denervated and in the adjacent innervated, but impulse-blocked fibres. This was in striking contrast with the behaviour of impulse-blocked fibres of control muscles not containing denervated fibres which, at this early time, showed little or no membrane changes. These results appear difficult to reconcile with the hypothesis of "neurotrophic" factors contributing with activity to the control of muscle membrane properties since such factors should not be missing from the impulse-blocked but innervated fibres of the partially denervated muscles. The results are on the other hand consistent with the interpretation that nerve degeneration consequent to denervation sets free the action of some factor that summates with inactivity in producing high values of ACh hypersensitivity and TTX resistance.

2571 MEMBRANE PROPERTIES OF THE SKELETAL MUSCLE AFTER BLOCKADE AND RECOVERY OF FAST AXONAL TRANSPORT. S. S. Deshpande*, R. J. Boegman, and E. X. Albuquerque. Dept. Pharm. & Exptl. Therap., University of MD, Sch. Med., Baltimore, MD 21201.

A single subperineural injection of batrachotoxin (BTX, 9.3 pmoles) into the peroneal nerve of rats causes immediate block of fast axonal transport and subsequent membrane depolarization of the surface fibers of the extensor digitorum longus (extensor) muscle. The onset of membrane depolarization is dependent on the site of BTX injection - the more distal the site, the earlier the onset (Neurosci. Abs. 4: 1978). BTX injection into the peroneal nerve (about 10 mm from the nerve's entrance into muscle) in female Wistar rats paralyzed the limb only for 3–4 days. The fast axonal transport in the nerve was determined by measuring migration of ^{3}H-leucine labelled proteins and showed complete recovery at day 7 at a time when the extensor muscle was still depolarized by about 14 mV. By day 22 the resting membrane potential (RMP) returned to near control levels (–76 mV vs. –79 mV). Between 4 and 14 days extrajunctional sensitivity to microiontophoretically applied acetylcholine was high (200–400 mV/nC) and spread over the entire surface of the muscle fiber. In addition, muscle action potentials were resistant to TTX (3 μM). Spontaneous miniature endplate potentials (mepps) and neurally elicited action potentials in muscle were totally absent up to day 7 but these showed gradual recovery by day 22 however, when significant recovery in RMP had occurred the mepp frequency was still lower than the control (1.27 ± 0.29 sec^{-1} vs. 2.40 ± 0.22 sec^{-1}). Muscle atrophy was prominent between day 4 and 14. Collateral sprouting was not observed after BTX injection. In addition, after degeneration of the distal stump produced by BTX, the newly grown nerve terminal reinnervated the former endplate. It is concluded that in spite of the early clinical recovery of the affected limb and its use in locomotion, the membrane properties of the extensor muscle do not return to control levels for at least 3 weeks after a single subperineural injection of BTX. The delayed recovery in RMP and of the physiological properties of the neuromuscular synapses were most likely a consequence of degenerative processes such as proliferation of Schwann cell (which is known to occur following denervation) interposed between pre- and postjunctional membrane. Apparently in spite of the recovery of the fast axoplasmic transport, the pattern of muscle recovery is similar to that seen after transection of nerve (Supported by USPHS Grants NS-12063, and funds from Muscular Dystrophy Association and the Paralyzed Veterans of America Inc..)

2572 EFFECTS OF DENERVATION AND REINNERVATION ON CHOLINERGIC ENZYMES IN SCIATIC NERVE, SLOW AND FAST MUSCLE OF RAT. Wolf-D. Dettbarn. Dept. Pharmacol., Sch. Med., Vanderbilt Univ., Nashville, TN 37232

The changes induced by loss and reestablishment of functional connections between nerve and muscle offer a suitable model for studying the mechanisms that control nerve and muscle enzymes such as acetylcholinesterase (AChE) and choline acetyltransferase (CAT). A difference in the rate of loss and recovery of these enzymes may give us insight into the mechanisms by which these enzymes are controlled in muscle. The sciatic nerve was crushed in the mid-thigh region, and 1-6 weeks following the surgery, the loss and recovery of function and the changes in activity of AChE and CAT in fast extensor digitorum longus muscle (EDL) and in the slow soleus muscle (SOL) were compared with those in sciatic nerve segments (1 cm long), one proximal (PN) and one distal (DN) adjacent to the crush. Enzyme activities were calculated as activity/muscle or unit length (cm) of nerve. Within 24 hours after nerve crush, neuromuscular transmission was blocked. Maximal loss of AChE and CAT activity was seen by the end of the second week. AChE activity in EDL, SOL and DN was reduced to 15-25% of control while the loss of AChE in PN was 50%. CAT activity was reduced to 40% in EDL, 20% in SOL, 25% in DN and to 80% of control in PN. During the third week, functional reinnervation of individual muscle fibers was observed and full innervation was reestablished by the end of the sixth week. During this period, AChE activity in SOL recovered rapidly, with maximum enzyme activity at 4 weeks after crush (200% of control) returning to control values by the end of the sixth week. AChE activity in EDL, DN and PN recovered at a much slower rate than that of SOL. None showed an increase over control activity nor was AChE activity fully restored during the observation period. By the end of the third week, CAT in PN had reached control activity, while in EDL (80%), SOL (60%) and DN (80%), CAT activity was still below the control value. The data indicate that recovery of AChE and CAT in regenerating nerve and self-reinnervated fast EDL proceed at a similar rate, indicating that the motor nerve has some regulatory function. In slow SOL, however, AChE activity recovers at a much faster rate than in the PN and DN leading to a temporary increase of AChE activity (200%) over that of the normal control muscle. The direct influence of the nerve does not seem to be the only factor controlling the recovery of AChE in reinnervating muscle. The observed difference may in part result from the difference in muscle fiber types in these two muscles.
(Supported by NIH Grant #NS-12438 and a grant from the Muscular Dystrophy Association of America, Inc.)

2573 THE PROPERTIES OF WHOLE FDL MUSCLE AND OF INDIVIDUAL FDL MUSCLE UNITS AFTER CROSS-REINNERVATION BY SOLEUS MOTONEURONS IN CAT. R. P. Dum, R. E. Burke, M. J. O'Donovan* and J. Toop*. Lab. of Neural Control. NINCDS, NIH, Bethesda, MD 20205.

The properties of the heterogeneous "fast twitch" flexor digitorum longus (FDL) muscle and of individual muscle units were studied 7 to 12 months after reinnervation by motoneurons of the homogeneous "slow twitch" soleus (SOL) muscle, using adult female cats. Intracellular methods were used to isolate single motor units and to identify their motoneurons as belonging to the SOL nucleus. Data from cross-innervated (XIN) muscles were compared with contralateral FDL, self-reinnervated (SI) FDL, and with results from size-matched normal cats (NL FDL; see Dum et al., Neurosci. Abstr. 4:294, 1978). XIN FDL muscles varied between cats (3/11 were very small with atypical histochemistry but apparent functional innervation), but tetanic force/gram wet weight was normal in 7/11. Histochemical fiber compositions in XIN FDL also varied but in the "best" cases, over 95% of the muscle was type I (low ATPase) with uniformly high oxidative staining. Further studies of myosin subtypes are in progress.

WHOLE MUSCLES	WET WT.(gm)	TET. TENS. (kg)	TW TIME (msec)
Normal & SI FDL	0.8 - 1.3 (1.0)	1.5 - 2.1 (1.7)	25 - 40 (33)
XIN FDL	0.2 - 0.7 (0.5)	0.08 - 1.0 (0.6)	34 - 137 (76)

We studied 32 motor units in 11 XIN FDL muscles, some of which were also studied histochemically by glycogen depletion. All were type S by physiological criteria and resembled NL SOL units in having a high incidence of post-tetanic twitch depression (PTD). The table below shows a comparison of properties (C.V. = axonal conduction velocity; AHP = afterhyperpolarization duration) for normal (NL) FDL type S, normal SOL type S and XIN FDL (FDL muscle units innervated by SOL motoneurons) motor units. Ranges are shown, with mean values in parentheses.

MOTOR UNITS:	NL FDL (S)	XIN FDL (S)	NL SOL (S)
Tw Time (ms)	34 - 81 (56.2)	33 - 82 (56.7)	
Tw/Tet	0.2 - 0.7 (0.4)	.06 - 0.6 (0.3)	
PTD incidence	1/10	12/29	10/26
C. V. (m/sec)		63 - 85 (73)	58 - 91 (72)
AHP (msec)		69 - 105 (85)	75 - 134 (93)

Re-innervation of the FDL muscle by SOL motoneurons in adult cats produced variable results, but at best there was evidence for significant "conversion" of muscle units from the predominant normal "fast" types (FF and FR) to a slow type with similarities to the S units of normal SOL. We found no evidence for reorganization of group Ia synaptic connectivity patterns.

2574 BENEFICIAL EFFECT OF WATER-DEPRIVATION ON MUSCULAR DYSTROPHY OF THE CHICKEN. Richard K. Entrikin, Judith A. Mouritsen*, Gary T. Patterson and Barry W. Wilson. Depts. Avian Sci., Pharmacol., and Phys. Med. & Rehab., Univ. California, Davis, CA 95616.

Drugs that beneficially affect hereditary muscular dystrophy of the chicken often decrease body weights. The possibility that the dystrophy might be altered by water and food utilization was tested by depriving dystrophic chickens (UC Davis line 413) of water and/or feed for periods of up to 96 hours beginning on day 33 ex ovo. At this age dystrophic chickens exhibit decreased righting ability, abnormal electromyographic (EMG) activity, increased plasma creatine kinase (CK) levels, increased acetylcholinesterase (AChE) levels in plasma and muscles, and histopathological changes in fast-twitch muscles.

Data below are from a 72-hour study of normal control (412C), dystrophic control (413C), water-deprived dystrophic (413 -H_2O), and feed-deprived dystrophic (413 -feed) chickens at 37 days ex ovo. Values are means ± SEM (8-10 birds per group) of exhaustion scores (ES, consecutive number of times a bird can rise from the supine position during a single trial), plasma CK (mU/ml), and body weights (Wt, grams).

	ES	CK	Wt
412 C	22.5 ± 1.2	173 ± 11	534 ± 13
413 C	1.0 ± 0.4	5370 ± 525	549 ± 23
413 -H_2O	8.7 ± 2.0	466 ± 109	348 ± 23
413 -feed	0.6 ± 0.2	1399 ± 104	322 ± 14

Water-deprivation increased righting ability nearly 9-fold and decreased plasma CK levels by about 90%. Feed-deprivation reduced body weights to the same extent as water-deprivation, but had less effect on CK and no beneficial effect on righting ability. Further studies showed that water-deprivation also reduced abnormal EMG activity and AChE levels. Pectoralis major muscle fibers of water-deprived dystrophic birds had smaller diameters and less-rounded, "more normal" angular shapes. Although water-deprivation was shown previously to decrease muscle stiffness in congenital myotonia of the goat (Hegyeli and Szent-Gyorgyi, Science 133:1011, 1961), it alleviated symptoms of the avian dystrophy that are independent of myotonia. The magnitude and scope of the changes brought about by water-deprivation exceed those of any drug yet studied in the dystrophic chicken. A mechanism revealed by water-deprivation might explain the beneficial effects of chemically-dissimilar drugs such as phenytoin, methysergide, and penicillamine on the avian dystrophy. (Supported by the Muscular Dystrophy Association, Inc.)

2575 NERVE GROWTH FACTOR RECEPTORS ON CHICK SYMPATHETIC GANGLION CELLS. Earl W. Godfrey and Eric M. Shooter. Dept. Neurobiol., Stanford Univ. Sch. Med., Stanford, CA 94305.

It has previously been shown (Sutter et al., Soc. Neurosci. Abstr. 3:461, no. 1475(1977); J. Biol. Chem., in press) that sensory ganglion neurons from chick embryos have two distinct high-affinity binding sites for nerve growth factor (NGF) on their cell surfaces. One class of receptors (site I) had a K_d of about 10^{-11} M; the other (site II) had a K_d of about 10^{-9} M. Both steady-state and kinetic analyses showed these two classes of receptors did not interact in a negatively co-operative manner. The site II receptors were already present on the surface of sensory cells at day 4 of incubation, whereas the site I receptors first appeared between days 5 and 6. Several lines of evidence indicated that the neurite outgrowth response to NGF was mediated by site I receptors.

These studies were extended to the cells of the chick embryo sympathetic ganglion. The NGF receptors were measured using the specific binding of ^{125}I-βNGF as an assay. Steady-state analysis of binding to dissociated cells at 37° revealed two distinct sites with K_d's of about 10^{-11} and 10^{-9} M. The kinetics of association and dissociation of NGF and its receptor appear similar to those seen with the sensory neurons. Sites I and II were about equally sensitive to inactivation or degradation by trypsin. No evidence for negatively co-operative interactions between the two sites was found. The development of the receptors has been followed, and both sites I and II were found on sympathetic cells from day 6.5 to day 20 of incubation. There were about 10 times as many site II as site I receptors per cell (as measured by steady-state analysis at 37°); this ratio did not change significantly from day 9 to day 15 of incubation. After several days in culture, non-neuronal cells from sympathetic ganglia exhibited high levels of site II receptors, but site I receptors have not yet been detected on non-neuronal cells. The NGF receptors on chick sympathetic ganglion cells seem, in most if not all respects, to be similar to those found on chick sensory ganglion cells.

2576 EFFECTS OF SOUND DEPRIVATION ON PRIMARY AUDITORY SYNAPSES IN THE COCHLEAR NUCLEUS OF THE ADULT GUINEA PIG. R.L.Gulley,D.Mattox* and F.Ulrich*Depts. of Anat.and Surg.(Div.Otolaryng.),Univ.of Texas Health Sci.Cntr.,San Antonio,Tx. 78284

Primary auditory terminals in the rostral anteroventral coch lear nucleus were studied in thin-sectioned and freeze-fractured material from animals maintained for 24 hours in a sound-reduced environment. All animals had their external auditory canal occluded with bone wax. Some animals were maintained under ketamine and urethane anesthesia for 24 hours to minimize animal-generated noise. Awake and anesthetized control animals were kept in ambient noise for 24 hours. In thin-sections,three changes are seen in the primary auditory terminals of sound-deprived animals. 1)In anesthetized sound-deprived animals few enlarged channels of extracellular space surrounding the active zone contain glial processes. In anesthetized and awake animals in ambient noise, many of the enlarged channels contain glial processes. In awake, sound-deprived animals the number of channels containing glial processes is reduced compared to control animals; however,some channels containing processes are present. These deeply invaginate the terminal,and the glial processes are located within the channel,deeply embedded in the terminal. Frequently the sides of the channel beneath the glial process are parallel and separated by a 20-30nm gap. 2)Numerous smooth elongated or crescentric-shaped cisternae appear within the terminal after sound-deprivation. These cisternae may be transitional forms in the formation of large double membrane-bounded sacs which are also common in the primary terminals of the sound-deprived animals.These sacs, measuring about 0.2μm in diameter, are sometimes filled with vesicles which are the same size and shape as the synaptic vesicles in the terminal.The cisternae and sacs may be related to an acceleration of a generalized remodeling process in the nerve terminal,since similar structures have also been observed in frog neuromuscular junctions subjected to various regimens of stimulation (K.Lynch,personal communication). 3) There is a decrease in the number of synaptic vesicles in the terminal.In freeze-fracture preparations of awake,sound-deprived animals, the number of non-aggregate particles in non-junctional regions of the postsynaptic membrane is over 130 particles /μm^2 compared to 53 particles /μm^2 in awake animals kept in ambient noise. Thus,in the auditory system, sound-evoked activity is apparently necessary for the maintenance of the presynaptic terminal and is a factor in the trophic regulation of the organization of intramembranous particles in the postsynaptic membrane.

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2577 AXONAL CHEMOTAXIS TO NERVE GROWTH FACTOR AND ITS POSSIBLE MEDIATION BY CYCLIC NUCLEOTIDES AND CALCIUM. Ross W. Gundersen* and John N. Barrett. Dept. Physiol. Biophys., Univ. Miami Sch. Med., Miami, FL 33101.

This present work reports a fast chemotactic response of dorsal root axons to β-Nerve Growth Factor (NGF) and attempts to determine the physiological events contributing to this response. A local gradient of NGF was applied, via a micro-perfusion system, to single growth cones of cultured chick dorsal root axons (8-13 days approximate emryonic age). All axons tested turned and began to grow up the NGF gradient within 9-21 min, without any detectable change in growth rate. This directed growth was observed when the micropipette source contained 2-50 biological units (BU) NGF/ml and the background level was 1 BU NGF/ml. Directed growth was not observed toward sources of fetal calf serum, bovine serum albumin, or sources duplicating the background concentration of NGF. A decrease in the number of axons turning toward a 50 BU NGF/ml source was observed when the background level of NGF was increased to 25 BU/ml. This oriented growth is not due to effects on growth rate, survival, fluid movement, or general response to any protein, but rather appears to be a chemotactic response to NGF which may involve saturable NGF receptors on the growth cone.

Dorsal root axons also turned and grew toward 1 mM sources of mono- and dibutyryl cyclic adenosine monophosphate, 1 mM cyclic guanosine monophosphate (c-GMP), phosphodiesterase inhibitors (caffeine, theophylline, Roche 20-1724), 1 mM cyclic adenosine monophosphate (c-AMP) in the presence of 10^{-7} M Roche 20-1724, and 20 mM calcium in the presence of the calcium ionophore A23187 (10^{-7} M). These results suggest that the physiological mediators of the chemotactic response to NGF may be increases in the intracellular levels of c-AMP and/or c-CMP, and free calcium. Supported by NIH grants NS 12207 and NS 07044.

2578 Neurofibromatosis: Evidence of Genetic Heterogeneity Supported by Nerve Growth Factor Alterations. WILLIAM R. KANTER*, Albany, New York, ROSWELL ELDRIDGE, and THELMA KOERBER*, Bethesda, Maryland

Neurofibromatosis is a complex hereditary syndrome which includes the common peripheral form and a central form recently documented by us. The latter is an autosomal dominant disorder whose hallmark is bilateral acoustic neuroma. There are generally mild skin changes as well consisting of 1 to 4 cafe-au-lait spots or subcutaneous neurofibromatosis.

Nerve Growth Factor (NGF) has been analysed in 9 affected with central neurofibromatosis and 22 at risk from 3 kindreds personally studied. The laboratories of Ned Boyer, Johns Hopkins and George Todaro, NCI, participated in the assays. NGF in serum was measured for antigenic activity by means of radioimmunoassay employing anti-mouse NGF antibody and for receptor binding by competition radioreceptor assay employing formalin fixed human melanoma cells. central

NGF antigenic activity was significantly elevated in those with neurofibromatosis while those at risk consisted of two groups, one whose NGF antigenic activity was normal and one whose activity was elevated. Receptor binding was normal or low. In contrast, receptor binding is reported elevated in peripheral neurofibromatosis while antigenic activity is normal or low.

On the basis of these clinical and biochemical observations, we suggest that central and peripheral forms of neurofibromatosis are closely related but distinct diseases that may be associated with separate alterations in NGF activity. These findings may help explain the "maternal effect" noted by us in central neurofibromatosis and by Miller and Hall in pheripheral neurofibromatosis. They may also lead to a method for screening individuals at risk, and suggest that mechanism responsible for the neoplastic and other changes common in this syndrome.

2579 NEUROTROPIC SUBSTANCE AS MEDIATOR FOR AXON-SHEATH CELL INTERACTION. H. Mei Liu*. (SPON. Igor Klatzo). Brown University, Providence, Rhode Island. 02906.

The present study aimed at defining the neurotropic mechanism between axon and sheath cell by in vitro and chemical methods. Cultures of sheath cells (Schwann cells) were established from sciatic nerves of chick embryos after 16 days of incubation and fed with serum-free medium for three days. The conditioned medium was subjected to bioassay with chick embryo dorsal root ganglia (DRG) and found to have nerve growth promoting activity (NGF action). Gel filtration of the conditioned medium with Sephadex G-200 revealed three major components with the NGF action. Affinity chromatography using Con-A Sepharose abolished the NGF activity in the conditioned medium while purified β NGF was not affected by the same treatment. These findings indicated that β NGF molecules secreted by sheath cells form a complex with the Con-A binding material in the conditioned medium. Chick embryo DRG placed at a distance of 2-3 mm away from explants of sheath cells showed accelerated neurite growth oriented towards the explants. Adhesion between neurites and sheath cells was observed. The cell recognition and adhesion appeared to be mediated by a Con-A binding, PAS and colloidal iron positive material on the sheath cell and axonal surfaces. The location of Con-A binding material corresponded precisely with the distribution of β NGF molecules on the cell membrane as revealed by indirect immunofluorescent technique (incubation with rabbit antiserum to β NGF followed by fluorescein conjugated goat antiserum to rabbit IgG). The above findings suggest that β NGF molecules synthesized by the sheath cells exist as a complex with glycoproteins on the cell membrane. The macromolecule, tentatively called "neurotropic substance" (NTS) is constantly shed into the microenvironment as a result of membrane turnover. This results in the presence of free NTS molecules in the conditioned medium. In the in vivo system, a diffusable gradient of NTS surrounding the sheath cells may be envisioned. The biological action of NTS appears to be twofold: one of local action and involves cell recognition and adhesion between axon and sheath cell that is mediated by the glycoprotein molecules and the other is a well known NGF action following transfer of NGF molecules from NTS to the receptor on the axonal membrane. The neurotropic substance appears to be the guiding principle in the organization of the peripheral nerve during embryogenesis and regeneration.

2580 PURIFICATION OF A SCIATIC NERVE PROTEIN HAVING TROPHIC INFLUENCES ON SKELETAL MUSCLE IN CULTURE. G. J. Markelonis* and T.H. Oh. Dept. of Anatomy, Univ. of Maryland Sch. of Med., Baltimore, Maryland 21201.

It has long been known that the spinal motor nerve exerts trophic influences on the morphologic, physiologic and metabolic properties of skeletal muscle. Evidence has been accumulated to indicate that such trophic influences are mediated in part by a neurally-derived trophic substance(s). Citrate-soluble chicken sciatic nerve protein was fractionated biochemically and added to aneural chick embryonic skeletal muscle cultures in order to identify the component(s) with neurotrophic activity. Biological activity of fractions was assessed by the ability of the fraction to enhance morphological development of muscle cells and to stimulate the incorporation of ^{14}C-leucine into muscle protein. A protein fraction expressing trophic activity was obtained by ion-exchange chromatography on diethylaminoethyl-cellulose followed by gel filtration on Sephadex G-100 superfine. This protein fraction, when added to mature, muscle cultures at 3-day intervals, maintained muscle fibers in a well-differentiated state for at least 5 weeks, a time when control cultures had degenerated completely. Characterization of the active fraction by sodium dodecyl sulfate-polyacrylamide gel electrophoresis revealed a single major protein with a molecular weight (M_R) of 84,000. At low concentrations, the protein migrated as a "doublet" when separated on standard, 7.5% polyacrylamide gels. Analytical isoelectric focusing revealed that the active protein was acidic, focusing as four species with isoelectric points (pI) of 5.74, 5.77, 5.92 and 6.15. Maximal stimulation of muscle protein synthesis was elicited by 20μg of active protein per ml. These data suggest that an acidic protein having trophic influences on muscle has been identified and purified.

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2581 AROMATIZATION OF ANDROGEN TO ESTROGEN MEDIATES INCREASED ACTIVITY OF GLUCOSE 6-PHOSPHATE DEHYDROGENASE IN RAT LEVATOR ANI MUSCLE. Stephen R. Max and James F. Knudsen*. Depts. Neurology & Pediatrics, Sch. Med., Univ. Md., Baltimore, MD 21201.

The mechanism of action of androgens on striated muscle is not understood. One approach to this problem is to study the production of specific proteins in response to androgen administration. We studied glucose 6-phosphate dehydrogenase (G6PD), the rate-limiting enzyme of the oxidative pentose phosphate pathway, as a biochemical marker, in the hormone-sensitive levator ani muscle of immature male rats. Sixteen h. after one i.p. injection of testosterone propionate (2.5 mg/100 g. body weight in DMSO), G6PD specific activity increased by 58% (Table). However, other androgens, e.g., fluoxymesterone and 5α-dihydrotestosterone, were without effect. These latter substances have a common characteristic, viz., they cannot be aromatized to estrogen. On the other hand, androstenedione, a metabolite of testosterone which is a good substrate for aromatization, increased G6PD by 98%. We therefore assessed the effect of estrogens and found that estradiol-17β and diethylstilbestrol increased G6PD by 58% and 44%, respectively. The response is stereospecific since the biologically inactive isomer, estradiol-17α, produced no effect. The increase in G6PD by testosterone was blocked by the anti-estrogen, MER-25, and by 4-hydroxyandrostenedione, a potent inhibitor of "androgen aromatase".

Experiment	G6PD, % Control
Vehicle	100
Testosterone propionate	158*
Fluoxymesterone	97
5α-dihydrotestosterone	90
Androstenedione	198*
Estradiol-17β	158*
Estradiol-17α	96
Diethylstilbestrol	144*
Testosterone propionate + MER-25	112
Testosterone propionate + 4-hydroxyandrostenedione	110

*$p < 0.05$

These results demonstrate that the conversion of testosterone to estradiol mediates the increased G6PD following testosterone injection. This phenomenon represents a direct effect of estrogen which is probably distinct from the general myotrophic actions of androgens. (Supported by MDA, Inc., and by the National ALS Foundation, Inc.)

2582 HYPERSENSITIVITY TO ACh IN INNERVATED MUSCLE FIBERS. E. J. Muñoz-Martinez, Jesús Cueva* and Pedro Joseph-Nathan*. Departamentos de Neurociencias y de Ingenieria Química. CIEA del IPN. México 14, D.F.

Ingestion of the fruit of Tullidora (Karwinskia humboldtiana) produces flaccid paralysis and segmental demyelination of peripheral nerves in cats and rats. Demyelination leads to conduction block of the nerve impulses and, consequently, to functional denervation when motor axons are affected. However, it cannot be excluded that paralysis results in some cases from axonal degeneration.

We investigated whether the soleus muscles of rats treated with extracts from Tullidora showed the alterations which are found after denervation; the extracts were administered through a gastric probe. After a latent period of 3-5 weeks, rats treated with a single dose (1.5 gr/Kg) showed paralysis. The soleus muscles were then removed under anesthesia and individual muscle fibers were tested in vitro for sensitivity to ACh (iontophoresis). The end plates showed the usual sensitivity but 90% of the explored fibers (215) showed hypersensitivity outside of the end plates as in the case of denervation. However, only 30% of these fibers were functionally denervated as judged by lack of response to nerve stimulation; in one fully innervated muscle, 87% of the fibers were also hypersensitive to ACh. In contrast, fore limb muscles from the same treated rats did not show hypersensitivity. On the other hand, non-treated rats but with hindlimb paralysis after chronic transection of the spinal cord showed normal sensitivity to ACh. Therefore, hypersensitivity to ACh in soleus muscles of treated animals does not result from denervation or from direct action of Tullidora on muscle fibers. Also, muscle inactivity cannot be implicated. We propose that increased sensitivity to ACh in the animals treated with Tullidora results from lack of a trophic substance which normally controls the synthesis and distribution of ACh receptors.

The same type of experiments were repeated in rats treated with a purified poliphenolic compound isolated from the fruit of Tullidora and the results were esentially the same as those described above.

2583 CELL PROLIFERATION IN DENERVATED MOUSE MUSCLE. Marjorie A. Murray* and Norman Robbins (SPON: Vernon Rowland) Dept. Anat., Sch. Med., Case Western Reserve Univ., Cleveland, OH 44106

Nerves exert control over cell division in many target tissues. In adult skeletal muscle, cell division increases soon after nerve section, but it is unclear which cell types proliferate or whether the response is spatially related to degeneration of nerve terminals. Indeed, some authors suggest that early membrane responses to denervation are causally connected to a cellular reaction at the endplate region.

In the present study, cell division in the extensor digitorum longus muscles of young adult mice was studied by injecting ^{3}H-thymidine (^{3}H-TdR) after denervation. Scintillation counting of muscle homogenates showed that ^{3}H-TdR incorporation into DNA in the denervated muscle was higher than in the sham operated muscle for the first week after nerve section, with the peak response at 4 days. Autoradiography revealed that most of the label was in cells extrinsic to muscle fiber basal laminae, although a few satellite cells were labeled. Some of the extrinsic cells had characteristics of fibroblasts, while others appeared to be macrophages. Although several cell types incorporated label, they were not preferentially located near certain structures such as small venules or nerve terminals. The muscle was not edematous and no polymorphonuclear leukocytes were seen.

In a series of reinnervation experiments, scintillation counting during recovery showed that 90-95% of the label injected at 3 days did not remain in the muscle 6 weeks later.

Several reports indicate that the number of satellite cells increases after denervation. In this study approximately 1% of the labeled cells were satellite cells, as confirmed by electron microscopy. This small number is not surprising since these cells comprise only 2-4% of the nuclei in the tissue. Satellite cell increase may result from budding off of myonuclei rather than mitosis. Experiments to test this hypothesis are under way.

The stimulus for cell proliferation after denervation seems to be a generalized signal throughout the muscle. Since the signal is reaching cells that are not synaptically connected to the nerve, it may be a secondary message, resulting from an earlier change in muscle fibers. Alternatively, cutting the nerve may alter primary mitotic influences that are normally present.

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2584 PERSISTENCE IN DEGENERATING SCIATIC NERVE OF SUBSTANCES HAVING TROPHIC INFLUENCES ON CULTURED MUSCLE. T.H. Oh, G.J. Markelonis* P. J. Reier and A.A. Zalewski. Dept. of Anatomy, Univ. of Maryland Sch. of Med., Baltimore, Md. 21201 and NIH, Bethesda, Md., 20014.

It is well known that cholinergic innervation exerts trophic influences on skeletal muscle and that such influences are mediated in part by trophic substances. Evidence for the trophic substances has been obtained from "nerve stump" experiments which suggest that there is a progressive depletion of trophic substances in the transected distal nerve stump. In the present study, we investigated whether depletion of trophic substances actually occurs in transected degenerating nerves by testing the effects of soluble protein obtained from normal and degenerating nerves on protein synthesis, maturation and maintenance of cultured muscle. When soluble protein obtained from normal or 21-d denervated chicken sciatic nerves was added to chick embryonic muscle cultures, the incorporation of ^{14}C-leucine into muscle protein was significantly greater than in control cultures. Addition of these proteins to muscle cultures markedly enhanced the rate of morphological maturation of muscle cells. By 6 d, cross-striated myotubes were present in cultures treated with normal or degenerating nerve protein, whereas control cultures showed thin immature myotubes. Under our culture conditions, control cultures began to degenerate after 2 w. By contrast, addition of normal or degenerating nerve protein to mature, muscle cultures maintained these cultures for at least 4 w. Recently, we have identified and purified a protein (MW 84,000) from normal chicken sciatic nerves which has trophic influences on cultured muscle (Proc. Nat. Acad. Sci., in press). To determine whether this protein persisted in degenerated nerves, we separated soluble proteins from normal, 3-d or 21-d denervated chicken sciatic nerves by SDS-polyacrylamide gel electrophoresis. The resulting gels showed that an 84,000 dalton protein was present in the degenerating nerves. The present study shows that soluble protein from normal or degenerating nerves was equally effective in promoting synthesis, maturation and maintenance of cultured muscle and that the electrophoretic pattern of normal and degenerating nerve proteins contained a protein which migrated in a manner identical to the trophically active, 84,000 dalton protein. We conclude that the in vivo changes in muscle following denervation are not due to the depletion of trophic substances following nerve transection. Instead, these results indicate that a trophic substance is still associated with the degenerating, distal nerve segment and possibly with non-neuronal cellular elements. Supported in part by grants from the NIH, NSF, PVA and MDA Postdoctoral Fellowship (GJM).

2585 A NERVE STUMP DEPENDENT APPEARANCE OF ACH RECEPTORS IN ORGAN CULTURE. Anthony Olek* and Norman Robbins (Spon: F. Boller). Dept. Anat. Sch. Med. Case Western Reserve Cleveland, O. 44106

The presence of a 2-3 cm. nerve stump retards changes in the numbers of acetylcholine receptors which occur after denervation (Uchitel and Robbins, Br. Res. 153.539, 1978). In order to determine whether the nerve stump effect depends on systemic influences and to develop a system for analysis of this effect, phrenic nerve-diaphragm preparations were studied in organ culture for up to 48 hrs.

Strips of rat diaphragm cultured for 42-44 hrs. after denervation demonstrated nerve stump dependent differences in the number of acetylcholine receptors (assayed by I^{125}-alphabungarotoxin binding) in endplate regions, but not in extrajunctional areas. The endplate region is defined as a 2mm. wide piece around the myelinated nerve trunk found to contain 95% of the endplates. The number of receptors specific to this region is expressed as that found after subtracting, on a weight basis, the number of receptors in far extrajunctional regions.

Paired diaphragm strips, one ("+N") with a long nerve stump, the other ("-N") with a short stump, showed no differences in receptor number until 42 hrs. of denervation in culture, at which time (-N) strips contain 2.0±.6 fm/mg more receptors than (+N) strips ($p < .002$). The receptor number specific to the endplate regions of (+N) strips at 42 hrs. of culture is not significantly different from that of fresh innervated muscle. Autoradiographic examination of the endplate region is under way to determine the locus of the increased endplate regional binding found in (-N) strips. Strips with the nerve stump present but cut close to the muscle demonstrated no difference in receptor number compared to (-N) strips. In non-endplate areas up to 48 hrs. of culture, both (+N) and (-N) strips showed increases of 3.3± 1.5 fm/mg, comparable to muscles denervated in vivo for the same time.

One interpretation of these results is that the nerve acts by some non-activity related mechanism to maintain numbers of receptors in the endplate region at the level found in innervated muscle. In vitro, this neurotrophic regulation by the nerve stump can be dissociated from effects on far extrajunctional receptors, i.e., distinct regions of the muscle may respond differentially to neural control and the regulatory mechanisms may differ.

(Supported by Muscular Dystrophy Assoc. of America)

2586 TRANSNEURONAL CHANGES IN THE ECTOMAMILLARY NUCLEUS AND VENTRAL LATERAL GENICULATE NUCLEUS FOLLOWING EYE REMOVAL IN THE NEWLY HATCHED CHICK. J. D. Peduzzi* and W. J. Crossland (SPON: J. A. Rafols). Department of Anatomy, Wayne State University, School of Medicine, Detroit, MI 48201.

Previous investigations of unilateral eye removal in newly hatched chicks have shown that the ectomamillary (EMN) contralateral to the removed eye is reduced in volume (up to 50%) when compared with the EMN contralateral to the unoperated eye. The ventral lateral geniculate nucleus (GLv) is also reduced in volume by a smaller magnitude (30%). The three most likely explanations for the observed reduction in nuclear volume are: 1) cell death, 2) reduction of neuron volume and/or 3) arrest of neuron growth. To examine these possibilities, chicks were unilaterally enucleated on the day of hatching and allowed to survive from 2 to 81 days. Since all (or nearly all) of the optic fibers cross at the chiasm, comparisons could be made between the affected and unaffected sides of the brain. Comparisons were also made with unoperated animals. The brains were embedded in paraffin and 10 µm thionin stained sections were examined. Neurons containing nucleoli were traced using a camera lucida to obtain neuron cross-sectional areas and to determine neuron number.

There was a significant cell loss in both the EMN (30%) and GLv (20%) by the end of the first post-operative week. The neuronal loss remained constant in both affected nuclei despite an additional 10 week survival time. In the GLv the cell loss was greater in the neuropil lamina (25%) than in the internal lamina (5%). Measurements of neuronal cross-sectional areas revealed a 20% reduction on the affected sides in both nuclei which was apparent by the end of the first week. Changes in cell area paralleled changes in cell number: neuronal atrophy remained constant following additional survival time and neurons in the GLv neuropil lamina were more severely reduced in area (30%) than neurons in the internal lamina (5%).

Although the EMN and GLv have different patterns of volume reduction following eye removal, the EMN and GLv neuropil lamina show marked similarities. In both sites we have demonstrated transneuronal cell atrophy as well as transneuronal cell loss. The latter observation seems particularly interesting in view of the relatively few studies of anterograde transneuronal degeneration which have quantified cell loss.

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2587 TROPHIC EFFECTS OF MUSCLE ON NERVE II. ACETYLCHOLINE (ACh) SENSITIVITY AND RELEASE. Guillermo Pilar, Jeremy Tuttle*, and Ken Vaca*, Physiology Section, Biological Sciences Group, UConn, Storrs, CT 06268

During normal in vivo development, ciliary neurons do not form intraganglionic, neuron-neuron synapses. However, embryonic ganglion cells form contacts upon themselves having typical synaptic ultrastructure during the initial two weeks in culture. These anomalous interneuronal synapses are ineffective, due either to a lack of sensitivity to ACh or to the inability to release ACh at these synapses. These phenomena were further examined by: 1) testing cultures of these neurons for the release of ACh; 2) examining other culture conditions which might lead to the retention or induction of membrane sensitivity to transmitter.

1) Ciliary ganglion neurons take up the ^{3}H-choline (^{3}H-Ch) from the medium and convert up to 50% of the transported ^{3}H-Ch into ^{3}H-ACh. Also the ^{3}H-ACh synthesized is primarily derived from ^{3}H-Ch taken up by a Na^+-dependent high-affinity process. Cultures were then tested for ^{3}H-ACh release in response to high $[K^+]_o$ depolarization. In all cases, depolarization caused the release of a significant proportion of the neuronal ^{3}H-ACh into the medium. However, the ACh released by neurons cultured alone was only 50-60% Ca^{++}-dependent, with the remainder not requiring extracellular Ca^{++}. Thus, the neurons cultured under conditions which seem to favor interneuronal synaptogenesis are capable of ACh release and the synapses formed are ineffective due to a lack of sensitivity to ACh. 2) Most ciliary ganglion neurons from ST 32 embryoes lack sensitivity to iontophoretically applied ACh in the initial weeks of pure neuronal culture, but the frequency of sensitive neurons increases by 4 weeks. In addition, the neurons that respond to ACh have very restricted "spots" of higher sensitivity on the soma, with other areas showing little responsiveness. However, when we tested the effect of co-culture of ciliary ganglion neurons with striated muscle upon their ability to respond to ACh, all of the neurons tested depolarized in response to applied ACh, even if tested during the first two weeks in vitro. Furthermore, the neuronal somas displayed a relatively uniform and high sensitivity over their entire surface. As has been reported by others, the neurons in these co-cultures formed functional neuromuscular synapses. We conclude that under in vitro conditions, contact and synaptogenesis with muscle induces either the retention or more rapid restoration of neuronal ACh sensitivity. This behavior in vitro may be similar to the response to axotomy in vivo. Non-transmitting, but otherwise normal-appearing interneuronal synapses are not effective in this regard. Supported by NIH-NS10338, NS5382, and the Univ. of Conn. Research Foundation.

2588 NEURITE OUTGROWTH ACTIVITY (NOA) IN SOLUBLE EXTRACTS OF CHICK EMBRYO -- ONTOGENY, AND EMBRYONIC DAY (ED) 8 REGIONAL DISTRIBUTION. Richard J. Riopelle and Donald Cameron*. Queen's University, Kingston, Ontario, Canada, K7L 3J7.

A single cell bioassay for βNGF using 8 day chick embryo sensory ganglion neurons (Sutter, Riopelle, Harris-Warrick and Shooter, J. Biol. Chem. 1979, in press) has been used to quantitate endogenous factors in soluble extracts of chick embryo that promote neurite outgrowth.

NOA can be detected on ED1 and has increased by an order of magnitude by ED6 when expressed on a per embryo basis. When expressed on a per microgram soluble protein basis NOA rises rapidly from ED1 to 4 and begins to decline at the time of a growth spurt of the chick embryo (ED5-6).

At ED8, NOA is in highest concentration in muscle, heart, gut and sensory ganglia and is in lower concentration in structures of the neuraxis.

Only a fraction of NOA has immunological cross-reactivity with mouse βNGF when heat inactivated rabbit antibody to purified mouse βNGF is used. At concentrations of antibody that are four orders of magnitude (10^4) greater than those required to suppress βNGF neurite outgrowth by 50%, there is less than 20% suppression of extract-induced neurite outgrowth from whole embryo or from any organ.

NOA is reduced by approximately 50% following incubation at 56°C for 60 minutes, and by 40-50% when stored in a lyophilized form at -80°C for 6-8 months.

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2589 GLIAL SEROTONIN TRANSPORT IN THE FROG FILUM TERMINALE: POSSIBLE MODULATION BY DESCENDING SEROTONERGIC FIBERS. T. Ritchie, S. Glusman, and B. Haber. Marine Biomed. Inst., Dept. Human Biol. Chem.&Genetics & Dept. of Neurology, UTMB, Galveston, TX 77550.

The frog filum terminale (FT) is that portion of the spinal cord (SC) extending caudally beyond the level of the last spinal root. It is morphologically different from the SC, having few neuronal elements, and can be considered as largely a glial preparation. We have previously shown that the FT contains sodium-dependent, temperature sensitive, high-affinity transport systems for the uptake of GABA and serotonin (5HT), with respective Km's of 27μM and 2μM. The high-affinity 5HT transport system is inhibited in the FT by chlorimipramine, desipramine, Lilly 110140, and a variety of indoleamine analogs to extents comparing favorably with those seen for 5HT uptake in clonal cell lines of glial origin and in brain slices and synaptosomes. The accumulation of GABA and 5HT by the glial cells of the FT was confirmed by autoradiographic studies.

The accumulation of 5HT at all levels caudal to the last spinal root is higher than in the lumbar enlargement (LE), and is maximal in the conus medullaris (CM). This correlates well with measured values of 5HT along the frog cord, highest values being in the CM. In contrast to this, the uptake of GABA, while being higher at all levels caudal to the last spinal root than in the LE, increases in a rostro-caudal direction with maximal rate of uptake in the most caudal portion of the FT. Preliminary studies show a similar rostro-caudal increase in the rate of norepinephrine uptake along these same regions.

The presence of descending serotonergic fibers in the lumbar region of the frog cord suggested to us that the higher rate of 5HT uptake by the CM may be due to the presence of descending serotonergic nerve fibers ending in the CM. To test this, lesioning was performed by transversely sectioning the cord at the level of the last spinal root, and a period of 10 days was allowed for degeneration of nerve fibers. At the end of this period a drastic decrease in the rate of 5HT uptake was observed only in the CM. Intraventricular injection of 50μg of 5,6-dihydroxytryptamine, which results in selective destruction of serotonergic terminals, and allowance of 2 weeks for the degeneration process to occur, also resulted in a decrease in 5HT uptake by the CM with little effect being seen in the more caudal portions of the FT. These observations would suggest that the presence of descending serotonergic fibers in the CM modulates glial transport of 5HT. The specificity of this modulation is under investigation.

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2590 NEUROTROPHIC REGULATION OF MUSCLE GLUCOSE-6-PHOSPHATE DEHYDROGENASE IN VITRO. Norman Robbins, Dept. Anat., Case West. Res. Schl. Med., Cleveland, O. 44106

In vivo, glucose-6-phosphate dehydrogenase (G6PD) activity of rat diaphragm increases 12 hrs after denervation, but the increase is delayed if a 3 cm nerve stump is left attached to the denervated muscle (Robbins & Carlson, Br. Res., in press). In order to analyze this apparent neurotrophic regulation further, an in vitro system was devised in which 2 strips of rat diaphragm with and without a 2.5 cm nerve stump ("+N" and "-N", resp.) were organ cultured in parallel in Med. 199 at 36°. A control strip was taken from the same muscle at the beginning of culture.

In -N strips, G6PD activity increased progressively by 34% at 16½ hrs and 54% at 19 hrs in vitro, provided the culture medium was changed at 11 hrs. Without the medium change, the increase was slower, presumably because of leakage of factors from nerve terminals before they degenerate. In +N strips with medium change at 11 hrs, G6PD activity was not increased at 16½ hrs and differed significantly (34%, $p<0.001$) from paired -N strips. If the nerve was crushed or cut at the point of entry into the muscle and left in culture, no nerve stump effect was observed. The nerve stump effect persisted in paired +N and -N strips exposed continuously to curare (1.5-3ug/ml). Curarization did not alter the time course of enzyme increase. Addition of nerve extracts or effluents from stimulated nerve-muscle preparations did not affect the enzyme increase. Also, -N and +N strips cultured in the same vessel showed no mutual influence on G6PD activity. However, transfer of medium conditioned by +N strips to -N strips in the 11-16½ hr period had a small but significant effect, dimishing enzyme increase by 11% ($p<0.05$) compared to paired controls treated with -N conditioned medium.

Conclusions: The neurotrophic effect of the nerve stump on G6PD activity does not require in vivo factors such as stretch, blood flow, and circulating cells or factors. 2. The effect is independent of physiological acetylcholine action on nicotinic receptors. 3. A soluble factor, probably leaking slowly and spontaneously from nerve terminals, produces effects similar to those of a nerve stump.

(Supported by Muscular Dystrophy Assoc. of America)

2591 Physiological and anatomical consequences of sensory denervation of the cornea. B. Schimmelpfennig* and R. Beuerman, Stanford University Medical Center, Stanford, CA 94305.

A trophic action of the sensory nerves of the cornea and their target tissue, the epithelium, was first suggested by Magendie in 1824. However, convincing evidence for this type of interaction has not been obtained. The present studies show that deafferentation produces functional as well as morphological alterations in the epithelium.

Radiofrequency thermocoagulation, delivered by an electrode inserted through the soft palate, was used to produce lesions in the medial portion of the trigeminal ganglion in anesthetized albino rabbits. Although there was no behavioral response to punctate mechanical stimulation of the cornea following the lesion, normal blinking (4 to 5/hour) was retained. Nerve degeneration was confirmed by electron microscopy. Slit-lamp observations revealed that the corneal surface was free from defects. Permeability in the denervated corneas was found to be significantly greater ($p<.005$, N=11) when determined by measurement of aqueous humor fluorescence 30 min after the instillatin of 5% fluorescein. Re-epithelialization of a 4 mm centrally placed abrasion was examined in 13 animals. After 40 hr all control corneas were epithelialized, but the denervated corneas were only 72% covered. Complete counts of mitotic figures were made in 6 mm dia. central corneal buttons and stained by the orcein method. The denervated corneas contained significantly fewer mitoses ($p<.001$).

Electron microscopy of the denervated epithelium has revealed alterations in structures responsible for the maintenance of cell shape and adhesion. Transmission microscopy in 6 rabbits showed that the cytoplasmic density of the epithelial cells decreased due to a loss of tonofilaments. Both hemidesmosomes and desmosomes stained less densely; in addition a decrease in number and size of the hemidesmosomes was noted. Scanning microscopy showed an abnormal desquamation of the surface cells many of which had lost microvilli and were smooth. In conclusion, it is shown here that the sensory innervation of the corneal epithelium is important to the maintenance of its tissue properties.

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2592 EFFECTS OF NICOTINE ON NEURITE OUTGROWTH IN CHICK SENSORY AND SYMPATHETIC GANGLIA: COMPARISON WITH NERVE GROWTH FACTOR. Betty F. Sisken and Jesse E. Sisken.* Wenner-Gren Research Laboratory, College of Engineering and Department of Pathology, College of Medicine, University of Kentucky, Lexington, Kentucky 40506.

To date only a few factors (NGF, conditioned medium, dibutryl cAMP, electrical stimulation) have been reported to stimulate nerve fiber outgrowth from ganglionic explants. Previous observations that nicotine stimulates microfilament function in Hela cell cleavage furrows (Vedbrat, et al 1979) led to the hypothesis that nicotine may also stimulate neurite outgrowth since the latter might be dependent upon microfilament function (Yamada et al, 1971). In this report, we present evidence that nicotine, a sympathomimetic alkaloid, can produce a substantial stimulation of neurite outgrowth in both sensory and sympathetic ganglia. Nicotine in concentration ranges of 15-500 µg/ml was tested on 8 day chick trigeminal, dorsal root and sympathetic ganglia explanted onto collagen-coated Falcon culture dishes. They were cultured in Dulbecco MEM supplemented with 10% calf and 5% fetal calf sera, glucose and penicillin-streptomycin. Nerve fiber outgrowth was evaluated after 3 days in vitro by a scoring procedure which took into account number, length, degree of branching and distribution of neurites around the explant. This outgrowth was compared to untreated control cultures and cultures treated with 200 units/ml of 2.5 S NGF (gift of R. Bradshaw, Washington University). Findings will be presented to demonstrate that significant stimulatory effects were produced by nicotine in the concentration range of 80-250 µg/ml at a pH range of 6.8-7.2. Preliminary evidence suggests that sympathetic ganglia are more responsive to nicotine than either trigeminal or dorsal root ganglia. Studies are in progress to determine the mechanisms behind this effect.

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2593 REGULATION OF SODIUM ION EXTRUSION BY NERVE GROWTH FACTOR IN RESPONSIVE NEURONS: A COMPARISON OF DIFFERENT GANGLIONIC CELL PREPARATIONS. Stephen D. Skaper* and Silvio Varon. Dept. Biol., Sch. Med., UCSD, La Jolla, CA 92093.

Nerve Growth Factor (NGF) is necessary for maintenance and growth of neurons from sympathetic and dorsal root ganglia. Traditional responses to NGF are observed only several hours following presentation of the factor. Short-latency responses, occurring within minutes, are more likely to reflect primary events involved in the mode of action of NGF.

Cell suspensions from embryonic chick dorsal root ganglia lose the ability to take up hexose (and certain other small molecules) when deprived of NGF, but recover it promptly if NGF is supplied within 6 hr. The recovery occurs over one to several minutes, depending on NGF concentration. The dependence on Na^+ gradients of these NGF-regulated uptake systems has led to the discovery that NGF controls extrusion of Na^+ (and, thus, intracellular Na^+ levels) in these ganglionic neurons. Incubation of the cell suspension with $^{22}Na^+$ for 6 hr causes accumulation of radioactivity which is 6 times greater when no NGF is present. Subsequent presentation of NGF to $^{22}Na^+$-loaded cells causes extrusion of the $^{22}Na^+$ with a speed (minutes) which is dependent on NGF dose. This "sodium response" (i.e., accumulation in the absence of NGF and extrusion on its delayed presentation) has now been examined with various ganglionic materials known to be NGF-sensitive. Qualitatively similar results are found with 1) intact as well as dissociated chick embryo dorsal root ganglia, 2) intact and dissociated chick embryo sympathetic ganglia, and 3) dissociated, but not intact dorsal root ganglia from neonatal mouse. In these last ganglia, it has been previously reported that cultured neurons do not require exogenously supplied NGF when adequately supplemented with ganglionic nonneurons (e.g., within the intact ganglion). Chick sympathetic ganglia and mouse dorsal root ganglia also allow the separation of viable neuronal subpopulations. Data to be presented will attempt to correlate the occurrence of Na^+ responses with NGF requirements for neuronal survival in culture. (Supported by NINCDS grant NS-07606).

2594 TROPHIC EFFECTS OF MUSCLE ON NERVE I. ACETYLCHOLINE (ACh) SYNTHESIS. Ken Vaca*, Jeremy Tuttle* and Guillermo Pilar (SPON: W. Chapple). Physiology Section, Biol. Sci. Gp., Univ. of Conn., Storrs, Conn. 06268.

The terminals of the ciliary nerve of the chick initially form functional synaptic contacts with iris muscle at embryonic stages 34-40. At the onset of this period the nerve terminals do not exhibit a Na^+-dependent high affinity choline (Ch) uptake system, and the very low level of ACh synthesis from ^{3}H-Ch is insensitive to removal of Na^+. At St. 36 ^{3}H-ACh synthesis gradually increases, the increment being Na^+-dependent. However, ACh synthesis in the embryonic iris is insensitive to a conditioning $[K^+]_o$ depolarization even as late as ST. 43. Prior to hatching the nerve terminals show a small response to conditioning depolarization, an augmented ACh synthesis, but by 2 days ex ovo this response has increased by an order of magnitude. Ultrastructural examination of the developing ciliary terminals reveals few synaptic vesicles and other specializations at early stages, which may underlie the rapid fatigability of immature synapses. Near hatching (St. 44) clustering of vesicles in regions of synaptic contact starts to appear, eventually attaining a closely packed morphology in the ex ovo chick. This correlation between vesicular accumulation and responsiveness to conditioning depolarization supports the suggestion that regulation of Ch transport and ACh synthesis depends primarily upon the recompartmentation of cytoplasmic ACh into vesicles.

In the absence of other cell types, dissociated ciliary neurons (St. 33) form ineffective synapses with each other in culture. Yet a few days after plating they are able to synthesize considerable amounts of ACh. 25 to 35% of the ^{3}H-Ch accumulated by these cells is via Na^+-dependent transport. This Na^+-dependent uptake is the predominant source of Ch for ACh synthesis. After a conditioning depolarization, these cells respond with a small increase in ACh synthesis (15-20%). A few days after dissociated ciliary neurons are plated onto cultured myotubes, resting levels of ACh synthesis are several-fold higher than muscle-free neuron cultures plated at the same time (on a per cell basis). Furthermore, the neurons respond to a conditioning depolarization with a large increase in ACh synthesis (100%). These cells are able to synthesize ACh at a rate comparable to that in mature parasympathetic neurons. It is suggested that cholinergic characteristics are present at a low level prior to synapse formation and then become progressively concentrated at points of synaptic contact. Full expression of the mature phenotype may require inductive interactions with appropriate target tissues. Supported by NIH-NS10338, NS5382 and the Univ. of Conn. Research Foundation.

2595 CHARACTERIZATION OF SPONTANEOUS TRANSMITTER RELEASE AND THE EFFECTS OF TEMPERATURE IN NORMAL AND DYSTROPHIC CHICKENS. S. Yeagle*, J. E. Warnick and E. X. Albuquerque (SPON: C. S. Hudson). Dept. Pharm. and Exptl. Therap., Univ. MD. Sch. Med., Baltimore, MD 21201.

Miniature endplate potentials (MEPPs) were examined in posterior latissimus dorsi (PLD) muscles of normal (line 412) and dystrophic (line 413) chickens from 6 to 12 week ex ovo. At 23° C the MEPP amplitude in surface fibers of normal PLD muscle (0.37 + 0.02 mV) was greater ($P < 0.01$) than dystrophic (0.26 + 0.02 mV) whereas the frequency of MEPPs in normal fibers (0.23 + 0.02 sec^{-1}) was similar to that seen in dystrophic fibers (0.24 + 0.02 sec^{-1}). Increasing the temperature to 40° C produced a unimodal exponential increase in MEPP frequency in both normal and dystrophic fibers with a Q_{10} between 2 and 3 and energy of activation between 30-40 kcal. The difference in MEPP amplitudes were sustained at 40° C (0.40 + 0.03 mV in normal vs. 0.29 + 0.03 mV in dystrophic; $P < 0.05$), while the MEPP frequencies remained indistinguishable (0.93 + 0.20 sec^{-1} in normal vs. 2.44 + 1.04 in dystrophic). MEPPs from fibers of both lines had similar rise times at both 23° C (3.32 + 0.16 msec in normal vs. 3.57 + 0.30 msec in dystrophic) and at 40° C (0.42 + 0.12 in normal vs. 0.65 + 0.10 in dystrophic). Half-decay times of MEPPs in both lines were also similar at 23° C (6.81 + 0.24 msec in normal vs. 7.79 + 0.59 msec in dystrophic) and at 40° C (0.79 + 0.06 msec in normal vs. 0.88 + 0.13 msec in dystrophic). At 23° C input resistance at the endplate region in normal fibers (0.44 + 0.03 MΩ) was not significantly different from dystrophic fibers (0.41 + 0.06 MΩ). Addition of neostigmine (3 μM) to the bathing medium or treatment with diisopropylphosphorofluoridate (1 mM) at 23° C produced a similar degree of potentiation of MEPP amplitude in normal and dystrophic fibers. It appears unlikely that the observed differences in the amplitudes of MEPPs in normal and dystrophic fibers can be attributed to differences in input resistance or junctional acetylcholinesterase alone. It seems probable that the decrease in MEPP amplitude in dystrophic fibers is due to either some alteration in the transmitter release process as suggested previously (Exp. Neurol. 63:135, 1979) or to a reduced access of acetylcholine to the receptor site since it has been shown that junctional acetylcholine sensitivities of normal (line 200) and dystrophic (line 304) chickens are similar (Exp. Neurol. 43:21, 1974). In addition, at temperatures from 18 to 41° C the MEPP frequency in PLD muscle fibers of both normal and dystrophic chickens fails to show the bimodal response to temperature increases seen in some other vertebrate species (J. Physiol. 132:650, 1956). (Supported in part by USPHS Grant NS-12063 and a grant from the Muscular Dystrophy Association of America.)

VISION

2596 SPATIAL AND TEMPORAL PROPERTIES OF RECEPTIVE FIELDS IN MONKEY AND CAT VISUAL CORTEX. Duane G. Albrecht, Lisa G. Thorell* and Russell L. De Valois*. Department of Psychology, University of California, Berkeley CA 94720.

The spatial and temporal variations are the two most important aspects of a visual stimulus with respect to evoking a response in a visual cortical neuron in area 17. The structure of the receptive field demands a specific spatial and temporal distribution of light. To quantify these space-time contingencies, one would like to have a method of analysis which produces a concise, accurate, general description. Use of spatial-temporal sinusoidal grating patterns, within the context of linear systems analysis, can potentially provide such a description. In this study we used such a method of analysis to measure (a) the spatial tuning, (b) the temporal tuning, and (c) the interaction between two.

With respect to the spatial tuning, the results show that most of the cells have a band pass characteristic; each cell attenuates its response to both high and low spatial frequencies. The average width of the band at half the maximum response (averaged across all of the cells) is 1.2 octaves. There is, however, a great deal of variability in this regard from cell to cell; the narrowest bandwidth was 0.5 octaves and the broadest was greater than 2.5 octaves. There is also a great deal of variability, from cell to cell, with respect to the locus of the peak of the spatial tuning function.

With respect to the temporal tuning function, while most of the cells (75%) do show both low and high frequency attenuation, the tuning is extremely broad (in comparison to the spatial tuning). There is a great deal of variability from cell to cell with respect to the width of the tuning function and the locus of the peak. The mean bandwidth for the entire sample was 3 octaves and the range extended from 2 to 6 octaves. The mean peak for the entire sample was 3 Hertz and the range extended from 0.5 Hertz to 16 Hertz. Somewhat surprisingly, there were no obvious differences in the temporal properties of simple vs complex cells. Correlation (across cells) of the peak of the spatial tuning function with the peak of the temporal tuning function revealed a very low negative correlation (-0.2).

Human psychophysical experiments have shown a clear interaction between spatial and temporal variables of a visual stimulus. Parametric examinations of these two variables for area 17 cells showed that for the vast majority of cells no such interaction exists. That is, for most cells the spatial tuning remains invariant as a function of temporal frequency. A few cells, however, showed a systematic interaction between these two variables.

2597 REGIONS WITH CONVERGENT VISUAL INPUT IN AREA 18 OF THE CAT. K.Albus and D.Sanides*. Dept. Neurobiology, MPI Biophysic. Chemistry, 3400 Goettingen, FRG.

In normal adult cats three regions with callosal projections (callosal islands) have been identified at the lateral limit of area 18 by anterograde and retrograde axonal tracing techniques. Receptive fields of single units and multiunits have been recorded in the callosal islands and were found to reach an extremely large size. Their centers lay either on the vertical meridian or 10°-20° in the contralateral hemifield. However similar as in other regions with callosal input the fields reached across the vertical meridian and often considerably into the ipsilateral hemifield. The callosal islands were wedged in between acallosal parts of lateral area 18 which represent the periphery of the contralateral visual field(periphery islands). Thalamic input to these different two parts of lateral area 18 has been identified by retrograde tracing with peroxidase. The periphery islands have been found to receive heavy input from the main part of the lateral geniculate nucleus (LGN) and light input from the medial interlaminar nucleus (MIN). In both nuclei it was restricted to portions with peripheral retinal input. The callosal islands have been found to receive peripheral visual input from the LGN, and a rather heavy projection from portions of the MIN representing the contralateral retina, the midline of the retina as well as the ipsilateral retina. It thus appears that the extremely large receptive fields in the callosal islands of lateral area 18 are produced by converging afferent fibers with widespread retinal input.

In some animals large receptive fields covering nearly the entire visual field were found also at the representation of the vertical meridian in area 18. The pattern of thalamic connections of these medial parts of area 18 resembled that of the callosal islands in lateral area 18.

The callosal regions in area 18 fulfill the criterion of a separate visual area because they contain a representation of most of the visual field. Unlike the remainder of area 18 and other visual cortical areas the representation is not or only poorly retinotopically organized.

2598 BRIEF LIGHT DEPRIVATION ALTERS NEURAL ORGANIZATION IN HUMANS. Patricia Apkarian*, Dennis M. Levi*, Ken Nakayama* and Christopher W. Tyler* (SPON: P. Bach-y-Rita). Smith-Kettlewell Institute of Visual Sciences, San Francisco, CA 94115.

We have used the steady state visual evoked potential (VEP) to study the changes in neural responsivity in the human brain following very brief periods (e.g., 10 sec) in the dark. Under our recording conditions, the synchronous response to uniform field flicker and counterphase flickering gratings reaches a maximum after about one second of stimulation. Following brief light deprivation, the buildup of neural activity elicited by the same stimulus is delayed by as much as 20 seconds, after allowing adequate time for light adaptation prior to recording.

This effect, which is clearly present after only 10 seconds in the dark, asymptotes after 1 minute. Control studies rule out pupillary responses, accommodation and convergence as the source of the effect, and the time course dissociates it from previously described neural and photochemical effects of light and dark adaptation. In addition, the effect of brief periods of darkness is different for the fundamental (f) and second harmonic (2f) of various uniform field flicker frequencies. Similar effects also occur with counterphase flickering gratings; however the profiles vary depending on the spatial and temporal frequency of the stimuli.

The high degree of specificity found in these studies does not conform with conventional light adaptation mechanisms, but indicates selective modification of different neural mechanisms resulting from brief light deprivation.

2599 SMOOTH PURSUIT EYE MOVEMENTS. A. Terry Bahill and B. Todd Troost, Biomedical Engineering, Carnegie-Mellon University, Pittsburgh, PA 15213

The easiest target to track with smooth pursuit eye movements is one that is moving sinusiodally. To track such a target humans use the saccadic system, the smooth pursuit system, and high level prediction processes. In order to limit our study and eliminate the prediction process we used nonpredictable target motions. Gaussian white noise was too confusing and induced numerous saccades. We used a waveform with a series of constant velocity ramps each of which has an unpredictable velocity and duration. The bandwidth of the target waveform was important; a bandwidth of one hertz was optimal.

The recorded eye movements consisted of a combination of smooth pursuit and saccades---dual mode tracking. We modified this record by removing all of the saccades to yield a cummulative smooth pursuit eye movement record---single mode tracking. We visually inspected these time domain records, but did all of our data analysis in the frequency domain.

Fatigued subjects, and patients with progressive supranuclear palsy had large differences between the single mode and dual mode transfer functions, particularly around one hertz. Patients with unilateral posterior hemispheric disease had smooth pursuit movements in only one direction which caused a large low frequency gain for the single mode transfer function and a very low value for the coherence function at low frequencies.

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2600 RETINAL SENSITIVITY DEFECT IN THE MUTANT MOUSE PEARL. G. W. Balkema, Jr., and L. H. Pinto. Dept. of Biol. Sci., Purdue Univ., West Lafayette, IN 47907

We have previously reported the presence of two visual defects in the mutant mouse pearl. (1) Pearl does not show visually evoked nystagmus for any test luminance, but its post-rotatory nystagmus is normal. (2) In recording from tectal cells we found that at dim backgrounds a threshold stimulus had to be about 2 log units more luminous for pearl than for normal mice; at higher backgrounds pearl thresholds approach normal values. This sensitivity deficit does not seem to be caused by a defect in pearl's photoreceptors, since the neuronal mechanism responsible for the generation of the ERG is normal.

In a search for histological abnormalities we injected the eyes of pearl mice with tritiated proline and prepared the brains for autoradiography. This method did not reveal any defects in the projections to the superior colliculus or the primary visual cortex in pearl mice. Furthermore, when we examined 1 μm plastic sections that were embedded in epon/araldite and stained with toluidine blue, we could find no differences between the retinas from pearl and normal mice.

Next, we tried to narrow down possibilities for anatomical sites of pearl's sensitivity deficit by recording from the axons of single retinal ganglion cells in pearl and normal mice. Of the different types of ganglion cells we saw in both normal and pearl, we selected the ones with concentric receptive-field organization for a comparison of sensitivities. Incremental threshold measurements showed a deficit similiar to the one we observed in pearl tectal cells: with dim backgrounds (-5 to -3 log cd/m^2) pearl ganglion cells required a stimulus 1.7 log units greater in luminance than normal ($p < 0.001$); with more luminous backgrounds (0 to 2 log cd/m^2) pearl's sensitivity came close to wild type values.

Thus, we have found that the coat color mutant, pearl, has in addition to its lack of OKN, a retinal sensitivity deficit in the dark-adapted state. Furthermore, this deficit in pearl appears to lie between the site of generation of the ERG and the ganglion cells in pearl's retina.

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2601 **SOMATOSENSORY AND VISUAL RECEPTIVE FIELD CHARACTERISTICS OF SINGLE UNITS IN THE NUCLEUS INTERCOLLICULARIS OF THE PIGEON.** **Gary O. Ballam* (SPON: D. M. Schroeder). Indiana University, Bloomington, Indiana 47401**

Microelectrode recordings from the Nucleus intercollicularis (ICo) of the unanesthetized pigeon indicate the presence of neurons responsive to visual and tactile stimuli. The receptive field size of units driven by tactile stimuli varies from 5% to 100% of the feathered body surface area. The units driven by tactile stimuli can be divided into 3 distinct groups according to receptive field size. The somatosensory receptive field (SRF) size of units representing these three groups peaks at areas covering 15%, 48% and 100% of the body surface. Units representing the smaller size group are more likely to demonstrate somatonsensory adaptation characteristics, receive inhibitory visual inputs from the ipsilateral eye and non-adapting visual inputs from the contralateral eye. Units representing the two larger SRF groups (those which peak at 48% and 100% of the body surface area) have similar receptive field characteristics and contain a smaller percentage of units demonstrating somatosensory adaptation. They also tend to receive either an excitatory or no visual input from the ipsilateral eye and have a much larger proportion of visual inputs from the contralateral eye which demonstrate adaptation. The visual receptive fields (VRF's) of units driven by the contralateral eye range in size from 15° to 180° of visual arc and can be divided into 2 groups according to receptive field characteristics. Units representing the group having smaller VRF's peak at 90° of visual arc and contain a larger proportion of inputs from the smaller sized SRF group. The units with smaller VRF's do not tend to demonstrate adaptation. They receive very few excitatory inputs and a relatively large proportion of inhibitory inputs from the ipsilateral eye. The second visual group, comprised of units with larger VRF's, contains a greater percentage of inputs from large SRF's and more units tend to demonstrate adaptation. The units with larger VRF's also receive a greater percentage of excitatory and a lesser percentage of inhibitory inputs originating from the ipsilateral eye. These different groups of units driven by visual and/or tactile stimuli may represent populations of neurons in the ICo associated with different behavioral responses such as orientation, focused attention or alertness.

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2602 *LIMULUS* VENTRAL PHOTORECEPTORS: DO THEY SEND INFORMATION TO THE BRAIN? Ranjan Batra*, Mildred Behrens* and Steven Chamberlain*. (SPON: R. B. Barlow, Jr.). Inst. for Sensory Res., Syracuse University, Syracuse, NY 13210; and Masonic Medical Research Lab., Utica, NY 13503.

Although *Limulus* ventral photoreceptors have been extensively utilized for studies of basic photoreceptor mechanisms, little is known of their central connections or functions in the larger context of the animal's visual system. Because the ~1.5 cm long central axons do not conduct nerve impulses, it has often been suggested that ventral photoreceptors may not provide visual information to the adult animal.

We have stained single photoreceptors and their central terminations by intracellular injection of cobalt ions. We have recorded light-evoked depolarizing potentials intra-axonally at various points along the ventral optic nerve and in the brain.

The ventral optic nerve makes connections with the ganglion cell layer of the medulla along the ventral and anteromedial borders of the medullar neuropil. The axons of single cells terminate in a ramification of fine processes covering about 0.1 mm^2 of medullar surface.

Intra-axonal recordings reveal depolarizing potentials (2-15 mV) elicited by illumination of cell bodies in the distal tip of the ventral optic nerve. These depolarizing responses have been recorded both at various points along the nerve trunk and within the protocerebrum itself along the anterior margin of the medulla.

Taken together these findings support an alternate view that even in adult animals, the ventral photoreceptors of *Limulus* may make specific and functional connections with higher visual centers.

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2603 Orientation Anisotropy in Striate Cortex of Awake Monkeys. Roman Bauer*, Bruce Dow and Robert Vautin* (Spon. E. Koenig) Neurobiology Division, SUNY, Buffalo, NY 14226.

In a series of penetrations through foveal striate cortex in alert behaving macaque monkeys, we noticed that the first encountered striate cell typically had a preference for lines oriented at 45° to horizontal and vertical. This was a somewhat surprising result in view of Mansfield's (1974) earlier report of an anisotropy for horizontal and vertical orientations among foveal striate cells. Review of our own data from marked penetrations in acute, anesthetized animals suggested that the horizontal/vertical anisotropy was in fact most prevalent in middle and lower layers of striate cortex.

In order to pursue this issue more systematically, we have been examining changes in orientation at 200-250 μ intervals from pial surface to white matter in penetrations at various angles to the cortical layers. The angle of our electrode is determined by the angle of the recording cylinder base with the monkey's skull. Variations in recording angle within a given hemisphere are thus primarily dependent on the contours of the cortical surface. To avoid bias in our test procedure we routinely present a series of standard orientations to every cell and use computer plotted histograms as a measure of response magnitude.

In a typical penetration through foveal striate cortex, the first encountered cells have orientation preferences within about 15° of diagonal. Subsequent cells prefer orientations close to this, with slight progressions in either a clockwise or counter-clockwise direction until the middle of layer 4, when orientation tuning becomes much broader or disappears completely. Toward the lower part of layer 4 the orientation suddenly jumps by 45° or more and then remains about the same until we enter white matter.

The most parsimonious interpretation of our data is that there are alternating horizontal and vertical orientation dominance columns in foveal striate cortex. Each column must necessarily consist of 2 subregions, one responsible for clockwise rotation, the other for counterclockwise rotation. Clockwise rotation from vertical and counterclockwise rotation from horizontal would both lead to the same limit, namely +45°, suggesting that 2 such subregions should lie adjacent to one another. Similarly, horizontal clockwise and vertical counterclockwise subregions should also be adjacent. The anisotropy for diagonal orientations in upper layer cells bears some resemblance to the binocularity of upper layer cells (Hubel & Wiesel, 1968), except that inhibitory mechanisms would appear to play a greater role in generating diagonal orientations, and two different diagonals must be generated instead of a single cyclopean eye.

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2604 EFFECTS OF INFEROTEMPORAL COOLING ON PERFORMANCE OF A VISUAL RECOGNITION TASK. Richard H. Bauer, John P. Jervey* and Joaquin M. Fuster. Department of Psychiatry and Brain Research Institute, School of Medicine, University of California, Los Angeles, Calif. 90024.

Ablation studies in the monkey have implicated the inferotemporal cortex (area TE) in learning and retention of visual discriminations. Presumably lesions of this cortical region interfere with some aspect of visual memory. The present study examines the effects of reversible inferotemporal lesion on performance of delayed matching-to-sample, a behavioral task that demands short-term retention of visual stimuli. For this study the stimuli were circles of red and green light projected on a panel. A trial required from the animal two successive instrumental responses. The first occurred immediately upon presentation of one color, the sample, which initiated the trial. This response turned the sample color off and started a preset delay. The second response occurred at the end of the delay, when the two colors appeared simultaneously and the animal had to select the one that matched the sample. Correct choices were rewarded. The sample color and the position of the matching stimulus were changed randomly between trials. Cryogenic brain probes were implanted bilaterally in three monkeys trained to perform the task. The probes conformed to the convexity of the temporal lobe and were epidurally placed, covering a major portion--about 1 cm^2 --of TE cortex. Cortical temperature was controlled by implanted thermistors. Bilateral inferotemporal cooling to 20°C produced the following reversible effects: 1) An increase in the number of errors; this effect was more pronounced on trials with long delay (8, 16 or 32 sec.) than on those with short delay (1 or 4 sec.). 2) An increase in reaction time on presentation of the sample color. 3) An increase in reaction time on presentation of the two colors for delayed matching (choice reaction time). The results indicate a participation of the inferotemporal cortex in short-term retention of visual stimuli. The effects of inferotemporal cooling on reaction time, especially choice reaction time, may be secondary to the difficulty that inferotemporal lesion induced in mnemonic function.

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2605 VISUAL PROJECTIONS OF THE TREE SHREW CLAUSTRUM: AN ANTEROGRADE AND RETROGRADE TRANSPORT STUDY. Mark F. Bear* and Russell G. Carey* (SPON: P. Kaufmann). Dept. Psychol., Duke Univ., Durham NC 27706.

We have previously reported that the dorsal claustrum projects to striate cortex in the tree shrew, Tupaia glis (Carey, Fitzpatrick & Diamond, Anat. Rec. 193, 1979). Further investigations have shown that injections of tritiated amino acids in striate cortex lead to autoradiographic grains concentrated over this same region of the claustrum. The present study was undertaken to examine the laminar organization of these connections by making iontophoretic injections of either tritiated amino acids or HRP into the claustrum.

Injections of amino acids in the claustrum produce a heavy accumulation of transported label in area 17 and a considerably smaller amount of label in the extrastriate regions. In striate cortex the terminal grains are primarily localized over layer IV and to a lesser degree over layers IIIb, VI and I. In extrastriate cortex, terminal grains are again mainly over layer IV with lesser activity over layers I and VI. No activity is seen in layer III outside of striate cortex. After injections of HRP in the same region of the dorsal claustrum, labeled cells are found concentrated in layer VI of striate cortex with a few scattered cells being found in the temporal visual areas. Both the claustral projection onto area 17 and the reciprocal projection back to the claustrum are found to be topographically organized. Control injections in the cortex overlying the claustrum lead to a pattern of activity or labeled cells totally different than that seen after injections of the claustrum.

Examination of the activity seen in the thalamus after these claustral injections indicates that the claustrum is reciprocally connected with the visual intralaminar nuclei that were previously shown to project to cortical layer I of striate cortex (Carey, Fitzpatrick and Diamond, Science, 203, 1979).

These results indicate that the claustrum is unique in its anatomical relationship with the visual cortex. First, it is the only nucleus outside the thalamus that receives a projection from cortical layer VI. Second, the claustrum projects heavily to striate cortex and overlaps the terminal zones of all the thalamic nuclei known to project to striate cortex. These findings lead us to speculate that the dorsal claustrum may play a significant role in modulating activity in the visual system, both at the cortical and thalamic levels.

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2606 IDENTIFICATION OF RETINAL TERMINALS IN THE CAT SUPERIOR COLLICULUS: ELECTRON MICROSCOPIC AUTORADIOGRAPHY AND DEGENERATION. Mary Behan and John K. Harting. Univ. of Wisconsin, Madison, WI 53706.

We have used the EM-autoradiographic method to determine specific details regarding the terminations of retinocollicular axons in the cat. Our findings confirm and extend earlier studies which have shown that while the major portion of the contralateral projection terminates within the stratum griseum superficiale (SGSl of Kanaseki and Sprague, '74), retinal efferents also end within the more ventrally located SGS2 and SGS3. With a data tablet digitizer interfaced with a computer, we have measured the mean diameter of terminals in sublayers of the superficial grey. These data reveal that contralateral retinocollicular terminals can be grouped into two size classes, the smaller (1.40μm ± 0.33 S.D.) ending within SGSl, and the larger (1.73μm ± 0.67 S.D.) within SGS3. The mean size of terminals in SGS2 is intermediate between that of SGSl and SGS3. Furthermore, we have found that the ipsilateral retinocollicular terminals, which end primarily in the upper 100μm of SGS2, correspond in size with the larger terminals of the contralateral SGS3 (1.87 ± 0.72 S.D.). Thus, it appears that there is a gradient of retinocollicular terminal size within the superficial grey: small terminals are more dorsally located and large terminals more ventrally located. This suggests a correlation with the distribution of W and Y cell axons as established by electrophysiological methods. While we have been able to identify different sized retinocollicular terminals, we have not been able to correlate this difference with either vesicle shape or type of synaptic density. Thus, retinal terminals contain round vesicles and form asymmetric contacts and the morphology of these terminals appears consistent in all layers of the superficial grey. However, there is a correlation between the morphology of profiles postsynaptic to retinal terminals and depth: 40% of postsynaptic profiles in SGSl (W cell input?) contain round vesicles in contrast to 23% in SGS3 and 19% in ipsilateral SGS2 (Y cell input?).

Mean counts of degenerating terminals from 7 contiguous sample areas cross the medio-lateral extent of the ipsilateral colliculus indicate that 5% of retinal terminals are from the ipsilateral eye. This figure of 5% is also obtained from counts of labelled terminals. However, presumably as a result of the patchy distribution of the ipsilateral projection, the proportion of ipsilateral terminals in small areas varies considerably.

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2607 THE ANATOMY OF THE OPTIC NERVE OF XENOPUS. Jerry N. Blancato, Dept. Chem. Eng., Univ. of Del., Newark, DE 19711, James E. Fulbrook, and David M. Koester, Inst. for Neurosci. and Sch. of Life and Health Sci., Univ. of Del., Newark, DE 19711.

The optic nerve of adult Xenopus laevis was studied by both light and electron microscopy. Approximately 90% of the fibers are unmyelinated. Unmyelinated fibers were all less than 1.0 micron in diameter. About 3.5 x 10^3 myelinated fibers ranging from 0.8 to 8.0 micra in diameter were counted. Total fiber counts yielded an estimated 6.0 x 10^4 axons in optic nerve of about 200 micra in diameter.

The distribution of fibers appeared to be random in cross sectional areas. Sections of the same nerve, taken several micra apart showed the larger fibers readily changing relative positions, revealing a sinuous path for many cells. Neuroglial cell distribution appears uniform throughout and accounts for approximately 10% of the total area. Previous studies (Wilson, Quart. of Exp. Physio. 56:83, 1971; Reier and Webster, Neurocytol. 3:591, 1974) show comparable findings and some discrepancies attributable to differences in animal ages.

Blood vessels of varying sizes were found exclusively outside the neuronal elements, within the pial sheath. Data dealing with vascular relationships, neuroglial cells types, and distribution and counts of neuronal fibers will be compared to other amphibian species and to reptilian data presented last year (Fulbrook, Soc. For Neurosci. Abs. 4:628, 1978).

2608 SPECTRAL ANALYSIS REVEALS SEPARATE VEP SOURCES RESPONDING TO CONTRAST MODULATION IN THE HUMAN CORTEX. I. Bodis-Wollner, J. Camisa*, and C.D. Hendley*, Mount Sinai School of Medicine, New York, N.Y. 10029.

The amplitude of the steady state visual evoked potential (VEP) to modulation of the contrast of a grating pattern depends on both mean spatial contrast and modulation depth (Bodis-Wollner, Hendley & Kulikowski, Perception 1:341, 1972). In some observers the VEP waveshape recorded near the inion is dominated by the fundamental, in others by second harmonic components (Bodis-Wollner & Hendley, Chap. 9, Visual Evoked Potentials in Man, 1977). A second harmonic contribution may be enhanced by maintaining adaptation to the same grating (Bodis-Wollner & Hendley, Soc. for Neuroscience, 1978, p. 620). Now, in 8 observers we simultaneously recorded VEP-s over the inion and from an electrode 7 centimeters higher. Temporal frequencies were 6, 7.6 and 9.4 Hertz. Spectral analysis of the VEP showed that three observers had similar spectral composition at both electrode locations, but in five others the harmonic amplitudes were unequal with respect to electrode location. Furthermore, a reversal of the ratio of the first and second harmonic amplitude occurred between the two electrodes, irrespective of the temporal frequency of modulation. We conclude that separate sources may respond to the fundamental and to the second harmonic frequency of contrast modulation. The tuning of each source is wide with respect to temporal frequency, but relatively narrow with respect to harmonic ratio. It seems that first and second harmonic components in the human VEP represent separate responses of distinct neuronal ensembles of the visual cortex in which linear or nonlinear properties predominate.

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2609 DISTRIBUTION OF ENKEPHALIN AND SUBSTANCE P IMMUNOREACTIVITY WITHIN AMACRINE CELLS OF THE RETINA. N. Brecha and H.J. Karten, Department of Psychiatry and Behavioral Science, S.U.N.Y., Stony Brook, N.Y. 11794.

The distribution of enkephalin and substance P immunoreactivity within the retina was studied with immunohistochemical techniques. Pigeons were perfused with cold 6% dextran and 4% paraformaldehyde in 0.1M phosphate buffer. The eyes were removed, postfixed in 4% paraformaldehyde 2-4 hours and stored overnight in 30% sucrose-phosphate buffer at 4°C. The retinae were sectioned at 10 μm either perpendicular to the vertical axis of the eye or tangential to the retinal surface. Retinal sections were subsequently incubated in antiserum directed against either Leu^5-enkephalin or substance P and processed according to standard immunohistochemical techniques. Specificity to the antiserum was established by absorption of the antiserum with 10 μm concentrations of synthetic peptides.

Immunohistochemical positive staining for enkephalin and substance P was observed with morphologically distinct classes of amacrine cells and their processes within the inner plexiform layer. Enkephalin-like immunoreactivity was located within diffuse amacrine cells which ramify within the most superficial (lamina 1) and deep (laminae 3-5) laminae of the inner plexiform layer. The majority of enkephalin immunoreactivity cells were located within the second and third tier of the inner nuclear layer. Enkephalin immunoreactivity was distributed throughout the retina with the greatest density of labeled cells within central retinal regions including the red field of the superior retina. In contrast substance P-like immunoreactivity was located within unstratified amacrine cells which ramify only within lamina 3 of the inner plexiform layer. The substance P immunoreactive cells were located at the border of the inner nuclear layer and inner plexiform layer and the second tier of the inner nuclear layer. Substance P immunoreactivity was distributed within inferior and superior retinal regions. There were less substance P immunoreactive cells located within the red field when compared to non-red field regions of the retina.

These observations demonstrate the existence of two distinct populations of peptidergic amacrine cells within the retina. The localization of peptides to the retina suggest they play specific roles in retinal processing.

2610 TEMPORAL ASPECTS OF CODING IN SINGLE CELLS OF MONKEY STRIATE CORTEX. Bruce Bridgeman, Dept. Psychology, U. of Ca., Santa Cruz, CA 95064.

Responses of single cells in parafoveal striate cortex of two Macaca Fascicularis monkeys were recorded while the animals performed a simultaneous brightness discrimination (B trials). Monkeys were rewarded for pressing a panel corresponding to the brighter of two projected discs. Interspersed at random with the B trials were other trials in which one choice was a simultaneous disc and ring, while the other was an identical flashed disc followed by a delayed ring at a delay previously determined to be optimal for metacontrast masking in that animal (M trials). These trials were rewarded for either behavioral choice. Response histograms were collected separately for correct and incorrect B trials, and for responses to M trials on the simultaneous or delayed side. On the basis of other work on temporal analysis in cats and humans, histograms were divided into early (0 to about 150msec post-stimulus) and late (150-400msec) components.

Early post-stimulus activity was independent of behavioral choice for both M and B trials (M, $t=0.49$, N. S.; B, $t=1.44$, N. S.), reflecting only stimulus variables. Late activity in M trials, however, was significantly greater when the monkey pressed the "simultaneous" side (and presumably experienced metacontrast masking on the other side) than when he pressed the "delayed" side ($t=3.41$, $p<.005$). Similarly, late activity in B trials was greater when the monkey responded on the correct side than on the incorrect side ($t=2.36$, $p<.04$). Pre-response changes were assessed by calculating a linear regression line through a 250msec backward-averaged pre-response histogram and testing the statistical significance of its slope. 25% of the cells showed a pre-response slope significant at $p<.05$. Differences in overall cell activity had disappeared by this late epoch (M trials, $t=1.70$, N. S.; B trials, $t=1.11$, N. S.), though the pre-response histograms summed in each trial type appeared different for correct vs. incorrect B responses, or simultaneous vs. delayed M responses. Thus the overall difference in firing level in the late post-stimulus interval was recoded to a difference in temporal pattern just before the response.

These results show that the first burst of stimulus-evoked firings in visual cortex is related only to the stimulus; at a later interval in the same cells this information is combined with data about the meaning of the stimuli in the current context, and at a still later time the same cells receive information about the organization of the behavioral response.

2611 SPATIAL RESOLUTION IN THE LGN OF KITTENS REARED WITH ARTIFICIAL STRABISMIS. James L. Burchfiel, George Mower*, Frank H. Duffy, and David Berry*. Seizure Unit & Dept. of Neurology, Children's Hospital & Harvard Med. Sch., Boston MA 02115.

A characteristic deficit in strabismic amblyopia is decreased acuity in the deviating eye. Changes of spatial resolving capacities in single cells of the lateral geniculate nucleus (LGN) may underlie this visual deficit. Here, we compare several models of strabismic amblyopia in terms of their effects on spatial resolution of single cells in the LGN.

Kittens were reared in total darkness from birth except for daily one-hour visual exposure sessions. One group was surgically esotropic. Another group wore goggles with twelve diopter prisms which optically displaced the image to one eye. Normal and monocularly deprived kittens with similar rearing histories served as controls.

Single units in the LGN were classified as "X" or "Y" and the highest spatial frequency square-wave grating which elicited a modulated response was determined. Significant differences ($P< .001$, t test) in mean spatial resolving power between the normal and treated eye were found for X cells in monocularly deprived (X=2.7 c/deg vs 1.3), esotropic (2.3 vs 1.1) and prism (2.4 vs 1.1) kittens. The differences were most marked for cells close to the area centralis. There were no differences in X cells of the normal control (2.0 vs 1.9). Y cells showed significantly lower spatial resolving power than X cells in all animals, but there were no differences between Y cells devoted to one or the other eye in any animal. Histological studies of the LGN are in progress, as are physiological comparisons of fixed and variable prismatic displacements.

We conclude that interocular differences in spatial patterns are sufficient to produce deficits in spatial resolution of LGN cells.

2612 NEURONS IN THE NUCLEUS OF THE BASAL OPTIC ROOT (ACCESSORY OPTIC SYSTEM) OF BIRDS RESPOND PREFERENTIALLY TO VERTICAL STIMULUS MOVEMENT. Sheila Burns* and Josh Wallman. Dept. of Biology, City College of the City University of New York, New York, NY 10031.

The accessory optic system, which is found in all vertebrate classes, has been implicated in the visual control of eye and head movements. In birds, the accessory optic system contains the nucleus of the basal optic root (nBOR), which is also known as the ectomamillary nucleus and is homologous with the medial terminal nucleus of mammals. The nBOR has several interesting anatomical features: (1) its principal input is from the displaced ganglion cells of the retina, a clearly identifiable collection of neurons, (2) it projects directly to the oculomotor complex, providing a short loop for visual control of eye movements and (3) it also projects to the vestibulocerebellum both directly, as mossy fibers and indirectly, via the inferior olive, as climbing fibers.

Single unit recordings were made from nBOR in chickens. Preliminary results show that individual neurons have extremely large receptive fields and respond preferentially to stimuli moving vertically at slow velocities (about 1 deg./sec.). They respond best to large stimuli and poorly, if at all, to small or stationary stimuli. Neurons with a preference for movement in the horizontal direction appear to be absent from this nucleus. The physiological properties of the neurons are, thus, strikingly consistent with the anatomical findings of Brecha and Karten (Science 203: 913, 1979; pers. comm.) which show that projections of nBOR in the oculomotor complex are to regions which control vertical eye movements. (Supported by NIH Grant EY-2937).

2613 THE MONKEY FRONTAL EYE FIELDS HAVE A NEURONAL SIGNAL THAT PRECEDES VISUALLY GUIDED SACCADES. M. C. Bushnell* and M. E. Goldberg. Lab. Sensorimotor Res., National Eye Institute, NIH; Behavioral Sciences Dept., Armed Forces Radiobiol. Res. Institute, Bethesda, MD 20014; and Dept. Neurology, Georgetown Univ., Washington DC.

Electrical stimulation of the monkey frontal eye fields (FEF) elicits conjugate contralateral saccades, yet in this area the only neurons found to be related to saccades discharge during or after the eye movements (Bizzi, 1968). Other cells in FEF respond to visual stimuli (Mohler, Goldberg, and Wurtz, 1973), and half of these neurons show an enhanced response before visually guided saccades (Wurtz and Mohler, 1976). We now show that this enhancement is specific to saccades and independent of the on-response of the neurons. Activation of these neurons could account for the eye movements evoked by stimulation of FEF.

We recorded extracellularly from over 200 neurons in rhesus monkey FEF while the monkeys performed visual tasks. The monkeys learned to fixate a spot of light and then make one of several responses to peripheral visual stimuli presented both inside and outside the receptive field of the neuron being studied: to continue to fixate the spot and ignore the peripheral stimuli, to make a saccade to one of the stimuli, or to reach out and touch a stimulus without breaking fixation on the original spot.

Nearly half of the visually responsive neurons in FEF showed more vigorous responses to the stimulus onset when the monkey was preparing to make a saccade to the receptive field stimulus. This enhancement was specific to a saccade to the receptive field; it did not occur when the monkey made a saccade outside the receptive field or when he touched the stimulus in the receptive field without making a saccade to it.

FEF neurons responded phasically to the onset of a stimulus, and then their activity habituated to a background level even if the stimulus stayed lit. We trained monkeys to make saccades to constantly illuminated visual stimuli using the disappearance of the fixation point as a cue. When the constantly lit stimulus was in the receptive field of an enhanceable neuron, the neuron gave no significant response until the cue to make the eye movement, at which time the neuron would produce a large burst of discharge that preceded the eye movement. This burst was predictive of a visually guided saccade; it did not occur when the animal made the identical movement in total darkness. Thus there is a discharge preceding visually guided eye movements that can be dissociated from the on-response of the neuron. It occurs only when the animal prepares to make a saccade to the stimulus in the neuron's receptive field.

Constant current biphasic stimulation through the microelectrode at sites of visually responsive neurons gave saccades into their receptive fields at thresholds as low as 20 uA. Microstimulation at the sites of cells that discharge during or after eye movements did not evoke saccades.

The enhanced response of FEF neurons may provide a retinal error signal that drives the brain stem to produce visually guided saccades. This activity may provide the biological signal duplicated when electrical stimulation of FEF evokes saccades.

2614 THE EFFECT OF INTEROCULAR ADAPTATION ON CONTRAST MODULATION. J. Camisa*, I. Bodis-Wollner, and C.D. Hendley*. Mount Sinai School of Medicine, New York, N.Y. 10029.

A contrast modulated grating is the sum of a steady and a counterphase flickering grating of the same spatial frequency. Adaptation to a steady grating separates the two mechanisms subserving detection of this stimulus: as a result of adaptation, the threshold of the counterphase component becomes independent of the stationary contrast (Bodis-Wollner & Hendley, J. Physiol., 1979). In addition, adaptation "enhances" the sensitivity to the counterphase component above 10% mean contrast. This paradoxical result is presumably due to some sort of "adapting out" of the steady component which at high mean contrasts normally reduces sensitivity to the counterphase component. In the present study the effect of contraocular adaptation on contrast modulation sensitivity was explored for a 6 c/deg grating modulated at 8 Hz. Contrast modulation thresholds were established both before and after adaptation for mean contrasts ranging from detection threshold to 40%. There was little and often insignificant effect of adaptation on the modulation sensitivity near detection threshold, but in the range above 10% contrast we again found a paradoxical increase in sensitivity similar to the effect of monocular adaptation. These results indicate that the interaction of the stationary and counterphase components of the 6 c/deg grating are affected by interocular adaptation, and therefore the major site of this interaction between the stationary and counterphase components must be at or beyond the point of binocular convergence.

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2615 STIMULATION OF THE CAT SUPERIOR COLLICULUS EVOKES EAR MOVEMENTS WHICH PARALLEL EYE MOVEMENTS. H. Peter Clamann and Barry E. STEIN. Dept. Physio., Med. Coll. of Va., Richmond, VA 23298.

The superior colliculus is believed to be involved in orienting receptor organs to a variety of sensory cues. Yet, while the influence of the colliculus on eye movements has been studied extensively, relatively little information is available about its control of ear movements. These experiments were directed toward examining whether or not an ear movement "map" is present in the cat colliculus and, if so, what its relationship is to the eye movement map. A cylindrical steel chamber was implanted over a cranial opening in each of 10 cats 1 to 3 weeks prior to experimentation. During an experiment the animal was alert. Its head was rigidly held by a special mount attached to the cylinder, so that no wounds or pressure points were created during experimentation, and movement of the ears was not impeded. Electrical stimuli were delivered through tungsten electrodes lowered to the colliculus through visual cortex. Both eye and ear movements were evoked in most electrode penetrations, and their thresholds were usually lowest at the same depth in the colliculus. Initial ear position and the final position achieved during stimulation were photographed with three orthogonally placed cameras. Reliable, ballistic-like movements of the contralateral ear were evoked and were predictable on the basis of stimulus site. Evoked ear and eye movements paralleled each other with lateral sites in the colliculus producing downward movements and medial sites producing upward movements. Stimulation of the most rostral aspect of the colliculus evoked forward ear movements while stimulation of caudal sites evoked backward ear movements. Colliculus-evoked movements orient the ears and eyes toward the source of a natural sensory stimulus. However, the organization of ipsilateral (to site of electrical stimulation) ear movements appears to be complicated by the sound-shadowing effect of the head.

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2616 PROJECTIONS OF THE LATERAL GENICULATE AND LATERAL POSTERIOR NUCLEI TO POSTERIOR NEOCORTEX OF RAT. W. J. Clerici, J. Coleman and D. Clark. Depts. Psychol. and Physiol., Univ. of South Carolina, Columbia, SC 29208.

Most mammals studied conform to the classical view that the projection from the dorsal lateral geniculate nucleus (GL) is restricted to area 17 of cortex. Using the retrograde degeneration method Lashley (J. Comp. Neurol., 1934, 60, 57) reported such a projection confined to area 17 of the rat. More recently, results using neuronal transport techniques suggest that GL also projects to extrastriate cortex in rat (Hughes, J. Comp. Neurol., 1977, 175, 311). Our goal was to study thalamic input patterns to areas of posterior cortex of albino rat by making horseradish peroxidase (HRP) injections of limited spread by using microiontophoresis. HRP injection of lateral striate cortex produced a column of labelled cells at the dorsal border of GL; injection remote from the lateral border of striate cortex produced a column of labelled cells ventrally within GL. Striate injections also labelled cells in the lateral posterior nucleus (LP). Injection in Krieg's area 18a adjacent to the caudal-lateral portion of area 17 produced not only columns of labelled cells in LP, but also diffuse labelling within GL. An HRP injection placed more laterally in 18a labelled numerous cells in LP, and in GL more sparsely. Injection of area 36 lateral to 18a produced labelled cells in caudal LP without any GL labelling. In conclusion, GL projects topographically to striate cortex of rat and in a more diffuse manner to portions of area 18a, while LP connections are distributed in a systematic manner to striate and extrastriate areas. The projection of GL outside striate cortex of rat may differ in organization and magnitude from that observed in the cat.

2617 CORTICAL TOPOGRAPHY IN SIAMESE CATS. Michael Lee Cooper and Gary G. Blasdel*. Division of Biology, California Institute of Technology, Pasadena, Ca. 91125

In Siamese cats, many ganglion cell axons from the temporal retina are misrouted in the chiasm, so that they misproject to the contralateral hemisphere. As a result, the Siamese LGN contains a representation of much of the ipsilateral visual field. Two mechanisms have been described for the way in which the Siamese visual cortex processes this aberrant visual information. In the "Midwestern" pattern, the abnormal geniculocortical inputs are suppressed, resulting in a roughly normal topography, with the border between areas 17 and 18 representing the midline of the visual field. In the "Boston" pattern, the aberrant inputs are inserted in an orderly fashion between the maps of the contralateral hemifield in areas 17 and 18, so that the 17/18 border comes to represent a point up to 20^{o} into the ipsilateral field.

We recorded from four Siamese cats; by making several electrode penetrations in each animal, we tried to map the region of the 17/18 border in at least two widely separated AP levels. By determining the receptive field positions for single units at intervals of about 100 μm along each electrode track, we obtained data concerning the extent of the ipsilateral field representation at each cortical level. In all of our animals, tracks made at coronal levels corresponding to the horizontal meridian and those made at levels representing lower visual field showed substantially different maximum excursions into the ipsilateral hemifield. Thus, penetrations crossing the 17/18 border at levels corresponding to average elevations between -7^{o} and -13^{o} resulted in maximum excursions of 14^{o} to 19^{o} into the ipsilateral field. Therefore, these tracks conformed to the "Boston" cortical pattern. However, in these same cats, more posterior tracks traversing the 17/18 border at levels representing average elevations from $+1^{o}$ to -4^{o} yielded receptive fields which were never centered more than 6^{o} into the ipsilateral hemifield. These posterior tracks thus gave maps reminiscent of the "Midwestern" modification. These differences in the extent of ipsilateral field representation were not due to any outward cyclorotation of the eyes during paralysis, as shown by photographic determinations of eye rolling made during each experiment.

The applicability of our results is not restricted solely to cats from our colony, since one of our four experimental animals was obtained from outside sources. However, it appears that not all "Boston" cats follow the pattern we describe, since Shatz and LeVay (Science, 204, 1979) found typical "Boston" topographies near the horizontal meridian in animals from their colony.

(The authors thank J.D. Pettigrew for advice and support.)

2618 ELECTROCORTICAL RESPONSES TO VISUAL SPATIAL FREQUENCY IN BEHAVING MONKEYS. Richard Coppola and Richard K. Nakamura, NIMH, Bethesda MD 20205

Cortical potentials to visual stimulation by gratings of various spatial frequencies (SF) were obtained from alert rhesus monkeys to gain some insight into the processing of visual pattern information. Stimuli consisted of vertically oriented sine wave or square wave gratings in the range 0.125 to 8.0 cycles/degree. They were presented on a computer controlled CRT screen in both pattern appearance-disappearance and pattern reversal sequences. Contrast was held constant at 0.44 and luminance was constant at 0.2 ft-L. The animals had chronically implanted electrodes of stainless steel or platinum wire with ball tips placed on the dural surface at striate (S), prestriate (PS) and inferior temporal (IT) locations. The animals were trained to attend to the screen as follows. A trial was initiated by a lever press which caused the grating to appear-disappear or reverse in contrast each 250 msec. After random time periods the screen activity stopped and the animal had to release the lever to get a water reward. 500 msec from the start of the trial the amplified electrical activity from the electrodes was digitized with correct trials saved and averaged.

The appearance response at striate consisted of a positive component at 100 msec latency whose amplitude decreased with SF and a negative component at 150 msec which was relatively flat with SF. The prestriate response had three components: positive at 90-110 msec that peaked at low SF and then declined with increasing SF; negative at 130-150 msec that peaked with SF and; positive at 190-210 msec that increased slowly with SF. The IT response had a negative component at 100-120 msec and a positive at 190-210 msec both of which were relatively flat with SF. The largest difference between sine wave and square wave gratings was for the late positive component at PS and IT, which were smaller for sine wave.

The reversal response at striate was a large positive wave at 100 msec that showed a relatively sharp peak at 2-4 c/deg. The PS response at 100 msec had a less sharp variation with SF. IT had a more variable response that seemed to show a considerable latency change with SF. Square wave reversal responses were generally larger than sine wave but showed the same form relative to SF.

These findings are similar to those for human responses to checkerboard stimulation (Jeffreys and Axford, Exp. Brain Res. 16:1-40,1972). The components of the PS response in particular seem related to the CI, CII, and CIII responses. Further, these findings are consistent with the idea that CII and CIII are of extrastriate origin.

2619 METABOLIC PATTERNS INDUCED IN MONKEY VISUAL CORTEX BY STIMULATION WITH COLORED LIGHTS. M.L.J. Crawford, J.D. Fagan*, M. Borchert*, A. Heston*, and R.E. Marc. U. of Texas Graduate School of Biomedical Sciences, Houston, Texas 77025.

The visual cortices of young monkeys stimulated monocularly by monochromatic lights show interesting patterns as revealed by the labeled 2-deoxyglucose method. In the autoradiographs from the striate cortex there are narrow bands of increased metabolic activity which extend from the cortical surface to the white matter but, unlike the orientation columns described by Hubel, Wiesel, and Stryker (J. Comp. Neurol., 1978, 177, 361-379), cortical layers I and II contain the highest density of the label. The labeled column is significantly narrower than the adjacent non-labeled space (0.367mm vs 0.529mm, N=500) suggesting that only a subpopulation of the neurons of the eye-dominance hypercolumn was activated by the colored stimulus. In tranverse sections the superficial cortical layers appear as strings of labeled spots, similar to those described for orientation columns, but bearing a close relationship to the eye-dominance hypercolumn (within row period = 0.850mm). These results are consistent with the idea that, at the striate cortex, color information is processed in a mosaic of functional columns of neurons and subsequently relayed to more specialized pre-striate areas.

In the pre-striate cortex, a complex pattern is consistently found throughout the inferior occipital sulcus (IOS). In the vault of the IOS the columns are 0.650 to 0.750mm in width with a period of 1.8mm. Crossing the IOS vault at right angles, the bands of label extend down the posterior bank to terminate at the ventrolateral edge of the striate cortex. Similarly, the broad parallel bands run down the anterior bank of the IOS to become diffuse at the ventral edge. Zeki (J. Physiol., 1978, 277, p. 233) has described aggregates of color specific neurons found in this area of pre-striate cortex.

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2620 BINOCULAR INTERACTIONS ACROSS THE CORPUS CALLOSUM. Max Cynader, Allan Dobbins, Jill Gardner, Franco Leporé and Jean-Paul Guillemot. Dept. Psychol. Dalhousie Univ. Halifax, N.S. and Dept. Psych. Université de Montreal, P.Q. Canada.

We have examined the responses of cortical units to monocular and binocular visual stimulation in cats in which the optic chiasm had been surgically-sectioned. The effect of this manipulation is to interrupt the direct retina-geniculate-cortex pathway from the contralateral eye, leaving the connections from the ipsilateral eye intact. Quantitative study of over 100 cortical units located near the border between areas 17 and 18 (with receptive fields located near the vertical meridian) showed that some units were driven exclusively by stimuli presented through the ipsilateral eye. However the firing rate of more than half of the units encountered could also be influenced by stimulation through the contralateral eye. The effect of visual stimuli presented through the contralateral eye could be excitatory or inhibitory.

We tested cortical units for their sensitivity to the retinal disparity of binocularly presented stimuli (corresponding to different positions in depth) and also for sensitivity to different directions of motion in three-dimensional space. While many units did not respond differentially to visual stimuli with varied locations or trajectories in depth, a subset of the units encountered were highly sensitive to the position and/or direction of movement of stimuli in 3 dimensional space.

Units showing strong binocular interactions were clustered within what appeared to be hemicolumns in the visual cortex. Within these hemicolumns which extended from the cortical surface to the geniculate-recipient zone in layer 4, successively-recorded units were sensitive to particular characteristics of binocular stimuli. On a given penetration through the superficial cortical layers one might encounter cell after cell which responded optimally to stimuli moving toward the animal. Other penetrations yielded units which responded optimally to stimuli moving sideways at a given depth. Binocular interactions were most pronounced in units of the supragranular cortical layers and nearly absent in layer 4. In the infragranular layers the large-field complex cells were often binocularly-activated but displayed no selectivity for disparity. Some units in cortical layer 6 did however respond differentially to stimuli at different disparities.

The responses to stimulation of the contralateral eye were abolished or much reduced after inactivation of the visual cortex on the other side of the brain indicating that the major pathway mediating these responses is the corpus callosum.

2621 EFFECT OF A COMBINATION OF MONOCULAR AND DIRECTIONAL DEPRIVATION ON THE PERCENTAGE OF NON-SPECIFIC CELLS IN CAT VISUAL CORTEX. Nigel W. Daw and Michael Ariel*. Dept. Physiol., Washington Univ. Med. Sch., St. Louis, MO, 63110.

Two groups of kittens were reared with a combination of monocular and directional deprivation. Group A (3 animals) had one eye sutured shut at 2 weeks of age, with each kitten placed in a striped drum rotating continually around it in one direction for one hour per day, otherwise remaining in the dark. Group B (5 animals) was reared similarly, except that the direction of drum rotation was reversed at 5 weeks of age. These rearing conditions were continued until 12 weeks of age, then the animals remained in the dark until electrophysiological recording. Cells recorded from visual cortex were classified as unidirectional, bidirectional, and omnidirectional or "hard to drive"; according to their ocular dominance; and various other characteristics.

The percentage of omnidirectional or nonspecific cells ranged from 22% to 41% for the deprived animals, compared to 6% to 15% for normal animals. Omnidirectional cells were found in substantial numbers in all layers of the cortex in the deprived animals, interspersed with specific (unidirectional or bidirectional) cells. Very few cells were dominated by the closed eye: of those that were most were nonspecific. The results of the animals in Group A showed that the monocular deprivation had a much more powerful effect than the directional deprivation for these rearing conditions. The results, when displayed in a two dimensional array with ocular dominance along one axis and directional sensitivity along the other, suggest that these cortical cells either lose their input from one eye, or lose their directional specificity, or both.

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Withdrawn by Author

2623 NEURONS IN THE SUPERIOR TEMPORAL SULCUS OF THE MACAQUE STILL RESPOND TO VISUAL STIMULI AFTER REMOVAL OF STRIATE CORTEX. R. Desimone*, C. Bruce* and C. G. Gross. Dept. Psychol., Princeton Univ., Princeton, N.J. 08540

Following total removal of striate cortex, monkeys (and man) retain an impressive degree of complex visually-guided behavior. Yet no cortical area has yet been found whose visual responsiveness survives striate removal or inactivation. We report on a cortical area which does retain visual responsiveness after striate removal. This area, the Superior Temporal Polysensory Area, lies in the upper bank and fundus of the anterior and middle portions of the superior temporal sulcus. Neurons in this area respond to visual, auditory and somesthetic stimuli, and usually have receptive fields encompassing virtually the entire visual field (including both half-fields). About half the units are sensitive to the direction of movement of a stimulus, usually preferring movement in depth or movement towards the fovea from any point in the periphery (or vice-versa) (Neurosci. Abs. 3, 554).

In three monkeys, striate cortex was removed unilaterally. Several weeks later the monkeys were paralyzed, anesthetized with N_2O, and single unit responses were recorded from the Superior Temporal Polysensory Area ipsilateral to the lesion. Each animal was repeatedly recorded from. Over two-thirds of the units responded to visual stimuli in the half-field contralateral to the striate lesion. In this "cortically blind" half-field, receptive fields were still very large but there was little or no sensitivity to the direction of movement. Some of the units which had no direction specificity in the contralateral field did have such specificity in the ipsilateral field. Preliminary experiments in two monkeys indicate that the superior colliculus contributes to the visual responsiveness that remains after the striate lesion.

2624 MOVEMENT-SENSITIVITIES OF VISUAL INTERNEURONES IN THE MEDULLAE OF FLIES. Robert D. DeVoe. Dept. Physiol., Sch. Med., Johns Hopkins Univ., Baltimore, MD 21205.

Intracellular recordings from the medulla (second optic ganglion) were made in intact, restrained flies that faced a hemispherical surface upon which moving patterns and spots were projected from the rear. Most cells responded to light and movement with slow-wave depolarizations or hyperpolarizations; some had spikes in addition or alone. In the distal medulla, noisy hyperpolarizing responses to light alone were characteristic of incoming axons of second-order, laminar cells. These cells responded with flicker-like oscillations to black-white alternations within moving gratings of 15°/cycle but not of 2.5°/cycle. Medullary cells penetrated nearby responded to grating movements with similar but depolarizing oscillations that were 180° out of phase with the laminar cell responses and may have been driven by the laminar cells. These simple, oscillating responses to gratings are quite likely indicative of the inputs to the motion detectors.

Most medullary cells had abrupt, maintained depolarizations in response to moving gratings. When the direction of movement was reversed, there were short, rapid, partial-repolarizations, but when movements stopped before reversals, the cells would partially-repolarize much more slowly. The depolarizations to movement were non-directional, but directionally-biased oscillations superimposed upon the depolarizations were sometimes recorded, either with increasing speed of movement or when the moving grating was restricted to within a small projected spot. A few, spontaneously-active spiking cells that were inhibited (hyperpolarized) by light were probably not purely OFF cells. Rather, these cells might have been inhibited by other, movement-excited cells, since one cell inhibited by light during movement resumed firing when movement was stopped.

Receptive fields of medullary cells were tested with small spots and were found to be simple, non-opponent and generally elliptical. It was usually possible to match a response elicited at any point in the receptive field to a response elicited by a suitable intensity at the center of the receptive field. Maximum extents of receptive fields ranged from 40° to 60° of visual field. Edges and spots elicited their largest responses when they moved across the borders of the receptive fields. These results indicate that large numbers of medullary cartridges contribute to the movement responses recorded.

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2625 A DEMONSTRATION OF COMPLEX PATTERN VISION IN THE MONKEY COMPLETELY LACKING STRIATE CORTEX. John Dineen* and E. Gregory Keating. Dept. Anat., Upstate Medical Center, Syracuse, N.Y. 13210 and Neurology Service, Veterans Hospital.

We developed a series of discrimination tasks to ascertain the types of visual cues that can be used by de-striate monkeys. After long periods of shaping and postoperative training, three of our five monkeys were able to discriminate a number of patterns that were equated for luminance and luminous flux.

Our results verify that the de-striate monkey can discriminate multi-element patterns differing in spatial organization but equated for amount of contour, number of corners and number of elements. The performance of our animals on several control tasks indicated that the monkeys were not simply using specific local cues but were using the overall organization of each stimulus to distinguish between patterns.

Histological examination of one of our animals revealed complete bilateral removal of area 17. No cell clusters were present throughout the extent of both lateral geniculate nuclei.

We interpret these results as evidence that the de-striate primate can realize the overall spatial organization of light falling on its retina, i.e., pattern. Our animals were not, as previous research would have suggested, limited to merely summating the amount of various visual features (e.g., contour or corners) irrespective of their spatial distribution. These results support Schilder et al's observation of basic form vision in the de-striate monkey and further extends the capacities of the primate extrastriate pathways to also include more complex pattern discrimination. The extensive postoperative training needed for our animals, however, suggests that the geniculo-striate pathway is the primary pattern vision system in the normal monkey.

(Supported by USPHS NS10576 & EY02941 and by Veterans Administration Research Funds)

2626 SPATIO-TEMPORAL INTERACTIONS IN CAT VISUAL CORTEX AND THE RESPONSE TO MOVING STIMULI. Robert M. Douglas* and Max Cynader (SPON: G. Melvill Jones) Psychology Dept., Dalhousie University, Halifax, N.S. B3H 4J1.

Many cells in the visual cortex respond much more vigorously to stimuli moving on one direction than in the opposite. We have examined how this property may be constructed from timed interactions between parts of the receptive field.

The receptive fields of units in area 18 of the cat were mapped and divided into 7 positions. Light bars 0.5° wide and 20 msec in duration were flashed onto one or two of these regions. Stimulation of two regions sometimes evoked a response considerably different from that expected from the sum of the responses to the 2 bars delivered separately. Both increases (facilitation) and decreases (inhibition) were observed. The pattern of these interactions was studied systematically in 15 cells. The 2 bars were flashed on all combinations of the 7 positions, and the delay between onsets varied between 0 and 640 msec. The 343 (7x7x7) stimulus conditions were presented in a randomized interleaved order, and the unit's responses compared with the linear prediction. At the same time, the directional properties of the cell were examined with bars of the same width moving between 2 and 512 degrees/sec.

Both facilitation and inhibition were widespread. Particularly common was a facilitation with 640 msec. intervals. However, it was the interactions at the shorter intervals (20-80 msec) that appeared to be most responsible for the directional properties. Stimulation of 2 positions in the same order as occurs when a stimulus moves in the preferred direction produced either a facilitation or an absence of inhibition. Movement in the opposite direction evoked inhibition. In the most directional cells the interactions were large and consistent. The consistency took 2 forms. In some cells, the spatial position of the major facilitatory or inhibitory feature was independent of the position of the first bar. In other cells, the position was relative. For any position used for the first bar, the facilitation occurred 1 to 2° away in the preferred direction.

Quantitative predictions for moving stimuli were obtained by first summing the responses to the single bars, and then adding those interactions corresponding to a given direction and speed. Without any further assumptions this measure was able to predict the preferred direction, although the degree of directionality was underestimated, particularly at the low velocities. Thus the directional properties of cortical neurons appear to be due to these interactions between receptive field areas.

2627 Extrastriate Color Cells in the Awake Monkey. Bruce Dow, Roman Bauer* & Robert Vautin*, Neurobiology Division, SUNY, Buffalo, NY 14226.

We have been examining the response properties of extrastriate cells in the foveal projection pathway of alert, behaving macaque monkeys (Rhesus and Cynomolgus), and have identified substantial numbers of color-selective cells. There is a concentration of such cells in the anterior bank of the lunate sulcus, confirming Zeki's (1973) earlier report of a color area (V4) in this region. Other color cells are found in the inferior occipital sulcus. Electrolytic lesions along with receptive field properties permit us to distinguish color cells in the various subregions of extrastriate cortex.

Our major concern has been to determine the color preference of extrastriate color cells. On the basis of cone inputs and spectral response peaks, there appear to be two distinct cell populations. The first group has opponent inputs from the long and middle wavelength cones (L/M cells) and excitatory/inhibitory peaks in the vicinity of 610-640nm (red) and 480-510nm (blue-green). The second group has synergistic input from long and short wavelength cones and antagonistic input from middle wavelength cones (LS/M cells). Excitatory/inhibitory peaks for this group are at the two ends of the spectrum (red-blue, i.e., purple) and at 530-570nm (yellow-green). Neutral points for L/M cells are in the same spectral region (530-570nm) as the central peaks of LS/M cells. LS/M cells have two neutral points, one toward each end of the spectrum. Some LS/M cells with very lateral neutral points can masquerade as non-color cells because of their midspectral response peak. Others with more medial neutral points can masquerade as either S/LM cells or L/MS cells. In all such cases we find responses to extraspectral purple (matched for brightness) to be more vigorous than responses to either blue or red alone. In instances where a neutral point was not immediately evident at one or the other end of the spectrum we have been able to uncover an opponent mechanism through the use of chromatic adaptation.

The two spectral loci of the opponent response peaks of any given color cell can be used to generate a line in CIE color space. Most such lines appear to pass close to the achromatic center of CIE color space. Color axis lines of L/M and LS/M cells are generally orthogonal in color space, though there is a considerable degree of scatter in each group. The data thus suggest anisotropy in the distribution of color axis lines. We are currently collecting further data to test this hypothesis.

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2628 RETINOFUGAL PROJECTIONS IN PIGMENTED, ALBINO AND ONE-EYED MICE. Ursula C. Dräger and John F. Olsen.* Dept. Neurobiol., Harvard Medical School, Boston, MA 02115.

Retinofugal projections were studied in black and albino mice, and in mice in which one eye had been removed at birth. One optic tract was cut at the level of the lateral geniculate nucleus and soaked in horseradish peroxidase. Most but not all retinal ganglion cells that projected into the tract were filled; from mouse to mouse filling varied. In the retina contralateral to the injected tract, cells were stained throughout. Ipsilateral to the injection all 3 types of mice had labelled cells mainly within a crescent-shaped area, which formed the lower and temporal margin of the retina and constituted about 20% of total retinal area. Displaced ganglion cells, located at the inner border of the inner nuclear layer, were concentrated in the peripheral retina, and were relatively much more common among ipsilaterally than among contralaterally projecting cells: for all 3 types of mice, 15.6% (sd 3.9) of the ipsilateral cells and only 0.95% (sd 0.3) of the contralateral ganglion cells were displaced.

Less ganglion cells were filled from the ipsilateral optic tract in albino than in pigmented mice: the best example from a black mouse had 978 filled cells in the ipsilateral retina, the best-filled albino retina 655 cells. The number of ipsilateral cells averaged approximately 2.8% of the total contralateral population in black mice, and 2.1% in albinos. This correlated with recordings in area 17 of albino mice: though the binocular region was normal in extent and most cells could be driven from both eyes, cells dominated by the ipsilateral eye were far less common than in black mice. Cell-area measurements in both albino and black mice showed that ganglion cells destined for the ipsilateral tract were on the average between 36 and 48% larger than contralaterally projecting ones. In unilaterally enucleated mice the number of ganglion cells projecting ipsilaterally from the remaining eye was much higher than in two-eyed mice: in the best-filled retina we counted 1592 such cells. A population of predominantly small cells filled from the ipsilateral optic tract was observed throughout the retina, outside the crescent region, in all 3 types of mice. In black mice these aberrant cells accounted for 4% of all cells in the ipsilateral retina, in albinos for 6.8% and in one-eyed mice for 14%.

The albino mutation in mice thus causes a homogeneous reduction in ipsilateral retinal projection, with much more subtle consequences for central visual pathways than described for Siamese cats. Eye enucleation at birth leads to an increase in the projection from the remaining eye to the hemisphere ipsilateral to it, in number of cells from topographically appropriate and inappropriate regions.

2629 THE LANDING REACTION (LR) OF FLIES: A MODEL REACTION FOR STUDYING THE NEURAL BASIS OF BEHAVIOUR. Hendrik E. Eckert, Dept. Animal Physiol., RUB, P.O. Box 102148, D-4630 Bochum 1, W.-GERMANY.

The landing reaction consists in flies of a simultaneous upwards throw of both foreleg tibiae obeying an 'all-or-none' rule. Employing stripes moving apart and periodic moving gratings the dependence of the LR - measured as the number of positive responses to 20 stimulus presentations - on the angular velocity, the extent and directions of pattern motion was tested for different regions of the eye. (1) Despite the 'all-or-none' rule of the behavioural reaction, the neural circuit is activated in a graded manner: the larger the extent of angular movement the higher the percentage of stimulus induced responses if ommatidia from the anterior to the posterior part of the eye (progressive motion) were stimulated; reversal of this direction of motion (regressive motion) exerts a graded inhibitory influence as can be shown by simultaneous and successive regressive and progressive motion. (2) The most effective direction of stimulus motion corresponded to progressive motion for all regions of the eye; the higher sensitivity of the frontal (binocular) region of the eyes to upwards motion is caused by binocular interactions. (3) The response to objects presented at different distances to the animal indicates a capability for depth perception. (4) The most effective angular velocity corresponded to a contrast frequency (i.e. the ratio of angular velocity and angular width of the pattern period) of 6-7 Hz, thus clearly separating the LR from the optomotor torque response whose peak lies at 1.4 Hz. The position of these peaks cannot be altered by subsequent filtering since the measured responses already represent the time-averaged outputs of the neural circuits. Thus separate neural pathways have to be postulated for mediating these two behavioural responses.

This behavioural investigation provides the means of studying the respective underlying neural circuits (e.g. depth perception, orientation of elementary movement detectors, excitation and inhibition).

Paper: Eckert, H.; Fligge, B.; Hamdorf, K.: Naturwissenschaften, in press (1979).

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2630 INCREASE IN MONOCULAR DOMINANCE IN AREA 17 OF THE CAT FOLLOWING NEONATAL SECTION OF THE POSTERIOR CORPUS CALLOSUM. Andrea J. Elberger. Dept. of Anatomy, University of Pennsylvania School of Medicine, Philadelphia, PA. 19104.

Extracellular single unit recordings in Area 17 were made in 8 adult cats that had received neonatal surgical section of the posterior corpus callosum. Callosal surgery was performed when the cats were 13-29 days of age; 2-3 years elapsed before the present experiments occurred. During the interim time period all cats had unrestricted visual experience.

Cells were examined qualitatively, using hand-held projected stimuli, and classified according to cell type (simple, complex, hypercomplex), ocular dominance (1-7, Hubel and Wiesel), receptive field size, and location from the vertical meridian. The cells were sampled across many ocular dominance columns, and were excluded from data analysis if orientation selectivity was not observed. For purposes of comparison, 3 normal adult cats were studied; in addition, reference to an additional normal population (Albus, 1975) was made. The extent of corpus callosum section was verified by subsequent histology.

There is a significant increase in monocularly driven cells (groups 1 and 7) throughout the visual region examined (receptive field centers 0.0-39.0°) following neonatal corpus callosum section. Forty-five percent of classified cells were monocularly driven, as opposed to 24% of the normal cats' cells studied. In the central region, 0.0-3.9°, the higher proportion of monocularly driven cells agrees with previous data for normals (Albus, 1975) Therefore, the observed increase in monocularly driven cells occurs mainly in cells with receptive field centers from 4.0-39.0°.

An additional comparison of the ocular dominance distribution of neonatal corpus callosum sectioned cats with adult corpus callosum sectioned cats (Payne, Elberger, Berman and Murphy, this volume) indicates that while neonatal, as well as adult, callosum sectioned cats show significant shifts in ocular dominance from normal cats, the neonatal results also differ significantly from the adult callosal results. The neonatal corpus callosum sectioned cats show a proportion of monocularly driven cells intermediate to that of normal and adult corpus callosum sectioned cats.

Cats with neonatal corpus callosum section have been shown to have a substantial loss of the extent of the binocular visual field, as well as exhibiting small, and varying, degrees of divergent strabismus (Elberger, Exp. Brain Res. 1979). The behavioral loss of the binocular visual field is reflected in the physiological loss of binocularly driven cells observed in the present study. These results suggest that the normal development of binocular vision, and binocularly driven cortical cells, depends in part on information transmitted by the corpus callosum early in life.

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2631 NEURONS SENSITIVE TO BINOCULAR DEPTH IN AREAS 17 AND 18 OF THE CAT VISUAL CORTEX. David Ferster* (SPONSOR: David H. Hubel). Dept. of Neurobiology, Harvard Medical School, Boston MA, 02115.

Several studies have suggested that neurons in area 17 are sensitive to retinal disparity and may form the basis of binocular depth perception. There are also reports that area 18, at least in sheep and monkeys, contains neurons even more suited to the task. The object of the present study was to assess the relative importance of these two areas for binocular vision in the cat. For 86 cells in area 17 and 187 cells in area 18 the optimal monocular stimulus was determined. This stimulus was then presented to both eyes, while retinal disparity was varied in a direction perpendicular to the receptive field orientaion with an adjustable prism in front of one eye. Eye movements were monitored with a binocular reference cell in area 17.

Two types of disparity sensitive neuron, similar to those in areas 17 and 18 of the monkey (G.F. Poggio and B. Fischer, J. Neurophysiol. 40:1392, '78) were found in the cat visual cortex. The first type resembled the tuned excitatory cells of the monkey in having a symmetrical peak in their disparity tuning curves. The position of the peak for each cell almost invariable coincided with the point at which the cell's own two receptive fields were superimposed; at this prism setting the receptive fields of the reference cell were also superimposed. This point was therefore taken to be the point of retinal correspondence, or 0° disparity. The zero disparity cells were usually binocular. The second type of disparity sensitive neuron resembled the near and far cells of the monkey and were usually monocular. In most cases they gave their smallest response (often no response at all) at 0°. On one side of 0° the response grew linearly for up to 5° towards its maximum (usually equal to the monocular response); on the other side it remained at the minimum for up to 2° before rising.

The two types of disparity sensitive neuron were present in both area 17 and 18 and in each area they were found in the same layers (2,3, upper 4, 6). There were, however, two important differences between the two areas. 1) the relative numbers of the two types of disparity sensitive cells were quite different: area 17 - 33 zero disparity cells, 9 near and far cells; area 18 - 18 zero disparity cells, 42 near and far cells. 2) The cells in area 18 were sensitive to much larger disparities than their counterparts in area 17. In area 17, near and far cells reached their maximum response within 2° of zero while in area 18 the response could change for up to 5°. The zero disparity cells of area 18 also had much broader tuning curves. It seems therefore that both areas 17 and 18 are involved in depth vision; area 17 is capable of discriminating small disparities close to 0°, while area 18 covers a larger range, but with less acuity.

2632 EXTRAVISUAL NEURONS IN THE OPTIC TECTUM OF A SIGHTED AND AN UNSIGHTED FISH. S.E. Fish* and T.J. Voneida (SPON. C.M. Sligar). Northeastern Ohio Universities College of Medicine; St. Rt. 44; Rootstown, OH 44242.

Extracellular recordings were made from single neurons in the optic tectum of the blind cave fish (Astyanax Hubbsi) and the goldfish. Cells were tested for responses to visual, somatosensory, auditory and lateral line stimuli. Although the cave fish has a vestigial optic nerve (Voneida and Sligar, J. Comp. Neur., 165:89, 1976), only somatosensory neurons were found. About two thirds of these cells were isolated in the periventricular layer with the remainder scattered throughout more superficial laminae. The somatosensory neurons were found to be organized with the rostral to caudal body axis represented from anterior to posterior in the tectum and the dorsal to ventral body axis represented from medial to lateral.

Normal visual responses, as reported by others, were recorded from the goldfish tectum. As in the cave fish the only extravisual responses recorded were somatosensory, although many fewer were found. The topography was similar to that of the cave fish but these neurons were rarely recorded outside the periventricular layer. In the goldfish a spatial correspondence was observed between visual and somatosensory receptive fields recorded from the same general location of the tectum. In mammals this correspondence is thought to facilitate visual orienting to non-visual stimuli (e.g. Chalupa and Rhoades, J. Physiol., 270:595, 1977). The significance of the (apparently more widespread) somatosensory representation in the blind fish optic tectum is under investigation.

2633 DISTRIBUTION OF ACETYLCHOLINESTERASE IN THE LATERAL GENICULATE NUCLEUS AND STRIATE CORTEX OF GALAGO SENEGALENSIS AND AOTUS TRIVIRGATUS. D. Fitzpatrick* and I.T. Diamond (SPON: G.C. Thompson). Dept. Psychol., Duke University, Durham, NC 27706.

This inquiry began with the discovery that just two layers of the lateral geniculate nucleus (GL) of Galago contain large amounts of acetylcholinesterase as demonstrated by the method of Karnovsky and Roots (J. Histochem. Cytochem. 12,1964). These two layers (3 and 6) are similar in cell size and staining characteristics, project to the same layer in the striate cortex and are thought to be homologous to the parvocellular layers of other primates.

This hypothesis has been strengthened by examining the distribution of acetylcholinesterase in the lateral geniculate nucleus of the owl monkey, Aotus trivirgatus. In this species, the parvocellular layers (3 and 4) stain darkly for cholinesterase while the magnocellular layers (1 and 2) are only slightly stained. The interlaminar zones as well as the "S" layers of the owl monkey GL also stain for cholinesterase.

In an attempt to determine the source of this cholinesterase staining in layers 3 and 6 of Galago, we made kainic acid injections into GL in some animals and lesions of striate cortex in others. Injections of .5 µg of kainic acid into GL, followed by survival times of 2-11 days, produce severe cellular destruction in GL,yet the cholinesterase staining of these layers is undiminished. In contrast, a small lesion of striate cortex, followed by a 9-day survival period, produces conspicuous gaps in the cholinesterase staining of layers 3 and 6. These gaps are restricted in width and their relative positions define a projection column that is perpendicular to the orientation of the layers of GL. These results indicate that the cholinesterase staining of these layers is not attributable to the cells within the layers, but is dependent upon descending projections from the striate cortex.

These results turned our attention to the distribution of acetylcholinesterase in the striate cortex. In Galago, cholinesterase positive cells can be seen in layer VI of striate cortex; and in both species, striate cortex is distinguished from other cortical areas by a prominent band of cholinesterase activity which coincides mainly with the lower tier of layer IV. This band ends abruptly at the 17-18 border. The precise origin of this cholinesterase staining within layer IV of the striate cortex remains to be determined. However, the fact that layer VI pyramidal cells project to GL (Raczkowski & Diamond, Br. Res.144, 1978) and that these cells send axon collaterals to layer IV in the monkey (Lund & Booth, JCN, 1975) suggests that the cholinesterase positive cells in layer VI may be responsible for the cholinesterase activity at both thalamic and cortical sites.

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2634 COMPARISON OF HEAD TILT BEHAVIOR DURING REARING IN STRIPED CYLINDERS WITH THE DEGREE OF MODIFICATION OF PREFERRED ORIENTATION IN VISUAL CORTICAL CELLS. Dorothy G. Flood*, Paul D. Coleman, and Robert C. Emerson. Dept. of Anatomy & Center for Visual Science, Univ. of Rochester, Rochester, NY 14642.

Rearing in striped cylinders has variably produced kittens whose visual cortical neurons have been modified to prefer the orientation experienced during rearing. We examined the influence of head tilt behavior during rearing on the modification of preferred orientation of cortical cells. Visual experience was limited to 100 or 300 hrs in horizontally or vertically striped cylinders, beginning at 4 wks of age. Head position was sampled by computer every second during the exposure. The cylinder light was turned off by the computer when the kitten's head tilted beyond a specified angular range. There were 3 groups of kittens with ranges specified as: continuous illumination or ±15° or 30° of erect. Kittens were then prepared for single unit recording using standard techniques of paralysis, anesthesia, and refraction. Tungsten-in-glass microelectrodes were angled to pass anteriorly down the medial bank of the lateral gyrus. A modified LINC-8 computer was used to present a square wave grating on a tangent screen (0.3 cycles/degree, moving broadside to and fro at 6°/sec) and to record in real time unit responses to the grating as a function of its orientation. Thirty-six to 91 units having a preferred orientation were recorded from 18 cats. All units that were encountered and isolated were studied, regardless of distance between units. The preferred orientation of each unit was plotted. Additionally the responses of all cells were summed at each orientation to produce the summed orientation preference of the sample of single units. Not only did some cats show a bias for the rearing orientation, while others did not show a bias, but some cats showed a bias for an orthogonal orientation. Generally, a subgroup of the inactive kittens formed the group which showed a bias toward the rearing orientation. Also restricted lighting conditions were somewhat more effective in producing biased cats. For kittens having a preferred orientation other than the rearing orientation, there is a preliminary indication that head tilt preferences during rearing were an important factor in producing a bias in the orientations of single units. Supported by PHS grants GM-01782, NS-07870, and EY-01440.

2635 DUAL RETINAL INNERVATION OF THE NUCLEUS RAPHE CENTRALIS SUPERIOR. Warren E. Foote. Dept. of Psychiatry, Harvard Med. Sch., Mass. General Hosp., Boston, MA 02114.

Prior work utilizing iontophoretic injections of horseradish peroxidase into the dorsal raphe nucleus revealed the presence of reaction product in the large "alpha" ganglion cells of the cat retina.

We now report the results of anatomical and electrophysiological experiments designed to determine if retinal afferents terminate in the nucleus raphe centralis superior. Applications of 4% horseradish peroxidase dissolved in .05m Tris-HCl buffer pH 8.6 were iontophoresed into the nucleus centralis superior in 4 cats. The animals were permitted to survive for 24 hours before being sacrificed and perfused with a 2.5% glutaraldehyde, 0.5% paraformaldehyde mixture. The brains and retinas were then removed and reacted with Hanker-Yates solution. Under oil immersion the presence of reaction product was detected in some of the large and small ganglion cells in all retinas. Ganglion cells containing reaction product were found scattered in all retinal quadrants but appeared to cluster in an area circumscribing the area centralis.

In an attempt to obtain confirmatory physiological data a series of animals were anesthetized and prepared for stimulation and unit recording. Bipolar stimulating electrodes were placed in the optic chiasm and superior colliculus and glass micropipettes were used for recording from cells in the nucleus centralis superior. Orthodromic activity was observed from both stimulation sites. The latency of activation from optic chiasm was dichotomous with one group of target neurons responding with a mean latency of 1.6 msec and another group with a mean latency of 6.6 msec. The combination of anatomical and physiological data suggest a dual retinal innervation of cells in the area of the nucleus centralis superior that appears to originate from the large "alpha" or y ganglion cells and the smaller "gamma" or w neurons.

2636 THE EFFECTS OF HEAVY METALS ON PHOTORECEPTOR FUNCTION. Donald A. Fox and Arnold J. Sillman. Dept. Animal Physiology, Univ. Calif., Davis, CA 95616.

Isolated perfused bullfrog (Rana catesbeiana) retinas, treated with sodium aspartate, were studied to determine whether or not heavy metals affect photoreceptor function. Sodium aspartate was present in the perfusing solution to suppress the PII and the proximal PIII components of the electroretinogram and to isolate the distal PIII or late receptor potential. Retinas were first perfused for 30 or 60 minutes with control Tris-Ringer and then for an equal duration with Tris-Ringer containing the chloride salt of either divalent lead (Pb), mercury (Hg) or cadmium (Cd). The concentration range of the heavy metals was 1 to 50 μM. Following each experimental perfusion the retina was again perfused with the control Tris-Ringer solution so as to compensate for any normal decay with time and to examine the reversibility of the effects. Rod and cone responses to brief light flashes were separated using previously described techniques (Sillman, Vision Res.14,1021,1974). Treatment of the retinas with either Pb, Hg or Cd resulted in decreases in the amplitude of the receptor potential of rods, but cones were never affected. The effects of Pb and Cd were reversible and concentration dependent. The effects of Hg were not reversible. On an equimolar basis Cd was two to three times more potent than Pb. Pb-treated photoreceptors exhibited a 0.7 log increase in absolute threshold. The results are consistent with reports describing scotopic vision deficits in human and non-human primates following exposure to either Pb or Hg. These observations suggest the involvement of rod photoreceptors as a primary lesion site in this deficit. The results predict that Cd exposure may produce clinical effects similar to those reported with Pb and Hg. (Supported by NIEHS ES05094 and PHS EY01839.)

2637 LINEAR FREQUENCY DOMAIN ANALYSIS OF PHOTOTRANSDUCTION IN THE FLY COMPOUND EYE REVEALS TEMPORAL RESONANCE BEHAVIOUR. Andrew S. French. Department of Physiology, University of Alberta, Canada.

The response of most photoreceptors to a brief flash of light consists of a slow graded depolarization or hyperpolarization. Although the early photochemical and biochemical stages of the process have been elucidated in several species, none of the mechanisms discovered so far can account for the time course of the response. The model originally proposed by Hodgkin and Fuortes to account for the dynamics of phototransduction in *Limulus* consists of a chain of exponential filters cascaded together. This model has been used with varying numbers of stages to describe the behaviour of several invertebrate and vertebrate photoreceptors.

We have shown previously that the frequency response function for light transduction is a useful method for testing the cascade model since the asymptotic gain and phase curves at high frequencies can be used to determine the number of stages involved. Unfortunately the signal to noise level of photoreceptors always drops progressively with ascending frequency so that it is difficult to determine the validity of the model and the number of stages with accuracy.

In the current work we have used an averaging technique to improve the signal to noise ratio at higher frequencies. Photoreceptors in the compound eye of *Phormia regina* were stimulated with repeated bursts of identical pseudo-random sequences of light fluctuations via a light emitting diode. The resultant membrane potential fluctuations recorded by microelectrodes were then averaged to reduce the inherent noise and the results processed as in normal linear systems analysis using a random input signal. This procedure resulted in significant improvement in the reliability of the frequency response function at higher frequencies, as measured by the coherence function. The frequency response function curves were fitted by the normal factorization of a polynomial into poles and zeros. All of the experimental results could be well fitted by a model which contained two second order poles and two first order poles. The presence of the second order poles indicates that there are temporal resonances occurring within the phototransduction mechanism and that the cascade model must be judged inadequate to account for the behaviour. In addition it was found that the phase portion of the frequency response function always lagged without limit as higher frequencies were approached. This strongly suggests that there is a pure delay element of several milliseconds present in the phototransduction mechanism.

2638 STRUCTURE OF GENICULATE X- AND Y-CELLS IN NORMAL AND MONOCULARLY DEPRIVED CATS. Michael J. Friedlander, C.-S. Lin, and S. M. Sherman. Dept. Physiol., Sch. Med., U. Va., Charlottesville, Va. 22908

At least 25 neurons in the dorsal lateral geniculate nucleus of normal adult cats were classified both physiologically and morphologically. To do this, we used intracellular recording and iontophoretic injection of horseradish peroxidase (HRP)[1]. Y-cells, morphologically, are Guillery's[2] class 1 cells, some are class 2, and the rest are intermediate between these morphological classes. Most X-cells, morphologically, are intermediate between Guillery's classes 2 and 3, while some are completely class 2 or 3.

Other morphological differences between X- and Y-cells were seen in our sample. 1) The X-cells have smaller somata. 2) X-cell dendrites tend to be elongated perpendicular to the lamination, while the Y-cells display no such asymmetry in their dendritic arborization. 3) Axons of most Y-cells issue collaterals within the region of the perigeniculate nucleus, while few X-cell axons have these collaterals. 4) Some X-cells issue intrageniculate axon collaterals, while none occur from Y-cell axons. 5) Dendrites of X-cells remain entirely within the lamina, while dendrites of Y-cells cross laminar boundaries.

In addition to the experiments on normal cats, preliminary data from monocularly deprived cats has been obtained. To date, only 7 cells from deprived animals have been analyzed. While soma sizes appear altered by the deprivation, the normal structure/function relationship, which relates receptive field type to dendritic morphology, appears unchanged. More detailed morphological studies of geniculate X- and Y-cells in monocularly deprived cats are in progress.

1) Friedlander, Michael J., C.-S. Lin, and S. M. Sherman, 1979, Science, (in press).
2) Guillery, R. W., 1966, J. Comp. Neurol., 128: 21-50.

Supported by N.I.H. grant EY01565 and N.S.F. grant BNS77-06785.

2639 **FUNCTIONAL ACTIVITY OF PIGEON OPTIC TECTUM REVEALED BY C-2-DEOXYGLUCOSE AUTORADIOGRAPHY.** **B. J. Frost and P. Ramm. Dept. Psych., Queen's Univ., Kingston, Ont., Canada, K7L 3N6.**

In the pigeon, single unit studies have shown that a majority of cells are optimally triggered by relatively small moving stimuli. Most of these same neurons however, do not respond to large textured patterns, and in fact have their response to small stimuli completely inhibited by patterns moved 'in-phase' (same direction and velocity)with test stimuli (Frost, 1978). These and other data suggest that one of the functions of the tectum is to 'notice' or respond to object motion and to ignore or veto self-induced motion produced by body, head or eye movements.

^{14}C-2-Deoxyglucose (^{14}C-2DG) autoradiography was used to reveal the relative responsiveness of tectal cells to small moving stimuli, to large textured patterns and to various combinations of these two types of stimuli. Lightly anesthetised birds were injected with 50 uci of ^{14}C-2-DG via the brachial vein. They were then rapidly inserted into a stereotaxic instrument and one eye opened to view a tangent screen upon which stimuli were projected, while the other eye was closed and occluded. One group of pigeons viewed for 45 minutes a single spot of light (<1° diameter) as it was repeatedly swept forward along the same 70° long horizontal path centered on the fovea. In other conditions birds viewed a large random dot pattern, with the same motion characteristics, and in-phase and anti-phase combinations of the test and background patterns. Autoradiographs were analysed using a computerized image processing system, so that the stimulated contralateral tectum could be compared to the ipsilateral tectum. The autoradiographs revealed no density increases in the region stimulated by the movement of the large random dot pattern, with or without the moving test spot. In contrast, forward motion of the small test spot alone produced a clearly visible and discretely localised area of increased density extending through all tectal laminae. This line of increased density ran posterior to anterior alons the tectum and was quite dorsally situated, thus suggesting that the lower visual field has a relatively larger tectal representation than the upper visual field.

2640 DISFACILITATION AND INHIBITION IN THE VERTEBRATE RETINA. Thomas E. Frumkes, Paul A. Coleman* and Nora Nicotera*. Dept. Psych., Queens Coll. of CUNY, Flushing, NY 11367.

The response properties of mudpuppy retinal neurons were examined by means of intracellular recording and current injection, and by studying receptive field properties. Most neurons with a predominantly depolarizing response show evidence of hyperpolarizing response components. For example, depolarizing bipolar cells often exhibit a small, hyperpolarizing prepotential (HPP) prior to depolarizing: in these cells, the HPP is more apparent with diffuse light stimulation than with a light flash restricted to the receptive field center. A similar HPP is also observed in the response of on-off amacrine and ganglion cells: hyperpolarizing current enhances the HPP while depolarizing current causes the HPP to diminish in amplitude. In contrast, the on and off IPSPs observed in on-off ganglion cells reverse when small amounts (less than 0.1 nA) of negative current are injected. Of interest, different amounts of current must be injected to reverse the on and off IPSPs of some on-off ganglion cells. Finally, ganglion cells can be categorized on the basis of their hyperpolarizing response components. For example, our analysis indicates the existence of three classes of off-center ganglion cells. Sustained off cells, which display a maintained hyperpolarization during photic stimulation and a small depolarization at light offset, show few obvious signs of any inhibitory input. Two classes of transient off ganglion cells respond with a phasic hyperpolarization at light onset and a depolarization-hyperpolarization sequence at light offset: in transient type I cells, the initial negative deflection is enhanced by hyperpolarizing current while the off negative deflection is reversed by hyperpolarization and appears to be a true IPSP; in transient type II cells, both on and off hyperpolarizations reverse with negative current injection and appear to be IPSPs. These results are consistent with the prior suggestions by Frumkes and Miller that different latencies of neurons in the outer nuclear layer play a major role in information processing, and that several different classes of amacrine cells supply the inhibitory input to ganglion cells.

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2641 V2 IN THE MACAQUE: VISUOTOPIC ORGANIZATION AND EXTENT. Ricardo Gattass*, Julia H. Sandell* and Charles G. Gross (SPON: B. G. Hoebel). Dept. Psychol., Princeton Univ., Princeton, N. J. 08544

The visuotopic organization of V2 was investigated in Macaca fascicularis. Six monkeys were studied with multiunit electrodes while immobilized and under N_2O/O_2 in repeated recording sessions. V2 surrounds striate cortex and contains a virtually complete representation of the contralateral half field. It corresponds closely to von Bonin and Bailey's cytoarchitectonic area OB and is myeloarchitectonically distinguishable by a dense and homogeneous fiber pattern in layers IV to VI. Dorsolaterally, V2 includes most of the annectant gyri and the posterior bank of the lunate sulcus. It extends dorsomedially into the posterior bank of the parieto-occipital sulcus and ventromedially it includes the collateral sulcus, the posterior portion of the occipito-temporal sulcus and moves into inferior occipital sulcus. On the ventrolateral surface it includes the posterior bank of the inferior occipital sulcus.

The representation of the fovea in V2 is located ventrolaterally, adjacent to that of the foveal representation in V1. The representation of the vertical meridian in V2 is adjacent to that of V1 and the isoeccentricity lines in V2 are continuous with those in V1. The representation of the horizontal meridian splits and forms the anterior border of V2, laterally, medially and in the calcarine sulcus. The lower visual field is represented dorsally and dorsomedially and the upper visual field is represented ventrally and ventromedially. Thus as in the cat and owl monkey, V2 is a second order transformation of the visual field.

2642 PROJECTIONS OF THE LATERAL SUPRASYLVIAN VISUAL AREA TO THE PONTINE NUCLEUS OF CATS. Mitchell Glickstein, Janet Lee Cohen*, George Mower*, Farrel Robinson*, and Alan Gibson*. Dept. of Psych., Hunter Lab., Brown U., Providence, RI 02912

The lateral bank of the suprasylvian sulcus of cats receives a direct visual input from the dorsal lateral geniculate nucleus and contains several independent representations of the visual fields. The receptive field properties of cells in these lateral suprasylvian areas resemble those of pontine visual cells in that they are insensitive to the orientation of targets but responsive to their speed and direction. In the present study, we hoped to determine: 1) Where in the pontine nucleus fibers from lateral suprasylvian visual areas terminate. 2) Which cells in the lateral suprasylvian areas project to the pontine nuclei. 3) Whether some lateral suprasylvian cells project both to the superior colliculus and to the pontine nuclei by way of bifurcated axons.

We made lesions in the lateral suprasylvian area of three cats and mapped degenerating fibers in the pontine nuclei and superior colliculus. In two additional cats, we injected radioactive amino acids into the lateral suprasylvain area. In one case, we injected only this area. In the other, the other lateral suprasylvian cortex was injected along with visual area 18 and 19. We also injected small amounts of HRP into the pontine visual area of five cats and the superior colliculus of one cat and reacted the tissue to label corticopontine and corticotectal cells.

The lateral suprasylvian areas send a dense projection to the pontine nuclei. The projection overlaps that from other cortical visual areas but is more extensive. Nearly all of the fibers terminate in a zone which is just medial and ventral to the pyramidal tract in the rostral pons. Unlike other cortical visual areas, the lateral suprasylvian cortex also projects to the pontine nucleus on the opposite side. These contralateral projections are confined to a small medial region which also receives fibers from the ventral lateral geniculate nucleus. The cells of origin of the corticopontine tract are layer V pyramidal cells. Indirect evidence based on the shape and size of corticopontine and corticotectal cells and direct evidence from a parallel study of antidromic activation suggests that many layer V cells send an input both to the pons and to the colliculus by way of a bifurcated axon.

The research reported herein was supported in part by National Science Foundation Grant BNS77-16884, "Visual Input to the Cerebellum."

2643 THE C-WAVE OF THE ELECTRORETINOGRAM IN LIGHT AND DARK-REARED ALBINO RATS. Adrienne L. Graves*, Daniel G. Green, and Maureen K. Powers. (Spon. T.E. Robinson). Dept. of Psychology, Univ. of Michigan, Ann Arbor, MI 48109.

The c-wave of the electroretinogram (ERG) is thought to be produced by the pigment epithelium in response to a light-induced decrease in extracellular potassium (K^+). The K^+ change is believed to be generated by the hyperpolarizing response of the photoreceptors. In view of this, the reports of failure to find a c-wave in albino rats are very surprising. Observations of our own suggested that the conditions of light-rearing might be an important confounding variable. Consequently, we recorded the ERG c-wave in 2 groups of albino rats. One group (N=14) was reared from birth in dim illumination (dark-reared) and the other group (N=20) was reared from birth in 12/12 cyclic light (light-reared). Rats were tested after birth from 22 days to about one year. In response to a one second flash (I= -1.2 log cd/m^2), all dark-reared animals showed c-wave responses ($\bar{x}$=301 μv). Rats reared in cyclic light typically showed no detectable c-wave; one young animal did show a very small (50 μv) c-wave in response to these flash conditions. Using a longer, brighter flash (5 sec, I=1.2 log cd/m^2), we were able to elicit small (<200 μv) c-waves in only 3 of the light-reared animals. This consistent difference, c-waves present in dark-reared animals but absent or severely diminished in light-reared animals, is probably not due to extensive light induced retinal damage. This could be ruled out because b-wave responses in the 2 groups of animals were indistinguishable. No differences were seen in b-wave thresholds for the 2 groups (I= -3.70 log cd/m^2 for dark-reared; I= -3.79 log cd/m^2 for light-reared). Maximum b-wave amplitudes were also comparable in the 2 groups ($\bar{x}$=994 μv for dark-reared; $\bar{x}$=987 μv for light-reared). No differences were seen in b-wave intensity-response functions in dark- and light-reared animals. Thus, we find that exposure to light can selectively interfere with the process of c-wave generation. Two possible mechanisms are: 1) light-rearing may cause photoreceptor debris to accumulate around the outer segments, so that ionic flow to the pigment epithelium is impeded; 2) light-rearing may damage the apical membrane of the pigment epithelium, so that it is unable to respond to extracellular K^+ changes. (Supported by EY00379).

2644 A PRETECTAL PROJECTION TO THE DORSAL LATERAL GENICULATE COMPLEX IN THE CAT. A.M. Graybiel, D.M. Berson*, T.P. Langer*, and C.L. Colby* (SPON: F.O. Schmitt). Dept. of Psychology, M.I.T., Cambridge, MA 02139

In the cat the pretectal region gives rise to an ascending extrageniculostriate pathway leading by way of the pulvinar-LI complex of the thalamus to parieto-occipital association cortex. This pathway is distinct from the tecto-thalamo-cortical channel leading from LPm to the lateral part of the Clare-Bishop complex. We here report that the pretectal region, like the superior colliculus, also sends a fiber projection to the dorsal lateral geniculate body (LGd). While the tectal projection is distributed mainly to the deep C-laminae, the pretectum projects mainly to the more dorsal laminae and to NIM. These observations confirm and extend earlier fiber degeneration findings described by Itoh (1977).

Pretectal efferents were studied by autoradiography in 28 adult cats. Without exception, ^{3}H-amino acid injections centered in the nucleus of the optic tract (NOT) elicited labelling of the LGd complex on the ipsilateral side. In several of the animals, labelling was dense enough to be seen at low power with dark-field optics. In the laminar LGd, labelled fibers formed a rich feltwork that was densest in layers A and A1 (including the monocular segment of A) and, variably, in lamina C. It is not clear that any labelled fibers terminated in laminae C1-C3, for labelling in these layers was weak and due at least in part to perforant fibers. Labelling of NIM was always as dense, and often denser, than that in the main LGd laminae. Extremely weak LGd labelling sometimes appeared contralaterally. It is striking that in the laminar LGd, the layers of densest labelling correspond to those richest in AchE activity and that in the LGd complex as a whole, the labelled regions correspond to those known to receive retinal Y-cell input.

In cases of thalamic or tectal injection sparing the pretectum, such a pattern of LGd labelling was absent, though in the thalamic cases labelled fibers passed through LGd in straight-line trajectories and after tectal injections a terminal field appeared in C_2/C_3. Pretectal deposits elicited labelling of LGd only when they involved NOT, save in one case (an olivary nucleus deposit with weak LGd labelling). Results in a case of HRP injection into LGd also suggest that the fiber projection arises in NOT, as most (but not all) HRP-positive cells in the pretectum were in NOT.

Since two amino acid deposits involving lateral NOT labelled mainly rostral LGd while 4 medial NOT injections labelled mainly caudal LGd, the pretecto-geniculate projection appears to have topographic order, at least along one main axis. Supported by NIH-1-RO1-EY 02866-01 and NSF BNS 75-18758 & 78-10549.

2645 EFFECT OF NEONATAL SECTION OF LATERAL RECTUS MUSCLE ON THE PROPERTIES OF RETINAL GANGLION CELLS OF CATS. D. I. Hamasaki* (SPON: S.H. Gruber). Bascom Palmer Eye Instit., Univ. Miami, Florida 33136.

At 10 days of age, the lateral rectus muscle was cut unilaterally in 6 kittens and bilaterally in one kitten. Experiments were conducted when the kittens were approximately 9 months old, and the properties of 468 ganglion cells were compared with the properties of 436 units recorded from normal cats.

The stimulus intensity required to elicit a firing rate of 200 spikes/sec was significantly higher for the units obtained from the experimental animals. This was found at all retinal loci tested. The maximum firing rate evoked by stimulating the receptive field center was significantly lower in the experimental animals at all retinal loci. The differences between the experimental and control animals were more marked for the units recorded in the area centralis. The correlation between the receptive field center size and conduction velocity found in normal animals was not found in the experimental animals.

These observations were made for units recorded from the operated as well as from the unoperated eye.

2646 ATTEMPTS TO MODIFY THE VESTIBULO-OCULAR REFLEX OF NORMAL AND DARK-REARED CATS. Laurence R. Harris* and Max S. Cynader (SPON: G.V. Goddard) Dalhousie University, Halifax, Nova Scotia, Canada.

We have developed a method by which the vestibulo-ocular reflex (VOR) may be modified within 1 hour in a normal adult cat and that maintains its effects for at least one hour after the adapting period. When a cat is rotated sinusoidally in the light, vestibular and optokinetic information combine to produce very accurate compensatory eye movements. If, however, an optokinetic drum is rotated continuously in one direction at a velocity equal to the peak velocity of the simultaneous sinusoidal oscillation of the cat, the visual information conflicts with the vestibular signal in one direction, while enhancing the vestibular signal in the other. The gain of the VOR is asked to double in one direction and simultaneously to decrease to zero in the other. Since it is the difference between the responses to the two directions that is of interest, any confounding effect of variation in the gain of the VOR with arousal etc... is minimized.

After one hour of this procedure in the normal cat the gain of the VOR (measured in the dark) goes to two in one direction and zero in the other. However, measurement of the VOR at different frequencies reveals that the effect of adaptation is to add a constant velocity (that of the drum) to the normal VOR. Thus, at lower frequencies (lower peak velocities), the gain increases above two in one direction and actually becomes negative in the other.

The VOR of a dark-reared cat normally has a very low gain (Harris and Cynader, ARVO, 1979). Attempts to modify the dark-reared's VOR with the same method as above revealed no modifiability, even though visual and vestibular information could combine to produce appropriate compensatory eye movements measured in the light.

Surprisingly, physical movement of the animal is not necessary for modification of the VOR in the normal cat. After one hour of drum movement, the subsequent optokinetic afternystagmus (OKAN) is sufficient by itself to explain the observed modification of the VOR. This explanation of VOR modification is supported by the observation that the dark-reared cat, whose VOR could not be modified, shows no significant OKAN.

2647 A HYPOTHALAMO-TECTAL PROJECTION IN THE CAT: EVIDENCE OF PATCH-LIKE TERMINATIONS WITHIN THE SUPERIOR COLLICULUS. John K. Harting, Michael F. Huerta, Joseph T. Weber, and G. James Royce. Dept. of Anatomy, University of Wisconsin, Madison, WI 53706.

Following an injection of tritiated proline into the dorsal part of the tuberal region of the hypothalamus, an extensive and dramatic pattern of transported protein is apparent within the ipsilateral superior colliculus. In one particular experiment the injection site of ^{3}H-proline lies primarily within the dorsal hypothalamic area (see figure). To a lesser degree the precursor spreads into the lateral hypothalamic area and the most medial tip of the zona incerta. The bulk of the label in the superior colliculus occupies the stratum griseum intermediale (SGI). Specifically, the silver grains are aggregated into: (1) a puff at the medial margin of the SGI which measures 700μ dorsoventrally and 450μ mediolaterally, (2) a tier of small clusters of label at the most dorsal boundary of the SGI, and (3) a lateral tier of label which follows the ventral border of the SGI and measures 1700μ mediolaterally and 300μ dorsoventrally. While there are other scattered areas of label in the SGI, the medial puff and the ventrolateral tier described above are continuous in the rostral-caudal extent of the colliculus. We emphasize that the SGI contains the greatest amount of transported protein (see figure). Layers ventral to the SGI contain only sparse label which is fibrous in appearance.

A second, extemely dense zone of label is located in the region of the central grey which lies immediately medial to the deepest collicular laminae. Although the distribution of transported protein does not cross the dorsal or ventral borders of the central grey, the lateral boundary is crossed by a flange of label which extends into the mesencephalic reticular formation at the horizontal level of the cerebral aqueduct.

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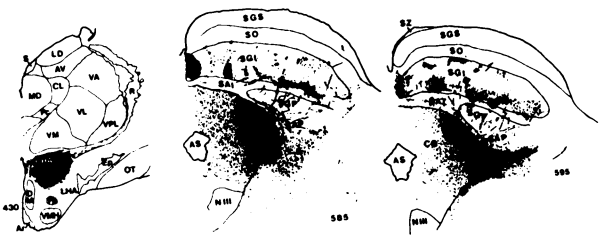

2648 DEMONSTRATION OF OCULAR DOMINANCE COLUMNS IN NISSL STAINED SECTIONS OF MONKEY VISUAL CORTEX FOLLOWING ENUCLEATION. E. C. Haseltine*, E. J. DeBruyn*, and V. A. Casagrande. Departments of Anatomy and Psychology, Vanderbilt University, Nashville, TN 37232.

Enucleation produces trophic effects in both the lateral geniculate nucleus (LGN) and visual cortex of mammals. We studied the geniculostriate system of a monkey following long term monocular enucleation in order to determine 1) if deprived cells in the LGN maintain connections with cortex, and 2) if any deprivation related changes can be demonstrated in visual cortex. The right eye of a wild adult cynomologus macaque was removed. Following a survival time of 29 months the remaining eye was injected with 500 µCi of ^{3}H proline and both cortices were injected with 30% horseradish peroxidase (HRP). Two days later, the animal was perfused and appropriate sections were processed for autoradiography, reacted with diaminobenzidine, or stained with cresyl violet. Examination of the LGN showed that although cells in the denervated laminae were 39% to 53% smaller in area than their non-deprived counterparts, the percentage (90%-95%) of HRP labelled cells from the cortical injections was approximately equal in all layers. This demonstrates that the deafferented neurons maintain cortical connections.

Inspection of the visual cortex revealed a striking and unexpected change. In Nissl sections, layer IV of striate cortex in both hemispheres showed alternating light and dark variations in staining that were comparable in width (300µ-500µ) to ocular dominance columns previously described in this species (Hubel et. al. '68, '76, 77). Although adjacent light and dark bands were of equal widths, there were 20%-30% more neurons in the dark bands, and cells in these bands were 7%-14% smaller than those in light bands. Because of differences in the degree of overlap in the bands, we were unable to determine if individual cells in the darker bands were more heavily stained. Comparison between the two hemispheres in the monocular region of cortex indicated that the darker columns represent the enucleated eye. With regard to these results, it is noteworthy that a decrease in cell size and an increase in cell packing density have also been observed in cats following early nerve crush (Cragg, '75). Taken together, these results demonstrate that in addition to transneuronal changes in the LGN, removal of one eye of a primate can result in dramatic changes in the visual cortex, even after maturity.

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2649 CELL BIRTH IN THE DORSAL LATERAL GENICULATE NUCLEUS OF THE CAT: A H^3-THYMIDINE STUDY. T.L. Hickey and N.R. Cox*. School of Optometry/The Medical Center and Department of Comparative Medicine, University of Alabama in Birmingham, Birmingham, AL 35294.

Single 'pulse' injections of H^3-thymidine have been used to define the birthdates of nerve cells in the cat dorsal lateral geniculate nucleus.

Using the following procedure dividing cells destined to form the dorsal lateral geniculate nucleus have been radioactively labeled in individual kitten fetuses of known gestational age. In order to gain access to the uterus a laparotomy is performed in each mother cat. Once the uterus has been exposed a single injection of H^3-thymidine (500 µCi in .05cc of sterile water) is made, through the uterine wall, into each conceptus. The intact uterus is then returned to its normal position in the abdomen and all surgical incisions closed. Following surgery the mother cat is housed in a metabolic cage for a period of two weeks during which time all waste products are collected and checked for radioactivity. At the end of the two weeks the mother cat is returned to her home cage and allowed to deliver her kittens normally. All kittens are reared for at least two months with either normal binocular visual experience or with the lids of one eye sutured closed. Each animal is then sacrificed and blocks of brain tissue embedded in paraffin and processed routinely for autoradiography. Since the radioactively labeled thymidine is injected directly into each conceptus it is necessary to expose the autoradiographs for only three-to-four weeks.

The results of these injections show that most dorsal lateral geniculate nucleus cells in the cat are generated during a period of rapid cell proliferation that begins during the fourth week of gestation and continues for slightly longer than one week. Although cell proliferation is most rapid between gestational days twenty-five and twenty-nine, a few dorsal lateral geniculate nucleus cells are born as late as gestational day thirty-three. Injections made at later times, including gestational days thirty-five, thirty-seven and fifty-five, have, in the animals processed to date, failed to label any cells in the dorsal lateral geniculate nucleus. However, other visual system structures do contain labeled cells with birthdates later than gestational day thirty-three.

Since, in the monocularly lid-sutured kittens, cells with the same birthdates can be found in both the deprived and non-deprived laminae of the dorsal lateral geniculate nucleus, deprivation induced changes in cell cross-sectional area can be determined for different populations of cells.

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2650 LAMINAR ORIGINS OF IPSILATERAL TECTOBULBAR PATHWAYS. Virginia Holcombe and William C. Hall. Departments of Anatomy and Psychology, Duke University, Durham, N.C.

As part of an ongoing investigation of the laminar organization of the superior colliculus in the grey squirrel, Sciurus carolinensis, the ipsilateral pathways from the superior colliculus to the brainstem have been studied with the following anterograde and retrograde techniques. Buffered solutions of tritiated leucine and/or lysine were injected electrophoretically into the superior colliculus 45-50 hours before sacrifice; series of 30-40µm sections were defatted, exposed to photosensitive emulsion for 4-5 weeks, developed, and counterstained. HRP (30% in saline) was injected electrophoretically into the brainstem 24-48 hours before sacrifice; series of 40-48µm sections were processed with 3,3',5,5'-tetramethyl benzidine and counterstained for Nissl substance.

When tritiated amino acids are injected into the superior colliculus, a prominent well-defined efferent pathway can be traced laterally and ventrally through the ipsilateral brainstem. The terminal fields of this pathway include the lateral tegmentum and the dorsolateral pontine nucleus.

When HRP is injected into the dorsolateral pontine nucleus and dorsally adjacent tegmentum, peroxidase-filled cells are located in all laminae of the superior colliculus with the exception of stratum zonale. In a typical case, the majority of labeled cells are located in stratum griseum superficiale while the remaining cells are distributed throughout stratum opticum, stratum griseum intermediale, and stratum griseum profundum. In stratum griseum superficiale, the number of labeled cells increases caudally.

Injections of tritiated amino acids, as well as lesions, which are restricted to stratum griseum superficiale suggest that the tectopontine projection arises in large part from superficial collicular laminae. Thus, the cells located in the deeper laminae, which are labeled following our HRP injections, may be the source of the projection to lateral tegmentum. We are currently seeking a more refined distinction between the cells of origin of these two projections by making a series of more restricted injections of HRP.

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2651 NEW PHOTOGRAPHIC METHODS FOR THE MEASUREMENT OF ACCOMMODATION AND REFRACTIVE STATE OF VERTEBRATE EYES. Howard C. Howland, Christopher Murphy* and Joanne Ballarino*; Division of Biological Sciences, 136 Langmuir Laboratory, Cornell University, Ithaca, New York 14853.

We have developed three new methods for the photographic measurement of accommodation and refractive state of vertebrate eyes. The first, applied to the great horned owl (Bubo virginianus) and the barred owl (Strix varia)involves the continuous binocular photographic measurement of degree of defocus relative to the plane of the camera, using a photorefractive method developed for single still pictures (Howland & Howland, 1974). The very large size of the owl's pupil provides enough light for continuous recording on Tri X cinematographic film. With this technique, we have been able to show that owls are normally focussed for distant vision, that the range of accommodation in the great horned and barred owls under semi-natural (tethered) conditions is no greater than 2 diopters and that their eyes are essentially non-astigmatic.

Secondly we have developed a method for the photographic determination of the axis of astigmatism in humans and the relative sign of defocus (in front of or behind) the camera. This method requires the use of simple attachment to a 35 mm camera consisting of a fiber optic light guide centered in an extension ring to photograph the pupils of the subject. The principle is the same as that of conventional photorefraction with the exception that the camera is focussed both in front of and behind the subject, and the two pictures are compared to determine the sign of defocus. Astigmatisms appear as an elliptical pupil reflex with the axes of the ellipse indicating the axes of the astigmatism.

Lastly we have developed a new photokeratometer, also an attachment to a 35 mm camera consisting of a ring of 8 light emitting diodes. The diode array is powered by a 9 volt calculator battery. The ring is used together with an extension tube set and the magnified image of the point source diodes is photographed on ASA 3000 speed recording film. By measuring the image diameters of the ring of lights it is possible to compute the corneal curvature and hence dioptric power from the known constants of the optical system in four meridia simultaneously.

Howland, H. C. & B. Howland (1974) Photorefraction: A technique for study of refractive state at a distance. J. Opt. Soc. Am. 64, 240-249.

2652 THE ORGANIZATION OF RETINOCOLLICULAR PATHWAYS IN MAMMALS. Michael F. Huerta, Joseph T. Weber and John K. Harting. Dept. of Anatomy, Univ. of Wisconsin, Madison, WI 53706.

We have made extensive use of the anterograde autoradiographic tracing method in order to analyze the organization of retinocollicular pathways in thirty different mammals. Representatives of the following orders were studied: monotremata, marsupialia, chiroptera, edentata, rodentia, lagomorpha, artiodactyla, carnivora, and primata.

Our data reveal several important features of retinocollicular organization across mammals. In several cases, exceptions have been omitted in order to generalize.

In all mammals thus far studied, retinal axons end exclusively within tectal laminae lying dorsal to and including the stratum opticum. The contralateral projection extends throughout the rostral-caudal extent of the colliculus. Terminations are most dense within the medial portions of the stratum zonale and the dorsal part of the stratum griseum superficiale. Caudal zones of the contralateral colliculus contain more label than do rostral levels. To a slight degree in carnivores and in all primates a patch-like distribution of contralateral endings are present within the binocular region of the colliculus, whereas lower orders have continuous contralateral input.

The ipsilateral retinocollicular pathway is less extensive than the contralateral in its overall projection (i.e., rostrocaudally and mediolaterally). In some of the lower forms this pathway is reduced, consisting of only one or two small patches of label. In contrast, primates possess an extensive ipsilateral pathway. The ipsilateral projection is also restricted in its laminar distribution, usually being confined to one of the sublayers of the superficial grey. In this regard, the ipsilateral input present in higher forms terminates within regions of the stratum griseum superficiale, while in lower mammals the stratum opticum is the locus of ipsilateral endings. Finally and quite importantly, the ipsilateral pathway terminates ventral to the region of densest contralateral input and always ends in a puff-like, discontinuous manner.

Additional points of interest are that: (1) in all primates the density of label within the collicular region containing the representation of central vision is reduced (but not absent) when compared with label present within the zone containing the representation of the peripheral visual field, (2) the greatest degree of ocular segregation, as revealed anatomically in our studies, is present in several marsupials and the prosimian Galago, and (3) the only mammals that do not possess an ipsilateral pathway are the echidna, the armadillo and the tree shrew.

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2653 PERCEPTUAL GROUPING AND PATTERN DISCRIMINATION IN THE DESTRIATE CAT. H. C. Hughes and J. M. Sprague. Dept.Anat.,Sch.Med.,Univ.of Pennsylvania, Phila., PA 19104.

Using a two choice discrimination apparatus, cats were tested for their capacity to polarize rectilinear arrays of pattern elements (dots or oriented line segments) into "rows" which appear oriented either at 45° or 135°. This apparent cohesion between figural elements is known as "perceptual grouping", and in this case, the association between elements is governed by their relative proximity to one another. These grouping effects are generally regarded as being importantly involved in one of the earliest stages of visual form and pattern perception, the division of the visual field into a figure-ground dichotomy.

A set of lattice-like arrays was generated through systematic variation of the spatial relationships between the pattern elements. The arrays were constructed so as to vary widely in the ease with which perceived orientation can be induced, that is, they vary in the magnitude of the proximity cue (i.e., relative spacing between elements). The patterns were then used to construct "frequency-of-seeing" curves which relate discrimination performance with the magnitude of the proximity cue. These psychometric functions were obtained before and after removal of areas 17 and 18.

In view of the large magnification factor associated with the striate and peri-striate representation of the visual field, it was hypothesized that the central representation of the spatial relationships between figural elements in these patterns would be most precisely elaborated within these areas. Accordingly, we expected that removal of areas 17-18 would impair the grouping process, especially when it was initiated by near-threshold proximity cues.

The results indicate that the formation of a linear gestalt in cats is immediate and unlearned, as judged by their near-perfect transfer of learned discriminations of obliquely oriented square-wave gratings to the rectilinear dot-patterns.

In contrast to our expectations, removal of area 17 and 18 had little effect on the grouping process, even under conditions of weak proximity cues. However, discrimination of randomly distributed arrays of similarly oriented line segments, a problem which requires a fine grain analysis of the geometry of the pattern elements, is adversely affected by the lesion.

The results thus indicate that processes which may mark the initial stages of form and pattern processing do not require the participation of striate-peristriate visual cortex, but that these cortices are important for more detailed spatial analysis, an interpretation that is consistent with parallel processing models of visual perception. (Supported by grants EY00577 and EY05130-02).

2654 Anatomical organization of layer IV in tree shrew striate cortex (area 17): evidence for two sublaminae. A.L. Humphrey & J.S. Lund, Dept. of Ophthalmology, Univ. of Washington, Seattle, WA 98195

The striate cortex of the tree shrew (Tupaia glis) does not have ocular dominance columns but it does have a highly organized orientation column system. The majority of cells in all cortical layers are binocular and/or orientation selective. As part of an investigation into the anatomical substrates for binocularity and orientation selectivity in the tree shrew striate cortex we studied the structural organization of layer IV, the site of major input to area 17 from the dorsal lateral geniculate nucleus (dLGN). Examination of the distribution of thalamic afferents to layer IV, and of the cell types within the layer, their dendritic spread and their axon trajectories outside of the layer leads us to conclude that layer IV of the tree shrew striate cortex consists of two laminar subdivisions.

Afferents to striate cortex were labeled using the method of anterograde transport of horseradish peroxidase (HRP) following injection of the enzyme into the optic radiations. Afferents from the dLGN previously have been shown to terminate in layers I, IIIB and IV in tree shrew striate cortex (Harting, Diamond & Hall, JCN, 150, '73). We examined HRP filled axons distributing in these laminae in the dorsal binocular region of area 17. Within layer IV, individual axons were seen to terminate only in the upper or lower half of the layer, with little encroachment upon the other half. The majority of the axon fields occupied from one half to all of the upper or lower subdivision of layer IV; axons with more narrow fields (≤1/3rd of a subdivision) were located adjacent to the layer IIIC-IV border. The average horizontal extent of single axon fields in both subdivisions was about 150 microns.

Examination of cells in the striate cortex stained using the Golgi rapid method further suggested a bipartite subdivision of layer IV. Three types of impregnated cells were seen in the layer: spiny stellate, non-spiny stellate, and pyramidal cells. Only stellate cells were present in the upper half of the layer; the lower half contained all three cell types. The dendritic trees of the spiny stellate cells tended to be stratified at the upper and lower boundaries of the two laminar subdivisions, while spiny stellate cells more centrally located in each subdivision had more spreading dendrites that spanned a greater thickness of the subdivision. The pyramidal cell somata and basal dendrites partially clustered at the upper border of the lower subdivision of layer IV. The apical dendrites of the pyramidal cells arborized in layer IIIB, as did the pyramidal cell recurrent axon collaterals and the axon collaterals of the spiny stellate cells of the lower division. In contrast, the collaterals of spiny stellate cells in at least the most superficial part of layer IV arborized in layer IIIC.

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2655 RESPONSES OF X AND Y LATERAL GENICULATE UNITS TO STATIONARY AND MOVING STIMULI. [1]Linda S. Ide* and Robert C. Emerson. Center for Visual Science, Univ. of Rochester, Rochester, NY 14627; [1]Dept. of Pharmacolog. & Physiolog. Sci., Univ. of Chicago, 947 E. 58th Street, Chicago, IL 60637.

Responses of 72 units recorded from the dorsal laminae of the cat's dorsal lateral geniculate nucleus were examined to determine whether units classified as X versus Y on the basis of linearity of summation in the receptive field differ in their patterns of responses to stationary and moving bar and edge stimuli. Several types of "null tests" were used to evaluate whether units summed effects of simultaneous luminance increments and decrements in an essentially linear manner. Thirty-five units were classified as X (linearly-summing) and 26 as Y (non-linearly-summing). Eleven units were incompletely classified or had ambiguous response properties. Stimuli of relatively low contrast were used, with luminances ranging from 0.3 to 0.6 log units above a background of 1.2-1.4 cd/m^2. Response patterns of both X and Y units could be interpreted as reflecting the interaction of antagonistic center and surround response mechanisms. Certain properties of the response patterns of Y units, however, support the hypothesis of Hochstein and Shapley that Y units have, in addition, nonlinear subunits within their receptive fields. Presentations of stationary stimuli to X units typically elicited responses whose character shifted abruptly between center- and surround-dominance as a function of stimulus position. Comparable abrupt shifts did not occur in responses of Y units; rather, mixed center-surround responses were elicited over a significant portion of the receptive field. Center and surround response components were more transient in Y units, with generally faster rise times. Further, surround response components were more pronounced in Y units. In responses to moving stimuli the greater strength of surround response components in Y units was reflected in more pronounced responses associated with stimulation of the distal surround by moving bars and edges. Some of the differences between responses of X and Y units appeared to depend on stimulus contrast. For example, a few X units tested at slightly higher contrasts showed stronger surround responses. Differences between X and Y units along a number of receptive field parameters support the idea that the two populations subserve distinct functions.

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2656 EFFECT OF PRETECTUM ABLATION ON DETECTION OF BARRIERS AND APERTURES BY FROGS. David Ingle, Brandeis University, Waltham, Massachusetts 02154.

Earlier studies in our lab demonstrated that ablation of optic tectum in frogs had no effect on the ability to negotiate barriers orapertures. Our recording studies in frog and those of Ewert in the toad revealed units in the "pretectal" region which may be called "stationary object detectors" in contrast to motion-dependent units of tectum.

The present study tested the hypothesis that barrier detection critically depends upon the integrity of the posterior thalamus or pretectal region. Eight frogs with ablation of the posterior third of the dorsal thalamus were severely or totally deficient in turning beyond the terminal edge of a hemicylindrical barrier. Control lesions in anterior or middle thalamus had no significant effect on this test. None of the lesions severely affected avoidance jumps elicited by visual threat.

Four animals who jumped randomly to the large barrier could rather consistently avoid a small (30° wide barrier) placed in a homogeneous white field. The same animals, however, also avoided a black or white aperture in a small compartment instead of jumping through to escape noxious stimuli. We conclude that a primitive mode of object vision survives some of these lesions, such that frogs can avoid a small area that is either darker or lighter than the homogeneous wide-field background, but fail to appreciate depth relationships. However, frogs which fail even the "small barrier" test can still avoid looming discs - an ability associated with the optic tectum.

Because tectum ablation in the tree-shrew can also dissociate deficits in food-localization from barrier detour capacity, we raise the possibility that mammalian pretectum mediates comparable "locomotor orientation" by mammals.

2657 CORTICAL BINOCULAR RECEPTIVE FIELDS AFTER VISUAL FIELD ROTATION IN DEVELOPING KITTENS: EFFECTS OF SUBSEQUENT NORMAL VISUAL EXPOSURE DURING ADULTHOOD. Michael R. Isley*, Kenneth R. Plummer*, and Paul G. Shinkman. Univ. North Carolina, Chapel Hill, N.C. 27514.

Previously (Shinkman, Bruce, & Isley, 1977) we showed that when kittens' early visual experience consisted of left- and right-eye visual fields optically rotated in opposite directions about the visual axes (16° of disparity between the two eyes), the distribution of interocular differences (IOD) in visual cortical cells' preferred stimulus orientations was found subsequently to be centered about the rotation experienced during early development.

The purpose of the present experiment was to assess the permanence of this effect, and its susceptibility to modification by subsequent normal visual experience during adulthood. Two kittens were reared in darkness except for two hours every day between the ages of 4 and 12 weeks, during which the kittens viewed a normal environment through goggles fitted with small prisms arranged so as to introduce a relative rotation between the visual fields in the left and right eyes, 8° counterclockwise in the left eye and 8° clockwise in the right eye. Both kittens showed good visual behavior: accurate orienting toward and pursuit of visual targets, good discrimination on a visual cliff, and so forth. Subsequently, the receptive field organization of visual cortex was studied, and the earlier results were confirmed. Except for the IOD distribution, the organization of visual cortex resembled that found in normally reared kittens, in terms of ocular dominance, orderly arrangements of orientation columns, and topographic representation of the visual field. The IOD distribution was centered about the experienced rotation ($\bar{X}$=19.7°), and the mean differed significantly from the expected value of 0° found in normally reared kittens.

The kittens were subsequently returned to the main colony without goggles where they experienced a normal visual environment for about 5 months, between the ages of 8 and 13 months. Each kitten was then retested to assess the effects of this exposure upon the physiological organization of visual cortex.

No changes were found. The pattern of results was the same as in the earlier measurements. The mean of the IOD distribution was 17.3°; this value differed significantly from 0° but not from the previously obtained mean of 19.7°. We conclude that the altered distribution of disparities between visual cortical cells' preferred stimulus orientations in the two eyes, induced by visual experience with prism goggles between the ages of 4 and 12 weeks, is permanent and not subject to modification by subsequent prolonged visual experience without the goggles during adulthood.

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2658 MODE OF PARASYMPATHETIC INNERVATION OF THE INTRINSIC MUSCULATURE OF THE EYE IN THE MACAQUE MONKEY AND RABBIT. R.J. Jaeger*and L.A. Benevento, Anat. Dept., Univ. of Ill. Med. Ctr., Chicago, IL.

The pathways controlling the intrinsic musculature of the eye are not fully understood. The most general view is based upon the observations that surgical removal of the short ciliary nerves in monkeys produced chromatolysis in 97% of ciliary ganglion (CG) cells while iridectomy alone caused chromatolysis in only 3% of the cells (Warwick, J. Anat. 88: 71, 1954). More recent physiological and pharmacological studies in monkeys challenge the view that a postganglionic pathway is involved in accommodation (Westheimer & Blair, Inves. Ophthal. 12: 193,1973). In the present study we investigated the mode of innervation of the intrinsic musculature by monitoring the retrograde transport of horseradish peroxidase (HRP). In both monkeys (Macaca iris) and rabbits the sclera was punctured with the needle of a 50 µl syringe and a series of 1 to 3 µl deposits of 20% HRP were made about the ciliary body and iris. The surface of the bulb was flushed with ophthalmic solutions immediately after puncture and withdrawal to minimize external flow of HRP. As a control for any seepage of HRP on to the eye or into the orbit, HRP was applied topically as well as injected subfascially in separate animals. After 24 hrs. the animals were perfused and the ciliary ganglia and brainstems were processed with the benzidene method. In the monkey, although it was not likely that we had the entire CG due to attrition of material during dissection, HRP reaction and processing, we have estimated that 20% of the cells were labeled. This percentage may represent the effectiveness of our intraocular application of HRP. The cells found in the brainstem were scattered throughout the dorsoventral extent of the midline of the oculomotor complex along the borders that the visceral nuclei (Edinger-Westphal, E-W) have with the somatic and accessory oculomotor nuclei. The cells were distributed in such a way that it was not possible to collectively assign them to a nucleus or nuclei. The cells did not have the usual appearance of the somatic cell types, but had smaller somas with sparce and often elongated dendritic fields. Some cells were clearly of the visceral type. In the rabbit, the results in the brainstem were similar. The labeled cells were of the visceral type and were, again, found at the edge of the E-W adjacent to the somatic and accessory nuclei. These projections appear to be unilateral. In the controls no cells were found in the brainstem. Although remote, the possibility of retrograde transsynaptic transport across gap junctions in the CG might explain our results and stay in harmony with the classic view. It might also be hypothesized that a ganglionic plexus in the intrinsic musculature exists in parallel with the CG. (Supported by grant EY2940)

2659 SEARCH FOR THE ORIGIN OF REPLACEMENT TERMINALS IN THE LATERAL GENICULATE NUCLEUS, PARS DORSALIS (LGd) OF THE ANOPHTHALMIC MOUSE USING HRP AND CORTICAL LESIONS: A LIGHT AND ELECTRON MICROSCOPIC ANALYSIS. I. R. KAISERMAN-ABRAMOF. Dept. Psych., Massachusetts Institute of Technology, Cambridge, Mass. 02139 and Dept. Anat., School of Med., Case Western Reserve University, Cleveland, Ohio 44106.

Using the anophthalmic mutant mouse (ZRDCT-An) as a model of primary deafferentation of the visual system, we have previously described a surprisingly normal anatomy of the LGd, visual cortex and oculomotor nucleus. Although these centers are somewhat reduced in volume and cell number, they contain the neuronal types that characterize the normal visual centers. Experimental analysis (HRP) of the circuitry of this mutant indicated that, despite complete absence of a retina, afferent and efferent connectivity exists between the LGd and striate cortex; in addition our study demonstrated a greater input from the thalamic LP and LD to the striate cortex. This quantitative difference in connectivity may represent a plastic and/or compensatory response that occurs during the differentiation of the visual system of the anophthalmic mouse.

A single distinctive feature of the mutant LGd at the ultrastructural level is the presence of a large terminal that replaces the retinal terminal in the glomeruli. The present study was directed toward identification of these large replacement terminals. The most likely origins were considered to be the cerebral cortex, thalamic nuclei or other subcortical centers.

The participation of other thalamic nuclei in providing replacement terminals was tested by microiontophoretic injection of HRP into the LGd of anophthalmic and normal mice. No HRP labeled cells were observed in any thalamic nuclei. Only the parabigeminal nucleus which is extra-thalamic was labeled; connectivity is known to exist between this nucleus and the superior colliculus. Cortical origin was tested in experiments on 31 animals in which discrete lesions were placed in area 17 and areas 17, 18, and 18a. In addition hemidecortication was performed. These lesions were made using suction. Also thermocoagulation was used to produce lesions extending beyond the visual cortex. Survival times varied from 2.5 to 14 days to allow detection of progressive degeneration. Most cortical lesions were made on the right side; the left LGd served as a control. The expected degenerative signs appeared in the ipsilateral LGd; however the replacement terminals persisted within the glomerular structure. This investigation indicates a subcortical origin of the large axonal terminals that replace the retinal afferents in the anophthalmic mouse.
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2660 DEVELOPMENT OF RETINOGENICULATE SYNAPSES IN THE DORSAL LATERAL GENICULATE NUCLEUS OF THE CAT. R.E. Kalil and G. Scott*. Department of Anatomy, University of Wisconsin, Madison, WI 53706.

Our previous light microscopical work has shown that afferents from the retina have reached the dorsal lateral geniculate nucleus (LGN) and are arrayed in an orderly laminar arrangement in the newborn kitten. It is of interest to know whether retinal axons have made synaptic connections in the LGN at the time of birth, but at present there is little information available concerning the developmental timetable for retinogeniculate synapse formation.

We have therefore used the electron microscope to study this problem in a series of cats ranging in age from newborn to adult (intermediate ages, 1, 2, 4, and 8 weeks). The animals were perfused with a mixture of paraformaldehyde and glutaraldehyde, and parasagittal slabs of the entire LGN were embedded in eponaraldite. Photographic montages were constructed from thin sections restricted to lamina A near the center of the LGN, and terminals from the retina were identified on the basis of their relatively large size, round vesicle shape and pale mitochondria (RLP classification of Guillery, '69).

In the newborn, RLP terminals make simple axodendritic synaptic contacts. The postsynaptic profiles do not contain vesicles, nor do any retinogeniculate synapses appear to be isolated from the surrounding neuropil by glial sheets. At one week, RLP terminals are somewhat more evident than in the newborn kitten, but synaptic organization remains essentially unchanged. The encapsulation of synaptic zones begins at around two weeks postnatal, but is not a prominent feature until four weeks. The end of the first month also marks a change in the complexity of retinogeniculate synaptic organization since many postsynaptic elements themselves contain synaptic vesicles. By eight weeks, synaptic complexes similar in configuration to those in the adult are common.

These results demonstrate that the morphological substrate for the relay of information from the retina through the LGN exists at birth in the cat. However, the development of mature retinogeniculate synaptic complexes does not take place until the second postnatal month, which may be related to the relatively late emergence of Y-cell responses in the LGN (Daniels et al., '78).

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2661 PENTOBARBITAL ENHANCES THE ACTION OF MUSCIMOL ON DOPAMINE TURNOVER IN THE RAT RETINA. Cylia W. Kamp* and William W. Morgan. Dept. Anat., Univ. Texas Hlth. Sci. Ctr., San Antonio, TX 78284.

Pentobarbital (PB) may exert part of its anesthetic action by potentiating GABAergic mechanisms. Recently, we demonstrated a GABAergic influence on the light-induced increase in DA turnover in the rat retina (Kamp and Morgan, Fed. Proc. 38:747, 1979). Muscimol (Mu), a GABA agonist, reduced the light-mediated enhancement of DA turnover in a dose-dependent fashion while picrotoxinin (Px), a GABA antagonist, blocked the effect of Mu on DA turnover. The present experiments were designed to determine the ability of PB to potentiate this GABAergic action. Five groups of rats were dark-adapted for 15 hr, and at the end of this time, one group was sacrificed without subsequent exposure to light while the other groups were exposed to light for 60 min before sacrifice. The first group of light-exposed rats was treated with a cumulative dosage of Mu (6.6 µmole/kg, i.v.), a concentration previously shown to be ineffective in altering DA turnover. The second group received an anesthetic dosage of PB (40 mg/kg, i.v., cumulative), and the third was treated with a combination of Mu and PB. The control group received saline injections at all time points. Half of the animals in all 5 groups were injected with α methyl-p-tyrosine (αMPT 250 mg/kg, i.p.) 60 min before sacrifice. The retinas were removed and frozen immediately after sacrifice. Later, DA contents were assayed radioenzymatically, and the rate of DA turnover was estimated from the depletion of DA following αMPT administration. Once again, light significantly enhanced retinal DA turnover in dark-adapted rats. Neither PB nor the low dose of Mu alone altered the dopaminergic response to light, but the combination of PB and Mu dramatically reduced the light-induced increase in DA turnover ($p<0.005$). In a separate experiment Px (1.88 mg/kg, i.v.) blocked the reduction in retinal DA turnover produced by the combination of PB and Mu. These data provide evidence for a GABA potentiating action of PB in the retina. The reliability of this retinal DA system as an in vivo test model of drugs which modulate the GABA system in the CNS is currently being investigated. (Supported by Grant No. 5 R01 DA 00755 and Research Development Award No. 5 K02 MH 00028 to WWM).

2662 RELATIONSHIP OF NEURONAL TO K-RESPONSES IN MUDPUPPY RETINA. Chester Karwoski, Hiroshi Shimazaki* and Luis Proenza. Depts. of Psychology and Zoology, Univ. Georgia, Athens, GA 30602.

Intracellular responses of on/off-neurons (probably amacrine cells) and of Müller (glial) cells, and changes in $[K^+]_o$, were recorded in the mudpuppy eyecup. A drop of Ringers was placed in the eye to retard drying.

Within a thin layer of the distal retina, an apparent light-evoked K-increase was observed in about 20% of the penetrations. The response was usually labile and its amplitude was typically small, but on two occasions it was greater than 0.6 mM. When observed, the distal K-increase always showed strong surround antagonism, but additional data on its behavior remain limited.

In the proximal retina, the light-evoked increase in K was quantitatively compared to Müller cell responses. This K-increase had maximum amplitudes of up to 0.6-1.0 mM, which should evoke glial responses of 5-9 mV. The rise time and latency of the K-increase were generally slower than glial responses, suggesting dead space may normally degrade the recorded K-response. In a few penetrations, however, the time course of the proximal K-increase was as fast as that of glial responses, so, on fortuitous occasions, dead space effects seem negligible. In these cases the half-decay time of K and glial responses was similar: 2-3 sec. Analysis of the spatiotemporal characteristics of the K-decay suggest both active reuptake and diffusion play roles in the clearance of K from extracellular space.

Müller cell responses show surround antagonism and decreased amplitude to high intensity stimuli, as previously shown in eyecups drained of vitreous. Müller cells in undrained eyes had large, stable resting potentials, but responses were small, averaging only 1-3 mV at maximum.

All on/off-neuron responses show initial transient and later sustained components. The sustained component shows surround antagonism and decreased amplitude to high intensity stimuli, which together with other evidence suggests that on/off neurons liberate much of the K recorded in the proximal retina. On/off responses and recorded K may be related as follows: (1) on/off neurons release K in proportion to their level of depolarization, (2) K accumulates in (is integrated by) extracellular space, and (3) the accumulating K is cleared by processes that have a half-decay time of ~2.5 sec. These three events can be modelled by passing on/off responses through a low-pass filter network having appropriate rise and decay times. The resultant "smoothed" neural responses bear striking similarities to K and Müller cell responses in all aspects yet examined.

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2663 EYE MOVEMENT-DEPENDENT ENHANCEMENT OF VISUAL RESPONSES IN THE PULVINAR NUCLEUS OF THE MONKEY. William Keys* and David Lee Robinson. Laboratory of Sensorimotor Research, National Eye Institute, Bethesda, MD 20014; Behavioral Sciences Department, Armed Forces Radiobiology Research Institute, Bethesda, MD 20014; and Department of Neurology, Georgetown University, Washington DC.

Cells in the superior colliculus and several cortical visual areas are excited by visual stimuli and show an enhanced response to a stimulus in their receptive field when a monkey uses this stimulus as the target for an eye movement. The behavioral contexts in which enhancement takes place vary for these regions of the brain. We have recorded from the pulvinar nucleus because its subdivisions receive inputs from these structures, and we wished to determine if enhancement were present and under which behavioral conditions it was demonstrable.

We have studied over 200 neurons in three rhesus monkeys trained on several tasks. The animals learned (1) to fixate a spot of light while visual stimuli were presented passively in the periphery, (2) to execute a saccadic eye movement from the fixation spot to another target, and (3) to maintain fixation while attending to a second light in the periphery. Extracellular single unit activity and eye movements were monitored while the monkey performed these tasks.

Ten percent of the cells in the pulvinar show a 50% increase in the response to a receptive field stimulus when the monkey uses that stimulus as the target for a saccade. This enhancement is spatially nonselective; the incremented firing rate is present when the animal makes saccades away from and into the receptive field. A similar proportion of cells in striate and prestriate cortices show enhancement that is likewise spatially nonselective but is nonspecific for eye movements (Wurtz and Mohler, 1976; Baizer and Robinson, 1974). The enhanced visual responses in the pulvinar are specific for eye movements. The modulation is present when the animal makes a saccade to a peripheral stimulus and is absent when the animal attends to but makes no eye movement toward the same stimulus. Enhancement in the superior colliculus and frontal eye fields is similarly dependent on eye movements but is spatially selective (Wurtz and Mohler, 1976; Bushnell, Robinson, and Goldberg, 1978). Thus the enhancement found in the pulvinar is unique. No other area of the brain is known to combine spatial nonselectivity with eye movement specificity.

The augmented firing of pulvinar cells in the enhanced condition indicates that a visually guided saccade is about to occur; it does not appear to encode the direction or size of the impending saccade. Such signals prior to eye movements may be useful to perceptual portions of the visual system in dealing with changes in the nature of the afferent stimulation caused by each eye movement.

2664 A SPECTRAL OPPONENT MECHANISM ENCODED BY ON-TYPE GANGLION CELLS OF RANA PIPIENS. Earl Kicliter and Yuzo M. Chino*, Laboratory of Neurobiology, University of Puerto Rico, San Juan, PR 00901, Illinois College of Optometry, Chicago, IL 60616.

We have previously shown that the major spectral opponent process on which the blue preference behavior of Rana pipiens is dependent, involves an interaction between the $\lambda_{max} \cong 432$ nm pigment of the green rods and the $\lambda_{max} \cong 580$ nm pigment of the principal and/or single cones (Kay and Kicliter, ARVO, 1978). We show here that some ON-type ganglion cells terminating in the anterior thalamus of these frogs encode the same spectral opponent process. Recordings were made from the anterior thalamus of Rana pipiens in or near the neuropils of Bellonci and the corpus geniculatum thalami, using tungsten-in-glass electrodes. Single ON-type units were isolated and were stimulated in the following manner. First, the receptive field of the unit was mapped. Then a spot of light was positioned within the receptive field center. The spot was composed of beams from 2 sources combined with a beamsplitter. Beam 1 was composed of short wavelength monochromatic light (dominant wavelength 450 nm). Beam 2 could be formed of any of 10 monochromatic stimuli varying in wavelength from 430-650 nm; these 10 stimuli were adjusted for equal quantal flux with a neutral density wedge. One sec stimuli were presented and total spikes in a 2 sec interval beginning with stimulus onset were counted. Activity produced by stimulation with beam 1 alone is defined as baseline activity. When beams 1 and 2 were combined, the following results were obtained. When the dominant wavelength of beam 2 was less than 500 nm activity was increased over the baseline rate. When the dominant wavelength of beam 2 was 500-640 nm activity was reduced as compared to baseline. Not all ON-type units appeared to respond in this manner, but at least some units encode the same spectral opponent process on which the behavior is based. This strengthens the evidence that the blue preference behavior of Rana pipiens is dependent on information transmitted to the anterior thalamus by ON-type ganglion cells.

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2665 RETINAL INVOLVEMENT IN THE ELONGATION OF NEONATALLY SUTURED CAT EYES. Albert W. Kirby and Harold Weiss*. Ophthalmology Dept., Kresge Eye Institute, Wayne State Univ. School of Medicine, Detroit, Mich. 48201.

Last year at this meeting we reported that the sutured eye of monocularly deprived (MD) neonatal cats and monkeys shows an increase in axial length compared to the fellow eye. In agreement with an earlier report by Wiesel & Raviola (1977) the deprived eye of a monkey is myopic. The deprived cat eyes are sometimes myopic and sometimes not. In the case of the non-myopic eyes the corneal curvature usually compensates for the increase in axial length. The amount of myopia differs considerably from that predicted on the basis of axial length differences alone, and the corneal changes, although in the right direction, are in most cases not sufficient to explain the absence of myopia in the non-myopic cats. Raviola & Wiesel (1978) further reported that dark rearing MD monkeys prevented the increase in globe length, seemingly implicating retinal absorption of light in the process. We sought to test this in cats by two methods: ablating all or part of the retina with laser photocoagulation, thereby destroying the involved retinal region and presumably preventing axial elongation; and by dark rearing MD cats.

Laser treatment alone apparently has no effect on eye growth. While we sometimes have trouble manipulating a small eye sufficiently to ablate the entire retina, we certainly ablate a large portion including the area centralis and surrounding regions. Lasered and fellow eyes develop in an identical manner. If we suture an eye which has previously been lasered, it elongates similarly to MD through suture alone, although it is certainly true that some critical retinal patch may have been missed by the laser. Finally, dark rearing seems to prevent axial elongation. While dark reared MD cats show equal axial dimensions in both eyes, their MD litter mates, reared under normal lighting conditions, show increased axial dimensions in the sutured eye.

Unfortunately we do not know the mechanism of axial elongation in MD cats or the reason the cornea sometimes compensates for the increase in axial length. It does, however, appear that some retinal absorption of light is required to provide visual feedback which somehow controls eye growth.

Wiesel, T.N. & Raviola, E. 1977. Nature 266:66-68.
Raviola, E. & Wiesel, T.N. 1978. Invest. Ophthal. Vis. Sci. 17:485-488.

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2666 BINOCULAR COMPETITION: ITS ENHANCEMENT BY NOREPINEPHRINE. Baruch Kuppermann* and Takuji Kasamatsu. Division of Biology, California Institute of Technology, Pasadena, CA 91125

It has previously been proposed that norepinephrine (NE) plays a role in synaptic plasticity in the cat visual cortex (Kasamatsu and Pettigrew, Science 194, 1976; Pettigrew and Kasamatsu, Nature 271, 1978). In the present study, cats given continuous local perfusions of 50 µM NE for seven days with a binocular environment were found to have a conspicuous lack of cells dominated by the ipsilateral eye, even though the ratio of binocularly:monocularly driven cells remained unchanged. This effect was not seen for periods of NE perfusion of less than three days. A slight innate contralateral bias in ocular dominance (OD) distribution has long been observed (Hubel and Wiesel, J. Physiol. 160, 1962). Therefore, NE is involved in exaggerating this natural bias in OD distribution. Several normal kittens were subjected to the same procedure, continuous local perfusion of 50 µM NE for seven days, but were simultaneously kept in the dark. The resulting OD histograms were quite normal, with none of the exaggerated contralateral skewedness observed. This suggests that the tendency for NE to induce a contralateral bias is activity-dependent. In current experiments, spontaneous retinal activity is being directly manipulated to further test this hypothesis.

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2667 SUBCORTICAL PROJECTIONS TO DORSAL LGN. Thomas P. Langer* and Carol L. Colby* (SPON: J.G. Malpeli). Dept. of Psychology, MIT, Cambridge, MA 02139.

We have examined the anatomical properties of those neurons in the superficial gray of the cat superior colliculus (SC) which project to the dorsal lateral geniculate nucleus (dLGN). In particular we have looked at their size, shape and position in relation to the known afferents and efferents of the superficial gray.

Small dense electrophoretic injections of HRP were made in the C laminae of dLGN. Sections were reacted with TMB which is essential for demonstrating the very small neurons in the dLGN projection.

After injections centered in the C laminae of dLGN, labelled neurons were found in a compact group at the corresponding retinotopic position in the ipsilateral SC. They were distributed throughout the depth of the zone of horizontal cells (0-250 µm below the collicular surface) and less densely within the upper margin of the zone of vertical cells (occasionally as deep as 350 µm). This region of the SC is known to receive a heavy input from the contralateral retina, the nucleus of the optic tract(NOT) and from both parabigeminal nuclei (PB) in addition to a lighter input from areas 17, 18 and 19 in visual cortex.

The HRP-labelled neurons were among the smallest encountered in the SC (mean cross-sectional area 33.4 $\mu m^2 \pm 15.2\ \mu m^2$ S.D.). There was little dendritic filling but the location, size, shape and proximal dendrites, where present, suggested that these were the stellate granule cells found in this region in Golgi impregnated material.

The collicular projection to dLGN is but one part of a network of interconnections involving SC, PB, NOT and dLGN. Labelled neurons were also found bilaterally in the parabigeminal nuclei, the NOT and in the parabrachial mesencephalic reticular formation along with the adjacent central gray ventral to the inferior colliculus. Ipsilateral neurons were more numerous in all these regions and the NOT group of labelled cells was almost entirely ipsilateral. The cells were of a variety of sizes but tended to be smaller than the average within each region. The labelled cells in PB were among the smallest neurons in that nucleus.

The collicular neurons labelled by HRP injections of the C laminae of dLGN are a discrete, well characterized population distinct from populations which project to other visual structures. The compactness of the labelled population suggests a tight retinotopic correspondence in this projection. Other subcortical projections to dorsal lateral geniculate nucleus appear to originate from equally distinct subpopulations of cells.

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2668 SPATIAL AND TEMPORAL CONTRAST SENSITIVITY IN MONOCULARLY DEPRIVED CATS. Stephen Lehmkuhle, Kenneth E. Kratz, and S. Murray Sherman. Dept. Physiol., Sch. Med., U. Va., Charlottesville, Va. 22908, U.S.A.

We measured spatial and temporal contrast sensitivity functions (CSFs), which plot the reciprocal of the contrast threshold as a function of spatial or temporal frequency, for a normally reared cat and two cats raised with monocular eyelid suture. Using the operant technique of conditioned suppression, we trained cats to suppress a licking response whenever a CRT-generated, counterphased, sine-wave grating replaced a homogeneous field of equal space average luminance. Contrast thresholds were estimated during a testing session using a staircase procedure.

The spatial and temporal CSFs of the nondeprived eyes of the monocularly deprived cats were similar to those of the normal cat. At lower temporal frequencies (e.g., 1 Hz), the spatial CSF showed an attenuation in sensitivity for spatial frequencies lower and higher than the peak frequency (0.5 c/deg). At higher temporal frequencies (e.g., 25 Hz), the spatial CSF showed reduced sensitivity for all spatial frequencies; however, there was no relative attenuation for low spatial frequencies. The temporal CSFs, which were measured at the most sensitive spatial frequency, showed a peak at 3 to 5 Hz, and attenuation for lower and higher temporal frequencies.

We found that contrast sensitivity of the deprived eye was nearly 1.5 log units lower than that for the nondeprived eye for all spatial and temporal frequencies investigated. The magnitude of this sensitivity loss was similar for both monocularly deprived cats, and has remained unchanged over a 9-month period of testing. Also, the shape of the spatial CSF did not change with temporal frequency.

These behavioral results indicate that the visual impairment of the deprived eye is more than a loss in acuity, but also represents a severe amblyopia that covers the entire spatial frequency spectrum. Moreover, these data complement the physiological result that there is a functional reduction of Y-cells in the deprived geniculate laminae.

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2669 RECEPTIVE FIELD PROPERTIES OF CELLS RECEIVING CALLOSAL INPUT. Franco Leporé, Jean-Paul Guillemot*. Dept. Psycho., U. of Montreal, Montreal, Canada.

The aim of the present experiment was to study the receptive field (R.F.) properties of cortical cells which receive part of their input from the contralateral hemisphere via the corpus callosum. Two groups of cats were used for recording unit activity: a normal group which served to gather normative data and an experimental group consisting of cats which had their optic chiasma split across the midline three weeks to three months prior to the recording session. Because of the surgery, the normal binocular input was abolished and any cortical cell manifesting this property must be receiving its input directly from the eye ipsilateral to the recording site via the geniculostriate pathway and indirectly from the contralateral eye via a callosal pathway. Acute recordings were carried out in the conventional way using tungsten microelectrodes and N_2O:O_2 anasthesia. The recording site was the 17-18 border. The stimulus consisted of thin bar generated on an oscilloscope screen by a computer. The bar, whose orientation was varied automatically from 0^o to 345^o in 15^o steps, was swept across the screen at constant speed orthogonal to the orientation. Three R.F. properties were studied using quantitative criteria: (a) R.F. position in relation to the vertical meridian, (b) ocular dominance, and (c) orientation tuning. Data from normal cats confirm the published results obtained under similar conditions: i.e. R.F. lie near the vertical meridian which they sometimes straddle, ocular dominance distributions were biased towards binocularity and orientation tuning varied from extremely fine tuned unidirectional cells, to bidirectional cells to untuned cells. The picture was somewhat different for chiasma sectioned cats: few cells were visually unresponsive, especially in recently sectioned cats; their R.F. were situated near or overlapping the vertical meridian; cells were all monocular during the first 200μ of the penetration and beyond about 600μ, between these two limits many of the cells were binocular although the ocular dominance distribution mainly favored the ipsilateral geniculostriate pathway; orientation tuning was similar for both the ipsilateral and contralateral (callosal) eye, the latter tuning curves being as fine or finer than the former.

2670 EFFECTS OF REVERSE SUTURE ON OCULAR DOMINANCE COLUMNS IN RHESUS MONKEY. Simon LeVay, Torsten N. Wiesel and David H. Hubel. Dept. Neurobiol., Harvard Medical School, Boston, MA 02115.

Monocular lid-suture in newborn rhesus monkeys redirects the development of ocular dominance columns in the striate cortex, so that the open- and closed-eye afferents to layer 4C segregate out into columns of unequal width. The effect is clear by 3 weeks of age, and by 6 weeks both the anatomy and physiology are very similar to what is seen in long-term deprived animals.

Can the deprived eye's afferents, once they have segregated out into their shrunken, fragmented columns, be induced to grow back into the territory they have vacated? To answer this question we studied animals in which the closed eye was re-opened and the seeing eye sutured shut. Ages at reverse suture were 3 weeks, 6 weeks and 1 year, and the animals survived for at least 5 months. Techniques included recording during tangential electrode penetrations and autoradiography of transneuronally transported 3H-proline.

In a monkey reverse-sutured at 3 weeks injection of the initially deprived eye gave rise to a surprising labelling pattern in layer 4C. The labelled columns were greatly expanded in the lower part of the layer (4Cβ) but shrunken in the upper part (4Cα). We interpret this pattern to mean that the afferents from the deprived parvocellular laminae of the LGN were induced to re-expand their territory even beyond their normal width, while the deprived magnocellular afferents were not induced to recover at all. Outside of layer 4C, neurons were overwhelmingly dominated by the initially deprived eye. With reverse suture at 6 weeks the autoradiography showed a recovery - in 4Cβ only - to a normal columnar width, but not an expansion beyond that; physiological responses in layer 4C matched the autoradiography. Again, the other cortical layers were dominated by the initially deprived eye. With reverse suture at 1 year there was no anatomical or physiological recovery.

The physiological results are similar to those of Blakemore et al. (J.Physiol.283,1978) in indicating a reversibility of eye preference after the effects of the initial deprivation have taken place. The autoradiography suggests that this process involves a contraction and later re-expansion of the afferents for the deprived eye in layer 4C. The alternative model, by which deprived-eye synapses are suppressed and later derepressed (Blakemore et al.,'78), seems less likely, but more precise anatomical methods will be required to resolve the issue. The period of anatomical plasticity seems to end earlier for the magnocellular than for the parvocellular afferents.

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2671 EVIDENCE FOR AN EXTRA-GENICULATE THALAMIC RELAY FROM THE RETINA TO CORTICAL AREAS 19 AND CLARE-BISHOP IN THE CAT. Audie G. Leventhal, Jeremy Keens* and Istvan Törk*. School of Anatomy, Univ. of N.S.W., Kensington, N.S.W., Australia.

A direct retino-pulvinar projection has been reported in several species, including the cat (cf. Berman and Jones, Brain Res. 134:237-248, 1977). We have investigated the afferent and efferent connections of the retinal recipient zone (RRZ) of the cats' pulvinar by 1) injecting tritiated proline into one eye and, in the same animal, injecting horseradish peroxidase (HRP) into the visual cortex, 2) comparing the distributions of labelled cells in the pulvinar following HRP injections into cortical areas 17, 18, 19 and Clare-Bishop, 3) removing one eye of adult cats, injecting HRP into their visual cortex and investigating terminal degeneration electron microscopically and 4) injecting HRP into the RRZ electrophoretically.

The results confirm a direct retino-pulvinar projection since 1) autoradiographic label was present in the RRZ after 72 hours survival, 2) degenerating terminals were found in the RRZ after monocular enucleation and 3) HRP injections into the RRZ labelled ganglion cells. We observed that cells of the RRZ project to cortical areas 19 and Clare-Bishop but not to areas 17 and 18. Labelled cells were observed in the C laminae of the lateral geniculate nucleus (LGNd) and in the medial interlaminar nucleus (MIN) following injections into each of the four areas. RRZ "relay cells" were small. They were similar in size to those of the parvocellular C laminae of the LGNd.

The retinal ganglion cells projecting to the RRZ all had medium sized somas and most were from 18 to 22 microns in diameter. The dendritic fields of over 200 ganglion cells with labelled dendrites were measured and found to be large: most were over 300 and many were over 500 microns in diameter even though complete "golgi-like" filling was not obtained in the HRP processed retinas. Thus, ganglion cells projecting to the RRZ had somas similar in size to those of beta cells but dendritic fields much more like those of gamma cells: no beta cell in Boycott and Wässle's (J. Physiol. 240: 397-419, 1974) sample had a dendritic field larger than 300 microns in diameter.

It has been suggested that gamma cells correspond to the physiological class of W cells (Boycott and Wässle, '74) and that the small relay cells of the parvocellular C laminae receive exclusively W cell afferents (Wilson et al., J. Neurophysiol. 39:1193-1209, 1976). Therefore, we suggest that 1) the RRZ is a region outside of the "conventional" limits of the LGNd via which the activity of W type ganglion cells is relayed to the visual cortex and 2) that the ganglion cells projecting to the RRZ represent a morphological class that has not yet been described.

2672 EFFECTS OF LESIONS OF PARIETO-OCCIPITAL ASSOCIATION CORTEX UPON PERFORMANCE OF OCULOMOTOR AND ATTENTION TASKS IN MONKEYS. James C. Lynch and Jay W. McLaren. Department of Physiology, Mayo Clinic and Foundation, Rochester, Minnesota 55901.

Parieto-occipital association cortex has been implicated in the functions of visual attention and oculomotor control by both clinical and electrophysiological studies. In the present series of experiments, four monkeys were trained to visually fixate a 0.1° target and to follow it as it moved smoothly across a 52° square screen at velocities of 6-30°/sec and also as it suddenly jumped 8-24°. Eye position was measured with chronic electrooculographic (EOG) electrodes. EOG signals were digitized and stored for later computer analysis. After several weeks of baseline data collection, each animal received either a unilateral lesion or a one- or two-stage bilateral lesion. The size of the lesions ranged from about 2/3 of Brodmann's area 7 to all of area 7 plus adjacent prestriate cortex and the anterior bank of the intraparietal sulcus (posterior area 5).

The three monkeys which received bilateral lesions were impaired in their ability to accurately track a target moving 20°/sec or faster. Deficits consisted of either reduced ability to match eye velocity to target velocity or increased latency of initiation of slow pursuit movements. Horizontal and vertical tracking were affected, though not always both in the same monkey. Three monkeys were tested after unilateral lesion and showed no tracking deficit. The ability to accurately maintain fixation of a stationary target did not appear to be affected by any of our lesions. Inspection of raw data plots and preliminary statistical analysis of about 4500 saccade trials showed increased saccade latency following both unilateral and bilateral lesions. Latency was most frequently increased for saccades from the center of the screen toward the side ipsilateral to a unilateral lesion. Detailed statistical analysis of horizontal and vertical saccades is still in progress.

No evidence has been found of contralateral neglect of unilateral visual stimuli following lesion. One monkey was trained on a visual extinction task and showed no tendency to *ignore contralateral stimuli in paired stimulus* presentations following lesion.

These results support the proposal that parieto-occipital cortex plays an important role in the initiation and control of visually-evoked eye movements, and suggest that damage to this cortical region alone is not sufficient to produce the contralateral neglect which has been reported to follow larger parieto-occipito-temporal lesions in monkeys.

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2673 RETINAL DIMENSIONS OF RECEPTIVE FIELDS INCREASE DURING GROWTH IN THE GOLDFISH. Alan Macy and Stephen S. Easter, Jr. (SPON: L.T. Rutledge). University of Michigan, Ann Arbor, Michigan 48109

The goldfish retina grows both by expansion and by the addition of new cells at its margins. As the fish lengthens from 70 mm to 200 mm, the diameter of its eye increases by 2.5X. This means that if a ganglion cell's receptive field diameter measured in μm on the retinal surface remains constant during growth, the angular subtense of its receptive field will decrease. The angular subtense will remain constant only if the receptive field diameter measured in μm on the retina, increases by 2.5X. To determine the manner in which the distribution of receptive field diameters changes with growth, we recorded intraocularly from retinal ganglion cells of small (<70 mm) and large (>200 mm) intact, paralyzed, submerged goldfish while spot stimuli were presented on a tangent screen. Area sensitivity functions were used to calculate receptive field diameters.

The mean angular subtense (± SEM) of receptive fields was $15.1^{o} \pm 1.3^{o}$ in small fish and $11.7^{o} \pm 0.8^{o}$ in large fish. This corresponds to an increase in receptive field diameters from 520 μm in the small fish to 1,040 μm in the large. Thus, neither angular subtense nor retinal dimensions of receptive fields remain constant during growth.

Receptive field diameters may change so as to maintain a constant amount of overlap with neighboring fields. To test this hypothesis, ganglion cell densities were determined from retinal area measurements[1] and optic nerve fiber counts in small and large fish. Calculated from these densities, the constant overlap hypothesis predicts a change in receptive field subtense from 15.1^{o} to 11.9^{o}, and is therefore supported by the data. Preliminary data indicate that the small observed decrease in angular subtense is not due simply to poorer image quality in the small fish. If dendritic tree size and receptive field diameter are related, as is widely believed, our results show that dendritic tree size increases during growth. (Supported by Training Grant 5 T32 EY-07022 to AM and Research Grant EY-00168 to SSE).

[1]Johns, P.R. and S.S. Easter, Jr. (1977) J Comp Neurol, 176, 331.

2674 SOME OBSERVATIONS ON THE ORGANIZATION OF THE RETINAL PROJECTIONS TO THE PRETECTUM AND SUPERIOR COLLICULUS IN THE MACAQUE MONKEY AS DEMONSTRATED BY THE COMBINED USE OF LASER BEAM LESIONS OF THE RETINA AND AUTORADIOGRAPHY. Debra J. Magnuson, Michael Rezak, Louis A. Benevento, Dept. Anat., Univ. Ill. Med. Ctr., Chicago, Ill. 60680.

Seven macaque monkeys received argon laser beam lesions of specified regions of the retina in one eye. Several weeks later an intravitreal injection of ^{3}H-proline was made in the same eye with the idea that the proline would be taken up by the surviving ganglion cells. Monkeys without lesions but with proline injections served as controls. Examination of Nissl stained whole mounts of the lesioned retinae provided confirmation of the distribution of surviving ganglion cells. In addition, the distribution of grains within the dorsal lateral geniculate nucleus, whose retinotopic organization is well known, served to further verify the extent of the lesion. The distribution of grains in the superior colliculus (SC) was in accord with previous workers and followed the precise retinotopic map previously shown to exist. Except in those cases which had lesions restricted to the papulomacular bundle, grains were seen in the appropriate portions of the SC including the anterior or foveal portion. These results may provide evidence for a direct projection from the fovea which was previously in doubt. However, that these results are due to transsynaptic transport of tritium cannot be dismissed. Within the pretectum the sublentiform nucleus (SL) and the olivary nucleus (ON) had a more dense concentration of grains on the contralateral side. In the SL no clear retinotopic organization was found and this region still contained a dense amount of grains in cases with foveal lesions. Within ON, however, the distribution of grains from individual cases suggested that the foveal representation occupies a major part of the nucleus. Although there was an excellent correlation between the boundaries of retinal damage and the distribution of grains in the geniculate and SC, such a correlation was not clear in the ON. The fovea appears to be centered about the ventral portion of the nucleus. A poor retinotopy along with a strong foveal representation, coming directly from retina, in the ON should not be surprising since this nucleus has been shown to project directly to the Edinger-Westphal part of the oculomotor complex which controls, in turn, the intrinsic musculature of the eye. In the case of the pupillary light reflex, for example, it would be necessary for the photopic receptors in the fovea to have rather direct access to the Edinger-Westphal nucleus and this is apparently possible via the olivary nucleus. In contrast a poor retinotopy with a strong representation from the periphery may not be surprising for SL since this region projects to regions of the pulvinar which project to parietal cortex. (Supported by NIH grant EY 2940)

2675 GOAL DIRECTED EYE MOVEMENTS BY INTRALAMINAR THALAMIC STIMULATION IN ALERT CATS. H. Maldonado*, J.-P. Joseph* and J. Schlag (SPON: M.L. Schlag-Rey). Dept. Anat. and BRI, Sch. Med. UCLA, Los Angeles, CA 90024.

Presaccadic neuronal activity recorded in the thalamic internal medullary lamina (IML) of cats suggests a participation of this cell population in the command of eye movements (EM). The elicitation of eye movements by electrical stimulation was studied in alert, intact animals (previous results were obtained in encéphale isolé). The head was fixed; vertical and horizontal electrooculographic recordings were made with implanted Ag-AgCl electrodes. Electrical pulses were applied through stereotaxically driven tungsten microelectrodes in and around the IML including nuclei ventralis lateralis (VL), lateralis posterior (LP), lateralis dorsalis (LD) and medial pulvinar (Pul). Pulses of 0.1 msec duration at a 250/sec rate were systematically used. Train duration was varied from 50 to 500 msec and intensity from 10 to 500 μA. Current threshold was defined as that sufficient to evoke EMs in 50% of the trials. All elicited eye deviations were conjugate, contraversive, and dependent on the alertness state of the animal. Three kinds of EMs were distinguished: (1) Goal directed i.e., the direction and amplitude depended on the initial eye position. The goal was an area about 5 deg^{2} located at least 10 deg away from the center. The greater the distance between initial position and goal, the more likely was a threshold stimulus to be effective, the amplitude was larger, and the latency of saccades was shorter. Minimum latency was 35 msec. Stimulus durations longer than 250 msec could produce double saccades of different directions, bringing the gaze to the goal. Head turning was obtained in experiments in which the animal's head was not fixed. Goal-directed saccades were observed exclusively with IML stimulations but only in 20% of the cases. (2) Direction and Amplitude Specific i.e., the direction was specific of the site stimulated and the amplitude was a function of stimulus current and train duration. Minimum latency was 55 msec. Stimulus durations longer than 250 msec could also produce double saccades but in the same direction. This type of saccade was the most commonly observed by stimulation of all sites explored except VL. (3) Centering i.e., whatever the initial eye position, the stimulation brought back the gaze to the center. These saccades were always accompanied by other movements (e.g. of face, ears, whiskers, neck, body). They were evoked only from VL. Minimum latency was 90 msec. The findings that goal-directed saccades were specific of the IML, that minimum thresholds (16μA) and minimum latencies were obtained with IML stimulation, may be related to the role of this structure in visual targeting. Supported by USPHS grant NS-04955 and CONACYT fellowship 16002, Mexico.

2676 INTRINSIC PROCESSING IN THE VISUAL CORTEX OF PRIMATES.
R. J. W. Mansfield and L. K. Simmons.
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The multiplicity of retinotopically organized, often reciprocally interconnected cortical areas in the primate brain raises the problem of analysing intrinsic properties apart from those resulting from extrinsic connections. One approach is the use of isolated preparations of cerebral cortex to facilitate the electrophysiological and pharmacological study of neuronal properties under more controlled conditions than are found in vivo.

To determine at a cellular level the nature of the intrinsic processing, we examined in vitro perfused thin slices (600 μm) of monkey visual cortex that were sectioned normal to the pial surface to retain functionally intact their columnar organization. The slices were mounted in a chamber on the stage of a microscope permitting direct visual placement of electrodes. In addition, cell soma and their processes could be visualized with Nomarski optics or viewed for epi-fluorescence after labelling with horseradish peroxidase conjugated with the fluorogen tetramethyl rhodamine isothiocyanate. A multiport perfusion system and small chamber volume allowed the rapid exchange of normal perfusate with perfusates containing various ions or pharmacological agents.

Of particular interest was the initial segment of the cortico-cortical pathway formed by the pyramidal cells in Layers II and III of Area 17, but similar results were obtained from slices from both the prelunate gyrus and the inferior parietal lobule. Previous work indicated that the pyramidal neurons in Layers II and III could be well activated by brief bipolar electrical stimulation in Layer I or IV. Their responses are reversibly abolished by low calcium perfusate or by atropine, but not by nicotinic antagonists, suggesting that the activation is mediated by cholinergic synapses of the muscarinic type. Low frequency stimulation reversibly depressed the response with exponential decay and recovery phases. The depression was not blocked by the gabinergic antagonist bicuculline, but rather this drug released a long latency component.

These results suggest a cellular basis for intracolumnar adaptation and pericolumnar inhibition. (Supported in part by NSF grant BNS75-08437.)

2677 MODULATION OF LGN CELL RESPONSIVITY BY VISUAL ACTIVATION OF THE CORTICOGENICULATE PATHWAY. R.T. Marrocco, J.W. McClurkin*, and Z.H.H. Farooqui*. Dept. Psychol. Univ. of Oregon, Eugene, OR 97403.

Corticogeniculate (CG) cells in layer VI of striate cortex have receptive fields similar to non-CG cells of the same layer. They are usually complex fields and require moving stimuli. Their receptive field areas in monkey average about 4 sq.deg. (Hubel and Wiesel, 1968). Receptive fields of monkey LGN cells average about 0.04 sq. deg., some two orders of magnitude smaller than the CG cells that may contact them. Moreover, there is a strict retinotopic correspondence between the field location of a geniculate cell and its CG feedback. By using a windmill-type (radial grating) stimulus that lacked a circular central disc, we were able to indirectly modulate an LGN cell's response to a flashing spot without directly stimulating its receptive field.

The activity of LGN cells in paralyzed, N_2O anesthetized macaque monkeys was recorded with microelectrodes and their receptive field properties measured with chromatic or achromatic spots on neutral or chromatic backgrounds, and with counterphasing sine wave gratings. Spike latencies to optic chiasm shock were also measured. Averaged responses to small and large spots (1) with and (2) without the stationary windmill were compiled. We also averaged responses to the spot in the presence of the same windmill (3) in radial motion, which by itself had no apparent effect on the cell's spontaneous activity.

Comparisons of conditions (1) and (3) showed that windmill movement produced net excitatory or inhibitory effects in different cells. Effects were seen as a change in the initial transient component of the LGN cell's response, or both the transient and sustained components, but not in the sustained component alone. Of a sample of 86 LGN cells, 18% showed net excitation, 47% net inhibition, and 35% were unaffected. Since the changes could be seen in the initial transient component, the modulation must be capable of acting at relatively short latencies. Effects were seen in opponent and non-opponent cells and in both X and Y types as well but not seen in LGN cells tested in one animal whose striate cortex had been removed. Although we have not conclusively ruled out contributions from the feedforward tectogeniculate pathway, we think it unlikely since most of our recordings were from parvo-, rather than magnocellular or interlaminar layers. These results suggest that the CG input serves to regulate the overall excitability of LGN cells across rather than between information channels. Supported by EY 01286-04.

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2678 RETINOTOPIC ORGANIZATION OF AXONS IN THE OPTIC NERVE AND TRACT OF NORMAL AND SIAMESE CATS. C.A. Mason, E.H. Polley+ and R.W. Guillery. Dept. Pharm. & Physiol. Sci., Univ. Chicago, Chicago, IL 60637 and +Dept. Anat., Univ. Illinois Coll. Med., Chicago, IL 60680.

The retinotopic organization of axons in the optic nerve, chiasm, and tract was studied in normal and Siamese cats. We wished to observe the degree to which retinotopy is maintained throughout the system and to determine where along the pathway the abnormally crossed temporal fibers of Siamese cats become misrouted. Fibers arising from specific parts of the retina were followed by making small photocoagulations and tracing degenerating profiles in sections of the pathways. Lesions were made within 5-10 mm of the area centralis in anesthetized cats. Ten days later, animals were anesthetized and perfused with 4% paraformaldehyde in 0.1M Sorenson's phosphate buffer. The optic nerve was transversely sectioned and the chiasm, including the adjacent optic tract, was horizontally sectioned at 35μ with a vibratome. Sections were treated with 1% buffered OsO_4, dehydrated and mounted on slides. The degenerating profiles are strongly osmiophilic, and degenerating fibers could be plotted under a 100x objective with an X-Y plotter.

Immediately behind the eye, degenerating axons produced by localized lesions form a well-localized group in both normal and Siamese cats. However, many individual axons are scattered away from the main grouping, and a considerable number of normal axons are mixed amongst degenerating fibers. These normal axon segments could be followed through the whole thickness of the section. In normal cats, this localized grouping is maintained in the optic nerve and can still be seen close to the chiasm, following either a nasal or temporal lesion. In contrast, after lesions of the Siamese cat's temporal retina, which gives rise to abnormally decussating fibers, sections from 1-2mm anterior to the chiasm show degenerating profiles which lie much more evenly scattered throughout the entire section of the nerve.

In normal cats, fibers crossing in the chiasm from the nasal retina occupy the entire cross sectional area of the chiasm. The small component that crosses from the temporal retina in normal cats tends to concentrate in the posterior part of the chiasm. In Siamese cats, the abnormally crossed axons that arise in the temporal retina also scatter throughout the cross-sectional area of the chiasm. Further, as described by others, there is little retinotopic order in the optic tract of normal cats, and the same is true for Siamese cats. In both, there is a segregation of fiber sizes in the tract, coarser fibers lying laterally. It is significant that at geniculate and tectal terminations, retinotopic order is re-established in normal as well as Siamese cats. (Supported by PHS NS 14283 and EY 02374).

2679 Development of Somal Synapses in Visual Cortex (Area 17) of the Macaque Monkey S. Mates* & J.S. Lund, Dept. of Ophthalmology, University of Washington, Seattle, WA 98195

As a continuation of previous studies on the developmental sequence of neurons in visual cortex (Area 17) of Macaque monkeys (Lund et al 1977) we have been studying the formation of somal synaptic contacts of identified cortical cell populations receiving thalamo-cortical axons. Cell bodies containing no synapses or only type 2 synapses were classified as spiny stellate or pyramidal neurons based on the shape of the cell and the laminae in which they were found. Intrinsic cortical neurons, the spine-free and sparsely spined neurons were found less frequently and were identified by their amount of cytoplasm and their mixed synaptic profiles, containing both type 1 and type 2 somal contacts. They are known to form type 2 contacts onto cells in the local milieu.

Type 2 somal contacts in 4cα increase until 5 weeks of age and then remain constant until some time between 6 and 9 months of age. During this time, a reduction in cell size causes the percentage synaptic covering of the neuronal surface length (mean covering) to increase until 12 weeks of age. Between 6 and 9 months a decline of synapses accompanies loss in cell size and by adulthood synapse number and somal size resemble that seen at birth. In 4cβ synaptic number and somal size increase during the first 12 weeks postnatal. From 12 weeks to adult there is a steady decline in both synaptic contacts and cell size. Pyramidal neurons of both upper and lower 6 show a rapid increase in synapses to 5 weeks of age. Between 8 and 12 weeks there is an increase in synapse number which remains constant until at least 9 months of age. Between 9 months and adult there is a further reduction in both somal size and number of contacts to reach the adult level. The mean covering is greatest in upper 6 at 8 weeks postnatal and lower 6 at 3 weeks postnatal.

In a monkey deprived of vision during the first 8 weeks of life, somal contacts and cell size resemble that seen at birth for 4cα, while in 4cβ the number of contacts is only slightly diminished from that of the normal 8 week animal. The differential effects seen in synapse formation in 4cα and 4cβ follows the same trend as the developmental sequence of dendritic spines onto cells of these laminae (Lund & Boothe, in press). Cells receiving primarily magnocellular influence reach a plateau in both spine number and somal synapses, and remain constant for a period of time, whereas cells receiving parvocellular information reach a spine and synaptic contact peak, followed by a rapid decline. Change in cell size alone does not account for synaptic and spine changes during this period. The differential effects seen in synapse formation may reflect functional differences in the establishment of intrinsic domains in area 17 during this time. Using Golgi EM techniques, we are examining the relationship between the formation of somal contacts and contacts seen on dendritic spines.

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2680 SYNAPTIC ORGANIZATION OF THE FROG'S OPTIC TECTUM. Dan E. Matsumoto* and Frank Scalia. Dept. Anat. & Cell Biol., Downstate Medical Center, Brooklyn, N.Y. 11203.

The terminals of retinal ganglion cell axons (OT) were examined in the optic tectum of R. pipiens by EM. OT were identified by four experimental markers. (1) Frogs were unilaterally enucleated to induce a terminal degeneration; (2) ^{3}H-proline was injected intraocularly to label OT for EM autoradiography; (3) OT were labeled by anterograde transport of HRP injected intraocular; (4) HRP was applied to the transected optic nerve to fill OT diffusely. The last method labeled OT in large numbers, and their morphology was relatively unaffected for usefully long survival periods. For details of methodology and background see Scalia and Colman, Br. Rs.'74, Colman et al., Br. Rs. '76, Matsumoto and Scalia, An. Rec.'79. Labeled axons and OT were observed in layers A-F and layer G (Potter, J.C.N.'69). The presence of OT in layer A confirms LM findings (Scalia, Br. Rs.'73; Scalia and Colman,'74). Labeled OT in layers B and D in frogs killed within 2 days of optic nerve transection and application of HRP were absent after survivals of 2 weeks. Labeled OT in layers C and E (and layer A) continuous with unmyelinated axons in the same layers were abundant after short survivals and were present in frogs surviving retinal ablation for up to 69 days before nerve transection and application of HRP. (This extends the potential for long-term survival of transected unmyelinated optic nerve axons beyond the time reported in Matsumoto and Scalia '79). Labeled OT in layers F and G were observed in continuity with large diameter axons in the same layers. Therefore, layer G axons terminate in their own layer. OT contained medium-sized, clear, spherical vesicles and relatively pale mitochondria. They made asymmetric contacts on dendrites, and on other unlabeled neurites, containing pleomorphic vesicles and dark mitochondria. No definite instances of axosomatic synapse were observed.

Szekeley et al. (J. Hirn.'73) distinguished four types of synaptic profile in material fixed by immersion in osmium tetroxide. OT identified in the present study resemble their optic, or type 1, terminals. However, type 1 was reported to be absent from layer A, whereas type 2 was prevalent there and was the most numerous throughout the superficial tectum 30 days after eye removal. Since OT labeled by HRP were present in layer A and remained intact in layers A,C and E in significant numbers for 30 days, it may be that some type 2 were OT. Few terminals having the given characteristics of types 2 and 3 were observed in the present study, while the impression was gained that the population of terminals is more highly differentiated than a four category schema would suggest. (Supported by NSF grant BNS 75-21308).

2681 THE WEBER-FECHNER RELATION IN RETINAL RODS. Gary G. Matthews, King-Wai Yau* and Denis A. Baylor. Dept. of Neurobiology, Stanford University Medical School, Stanford, CA 94305

The membrane current of a rod outer segment in a piece of isolated toad retina was recorded by drawing it into a glass suction electrode connected to a current-to-voltage transducer. The sensitivity to brief light flashes was determined in darkness and in the presence of various levels of background illumination. The relation between flash sensitivity, S_F, and background light intensity, I_B, was fitted by the Weber-Fechner relation,

$$S_F = \frac{S_F^D}{1 + I_B/I_o}$$

where S_F^D is the flash sensitivity in darkness and I_o is a constant with dimensions of light intensity. The constant I_o has been interpreted as representing the level of a "dark light" or intrinsic excitation present in photoreceptors in darkness: only when background illumination is appreciable relative to this dark light does it cause a significant reduction in sensitivity. For thoroughly dark-adapted toad rods, the value of I_o at 500 nm was 0.19 ± 0.05 photon μm^{-2} sec^{-1} (mean ± S.D., N=7). In comparison, Fain's (1976) intracellular recordings give a value for I_o of 0.43 photons μm^{-2} sec^{-1}. Our value for I_o is equivalent to about 3 photoisomerizations sec^{-1}. This rate is two orders of magnitude greater than the rate of thermal isomerization of rhodopsin, which was determined by suction electrode recording to be about 0.02 sec^{-1} under the same conditions. This indicates that the dark light does not represent spontaneous isomerization of rhodopsin, as has sometimes been assumed. Assuming that the dark light represents a finite resting level of the internal transmitter which regulates outer segment conductance, the dark light may be converted to a steady "response" in darkness from the values of I_o and dark step sensitivity. This steady dark "response" and the measured dark current then give the fractional closure, B_D, of the light-sensitive channels in darkness. In seven experiments B_D was 0.35 ± 0.11 (mean ± S.D.). This value is similar to the mean value found in cones by Lamb and Simon (1977), using a different technique.

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2682 THE MIDDLE TEMPORAL AREA (MT) IN THE MACAQUE: ARCHITECTURE, FUNCTIONAL PROPERTIES AND TOPOGRAPHIC ORGANIZATION. J. H. R. Maunsell*, J. L. Bixby* and D. C. Van Essen (SPON: M. Konishi). Division of Biology, California Institute of Technology, Pasadena, CA 91125.

We have used anatomical and physiological techniques to study the organization of MT, an area in the superior temporal sulcus (STS) of the macaque receiving direct inputs from the striate cortex. MT is situated in a region characterized by heavy myelination relative to that of adjacent cortical regions. The projections to the STS revealed by lesions and ^{3}H-proline injections are restricted to the heavily myelinated zone, and all parts of this zone receive striate projections. Thus, cortical myeloarchitecture provides an adequate basis for identifying the borders of MT. MT occupies an elongated region of cortex about 35 mm^2 in surface area, which is less than 3% of the size of striate cortex.

Physiological recordings within the STS confirm Zeki's (J. Physiol. 236, 1974) finding that MT contains a high percentage of directionally selective (DS) cells. The region immediately adjacent to MT along its dorsolateral border contains very few DS cells, and the transition in functional properties occurs over a distance of 0.25 mm or less. In contrast, the region medial to MT contains numerous DS cells which differ from those within MT mainly in terms of their larger receptive field sizes. Thus, there are at least two visual areas in the STS concerned with the analysis of movement in the visual field.

The topographic organization of MT in the macaque is similar to, but more complex than that found in the owl monkey and the bushbaby. The complexities are indicated by several observations. a) Receptive fields of cells encountered along the perimeter of MT are not always close to the perimeter of the visual hemifield (vertical meridian and far periphery), and fields recorded midway between the borders of MT are not always on the horizontal meridian. Also, relatively large shifts in receptive field locations sometimes occur over short distances within the cortex. b) Anatomical experiments demonstrate instances of single loci within striate cortex projecting to multiple sites within MT. c) Interhemispheric connections, which in the bushbaby and owl monkey terminate preferentially along the perimeter of MT (Newsome, ARVO Abstr., 1979), are distributed irregularly within MT in the macaque, without any noticeable increase in density along the border. Analysis of receptive field locations and anatomical inputs also suggests that the relative emphasis of central vs. peripheral visual fields in MT is comparable to that found in striate cortex.

2683 TEMPORAL SUMMATION IN GANGLION CELLS OF THE TURTLE. J. H. Maxwell, J. E. Fulbrook and A. M. Granda. Inst. for Neuroscience, U. of Delaware, Newark, De 19711.

Temporal summation characteristics were determined for single long-wavelength-sensitive ganglion cells in the turtle, Pseudemys scripta elegans. Extracellular recordings were made with tungsten microelectrodes in the optic nerves of curarized and locally anesthetized animals.

Initially, the receptive field of each cell was mapped with both moving spots and stationary flashes of monochromatic light projected on a hemispherical screen 1 m in radius centered on the turtle's left eye. Subsequent test stimulation was restricted to the receptive field centers which ranged in diameter from 2 to 6 degrees. Spike responses to three to five stimulus presentations were summed by computer. Intensity-response curves for individual wavelengths and flash durations were plotted using both latency and spike count response measures.

Close agreement was observed between spectral sensitivity curves derived from latency and spike count measures. In all cells, high criterion responses were associated with peak sensitivities in the area of 620 to 650 nm indicating red cone dominance. In some cells, thresholds determined with low criterion responses revealed significant gains in sensitivity to short wavelength light indicating multiple receptor mechanism input.

Temporal summation curves at each cell's peak sensitivity were determined using flashes 10 msec to 2 sec in duration. Summation curves derived from spike count and latency response measures were not always in close agreement. For some cells, latency-derived curves reflected no change in threshold as a function of stimulus duration while, for the same cells, spike-count-derived curves accurately defined the reciprocal relationship between threshold intensity and flash duration. Critical duration values fell between 130 and 200 msec but, for low criterion responses, cells with multiple receptor mechanism input summed beyond 500 msec. These findings are consistent with previously obtained behavioral data for this same species.

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2684 CONNECTIONS OF THE CLARE-BISHOP AREA WITH AREA 17 AND THE DORSAL LATERAL GENICULATE NUCLEUS IN THE CAT. A. J. McGrath,* G. R. Leichnetz, and J. Astruc, Department of Anatomy, Medical College of Virginia, Richmond, Virginia 23298.

The Clare-Bishop (CB) area of the cat cortex bears the names of the investigators who first (1954) recognized a region, located primarily on the lateral aspect of the middle suprasylvian gyrus, which electrophysiologically responded to both striate cortex and optic nerve stimulation. More recent studies (Palmer et al, 1978; Kennedy and Magnin, 1977) have supported its role in visual and/or oculomotor functions. Anatomical studies were performed in order to provide a morphological basis for its proposed function. In the process of this investigation it was found that a substantial reciprocity of connections exists between the CB area and Area 17, using both orthograde (silver impregnation) and retrograde (HRP) methods. Silver techniques (Nauta, Fink-Heimer) revealed that CB projects primarily to laminae III-IV of Area 17. However, some degenerating fibers were also observed to traverse lamina II and enter lamina I, giving rise to preterminal and terminal degeneration running parallel to the surface of the lateral gyrus. HRP studies, on the other hand, revealed that medium-sized pyramids located primarily in lamina III of Area 17 projected to the CB cortex. Anterogradely transported HRP also confirmed the presence of a terminal field in both laminae III and I of Area 17. In addition to finding these intimate connections of CB with Area 17, it was also observed that the Clare-Bishop area apparently receives substantial input directly from the dorsal lateral geniculate nucleus (dLGN). Whereas earlier investigations (Heath and Jones, 1971; Rosenquist et al, 1974) have held that CB only receives innervation from the medial interlaminar nucleus and lamina C of dLGN, this study demonstrated that all of the main laminae (A, A1 and C) have direct projections to the CB cortex. Thus it appears, based on the data presented in this study, that the Clare-Bishop area is indeed an intergral portion of the primary visual cortex in the cat, due to its receiving input directly from the dorsal lateral geniculate nucleus, and its heavy reciprocal connections with Area 17. The data from this study provide an important anatomical substratum for CB's proposed role in visual activities.

2685 QUANTITATIVE STUDIES OF OPTOKINETIC NYSTAGMUS IN MONKEYS BEFORE AND AFTER LESIONS OF PARIETO-OCCIPITAL ASSOCIATION CORTEX. Jay W. McLaren and James C. Lynch, Department of Physiology, Mayo Clinic and Foundation, Rochester, MN 55901.

Damage to parieto-occipital cortex in humans results in asymmetric optokinetic nystagmus (OKN) with the slow phase diminished toward the side of the lesion. In the present study eye position (electrooculogram) was recorded during OKN in rhesus monkeys before and after unilateral and bilateral lesions of parieto-occipital cortex. OKN was induced with either a large vertically striped drum which could be rotated around the monkey, or projected black and white stripes that could be moved across a 52° square screen in front of the monkey. Stimuli were presented at angular speeds which ranged from 20-160°/sec. In all cases the rotating drum was found to be a considerably stronger stimulus than the projected stripes. When sufficient data had been recorded to establish a baseline level of response, lesions were made under sterile conditions. Four monkeys received unilateral or bilateral lesions in one or two stages. The smallest lesion was limited to about two-thirds of Brodmann's area 7, while the largest included all of area 7, part of the immediately adjacent prestriate cortex (areas 18 and 19), and the anterior bank of the intraparietal sulcus (area 5).

Immediately after the unilateral lesion, monkeys showed a marked depression of OKN slow phase in the direction toward the side of the lesion. Following the second lesion (and in the single stage bilaterally lesioned monkey) slow phase velocity was depressed in both directions. In addition, two monkeys showed temporary spontaneous nystagmus following unilateral lesion.

OKN has been studied as a function of stimulus velocity in one monkey. Before lesion OKN slow phase velocity accurately matched angular velocity of the rotating drum at 40°/sec or less. When stimulus velocities were increased above 40°/sec, mean slow phase eye velocity increased at a lower rate than stimulus velocity. After unilateral lesion, the monkey's ability to maintain slow phase eye velocity close to the stimulus velocity was significantly reduced in the direction toward the side of the lesion at velocities of 40°/sec or higher. At 20°/sec, however, slow phase was not changed from the prelesion response. Slow phase in the direction away from the side of the lesion was also reduced slightly at velocities above 80°/sec. These results indicate that the parieto-occipital region in rhesus monkeys plays an important role in the generation and maintenance of the slow phase of OKN, particularly at high stimulus speeds.

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2686 SPATIAL AND TEMPORAL VISION OF MACAQUES AFTER CENTRAL RETINAL LESIONS. William H. Merigan, Tatiana Pasternak and Donald Zehl.* Univ. Roch. Med. Ctr., Rochester, NY 14642.

The spatial and temporal modulation transfer functions (MTFs) of two pigtailed macaques (Macaca nemestrina) were measured behaviorally before and after small laser burns were placed in their foveas. The spatial MTF was determined with stationary sinusoidal grating patterns. The temporal MTF (deLange function) was measured with both large (5°) and small (0.5°) unpatterned flickering targets. Preoperative sensitivity functions of both monkeys were similar to those of human subjects tested in the same apparatus. An argon laser was used to place small (2° radius) circular lesions in the center of the fovea of both eyes of each monkey. Fundus photographs confirmed the location, size and completeness of the lesions.

The post-operative spatial MTF revealed no loss of sensitivity at the lowest spatial frequencies, a loss of less than 0.5 log unit at middle frequencies and a loss approaching 1 log unit at the highest spatial frequencies. The temporal MTFs measured with the 5° stimulus showed no loss of modulation sensitivity. However, the temporal MTF for the 0.5° stimulus indicated a 0.3 log unit loss of modulation sensitivity at the low frequencies and no loss at the high temporal frequencies. These results will be discussed in relation to the spatio-temporal properties of human foveal vision.

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2687 A NEUROBEHAVIORAL INVESTIGATION OF ORIENTING BEHAVIOR IN THE SUPERIOR COLLICULUS AND VISUAL CORTEX LESIONED RAT. Glenda C. Midgley* and Richard C. Tees*. (SPON: J.P.J. Pinel). Psychology Department, University of British Columbia, Vancouver, Canada.

The orienting behavior of rats with variously sized lesions of the superior colliculus (SC) and visual cortex was assessed. Orienting behavior to auditory stimuli and 5 patterns of apparently moving light displays was measured by examining the disruption of ongoing behavior and the animals head and postural adjustments to repeated presentations of the stimuli.

Lesions of the SC did not result in visual agnosia or an inability to perform the motor responses involved in orienting. These rats were capable of orienting even in the complete absence of the SC. Rather, the orienting response was available in the behavioral repertoire of the lesioned animal, but, it was not always emitted in response to the visual displays that the intact animal treated as less salient. Rats with SC lesions oriented to and localized an apparently approaching and receding light display and lights which appeared to circle the entire visual field of the animal, but, did not orient to and localize lights which appeared to circle in smaller segments of the visual field. Deficits in orienting to the less salient light displays were reduced or eliminated by manipulating the amount of deprivation prior to testing and SC lesioned animals were capable of using the less salient displays as a signal of shock. The SC lesioned animals and animals with lesions of the cortical projections of the SC habituated more quickly than intact animals and did not dishabituate to changes in the visual displays. The results suggest a modulating role of the SC and cortex in orienting behavior and the stimulus parameters which must be considered in models of the neural basis of orienting behavior.

2688 EVOKED POTENTIALS TO DYNAMIC RANDOM-DOT CORRELOGRAMS IN MONKEY AND MAN: A TEST FOR CYCLOPEAN PERCEPTION. Francis M. Miezin*, Joel Myerson, Bela Julesz*, and John M. Allman. Biology, Caltech, Pasadena, CA 91125 and Bell Labs, Murray Hill, N.J. 07974.

Large evoked potentials are elicited in humans by dynamic random-dot correlograms of binocularly identical noise alternating with binocularly negatively-correlated noise (Julesz, Kropfl and Petrig, 1978). These correlograms contain no monocular cues; thus the evoked potentials are an objective indicator of a cyclopean perception (Julesz, 1971). We tried to elicit evoked potentials to these stimuli in alert macaque monkey so as to compare them with human evoked potentials and to develop a fast, objective test for cyclopean perception in animals. The monkey was trained to sit quietly in a chair placed facing a rear-projection screen. Dynamic random-dot correlograms, alternating at 0.5 Hz, were generated at 60 frames/sec. Stimuli for the left and right eyes were fed into the red and green channels, respectively, of an Advent projection TV set. Stereo separation was achieved by placing red and green Wratten filters over the red and green projection optics. In the cyclopean condition, goggles with red and green filters over the left and right eyes,respectively,were worn by monkey and human observers. After every even reversal,left and right dynamic noise was identical giving the percept of a flat depth plane; after every odd reversal, dynamic noise for one eye was the negative image of the noise for the other eye giving rise to binocular rivalry and an uncertain depth percept. To test for monocular cues, we used three control conditions: A) placing identically colored filters over both eyes; B) vertically shifting the green image relative to the red image; C) placing a 0.5 log neutral density filter in front of the green filter (this delays conduction from that eye (Julesz and White, 1969) thereby reducing the amount of time the two images are correlated). In a fourth control condition (D), 0.5 log neutral density filters were placed over both filters; this delays conduction from both eyes equally so that the images appear as in the cyclopean condition but dimmer. While the cyclopean condition and control D gave large evoked potentials, controls A and B gave none and control C gave a greatly diminished evoked potential. This proves that the evoked potential was a response to only the cyclopean aspects of the stimulus. Human subjects were told to look at the screen's center whereas the monkey's gaze wandered freely over the entire screen. Nonetheless, the monkey's evoked potentials were as large as those of the human, demonstrating that the method does not depend upon steady fixation of the stimulus. This result testifies to the robustness of evoked potentials to dynamic random-dot correlograms and extends their usefulness as a test for cyclopean perception. (Supported by NIH NS-00178, NS-12131, NSF BNS-77-15605, the Spencer Foundation and the Sherman Fairchild Scholars Fund)

2689 SENSORY REARRANGEMENT AND EYE POSITION. H.H.Mikaelian, M.Linton*, and M.Phillips*. Dept. of Psych., Univ. of New Brunswick, Fredericton, New Brunswick, Canada E3B-5A3

The generalizability of response alterations produced by sensory rearrangement was explored. Experiment 1 dealt with auditory rearrangement induced by pseudophones that rotated the interaural axis by 40° (right ear lead). The subject listened, for 20 min., to a hand held sound target emitting clicks while moving it from side to side in a semi-circle. Tests consisted of a)pointing to sound targets with the hand b)orienting the eyes towards the sound targets, and c)orienting the eyes to the straight ahead. All testing was done in the dark. Ear-hand coordinations were measured on a semi-circular target board in front of the subject, and eye position was monitored by infrared oculography. The results, measured as differences between pre and post-exposure responses, showed adaptive changes in ear-hand coordination of 30%. No systematic changes in orienting the eyes to the sound targets or to the straight ahead were found.

In a second experiment rearrangement was produced by prisms. The subject, moving the arm on a table, viewed it through 20 diopter wedge prisms. Testing consisted of a)pointing to visual targets with the hand, b)orienting the eyes towards the visual targets, and c)orienting the eyes to straight ahead. The results parallelled those of experiment 1. Up to 40% adaptive shifts in pointing with the hand were found; no systematic changes in orienting the eyes to the visual targets or to the straight ahead were observed.

These results indicate that sensory-motor changes produced by arm reafference during rearrangement are confined to responses in that arm. Following rearrangement, in both experiments, the subject locates the target in one position with the arm, and in a different position(same as pre-exposure) with the eyes. Recalibration of target directed responses, therefore, are limited to the system regulating arm movements, and adaptation does not represent a generalized change. It is possible to account for these results in terms of the proprioceptive change hypothesis(Harris, 1964), although previous studies have shown that response alterations tend to also be specific to the sensory modality that is rearranged, contrary to predictions of that theory.

2690 NEURONAL SPIKE TRAINS FROM SPONTANEOUSLY ACTIVE CELLS IN THE LATERAL GENICULATE NUCLEUS AND THE SUPERIOR COLLICULUS. Stéphane Molotchnikoff et Pierre Lachapelle. Dept. Sci. Biol. Université de Montréal, Montréal, Qué. Canada.

In anesthetized and immobilized rabbits, spike trains from spontaneously active neurons were recorded simultaneously with two tungsten microelectrodes aimed at the Lateral Geniculate Nucleus (L.G.N.) and the Superior Colliculus (S.C.). Autocorrelation and cross-correlation histograms were triggered by spontaneously occurring action potentials recorded from each location.

In the L.G.N. two patterns can be dissociated. Cells of the first group showed a tendancy not to fire after production of an action potential, and presented a slow recovery of their firing probability up to 250 msec. They were units with an initial decrease of excitation (I.D.E.). By contrast in cells of the second group the generation of an action potential led to an initial increase of excitation (I.I.E.) within 20 msec. Amongst this second group interneurons and relay cells must be distinguished according to standard criteria: response pattern to single optic nerve shock.

In the S.C. same patterns of histograms are found, that is: I.D.E. and I.I.E. However, the collicular depth profile is different for each class. Thus I.I.E. are almost exclusively encountered in the upper part of the Stratum Griseum Superficial. (Stratum zonal and upper third of the S.G.S.). While I.D.E. are more numerous deeper. Cross correlations between units of L.G.N. and S.C. suggest the possibility of mutual excitatory and inhibitory interactions. Such relationships may shed some light on the basis of visuomotor integration.

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2691 THE ANURAN ACCESSORY OPTIC SYSTEM: NEUROANATOMICAL ANALYSIS Neil Montgomery*, Katherine Fite & Lynn Bengston*. University of Massachusetts, Amherst, Massachusetts, 01003

The accessory optic system of Rana pipiens and Bufo Americanus was investigated using autoradiographic (^{3}H proline), horseradish peroxidase and Golgi techniques. Retinal afferents project via the basal optic root (BOR) to a "nucleus" (neuropil) of the basal optic root (nBOR) in the ventral mesencephalon, and are primarily large-diameter axons with well-defined terminal boutons, although some smaller caliber axons are also present. The major retinal projection is contralateral, with a small ipsilateral component. At least some nBOR optic axons appear to originate from giant and displaced ganglion cells.

The cellular constituents of nBOR resemble those previously described for the superficial layers of the optic tectum (Szekely & Lazar, 1976), and four morphologically distinct cell types were identified within the terminal field: 63% stellate, 19% amacrine, 14% elongate and 4% ganglionic cells. Both stellates and amacrines appear to be intrinsic neurons. In addition, pyriform neurons located in the region medial to nBOR send their dendrites into the terminal field and their axons to the anterior-ventral cell groups located paraventricularly. Optic afferents, as well as the dendritic and axonal processes of stellates, converge upon the dendrites of both ganglionic and pyriform cells in a glomerular configuration. Preliminary data suggest that the ganglionic cells project to the caudal thalamic-pretectal region, and elongate neurons project to the hypothalamus. Other neurons in the anterior-ventral cell group which send dendrites into nBOR include commissural neurons which project to the opposite nBOR, and pyriform neurons which interconnect nBOR and the oculomotor nucleus (n. III).

No evidence of direct efferent projections to the cerebellum was obtained despite numerous HRP injections of cerebellum. However, cells from the anterior-ventral cell group (whose dendrites penetrate nBOR) appear to project to the ventral medulla where several cell groups (n. raphe and n. reticularis) have been previously reported to give rise to cerebellar climbing fibers (Cochran & Hackett, 1977) in frog.

These observations strongly imply that the accessory optic system may play an important role in anuran oculomotor behaviors, a possibility first suggested by Herrick (1948) three decades ago.

2692 SINGLE UNIT AND BEHAVIORAL ASSESSMENT OF ECTOMAMILLARY NUCLEUS FUNCTION. B. Morgan* and B. J. Frost (Spon: M. Donald). Dept. of Psych., Queen's Univ., Kingston, Ont., Canada, K7L 3N6

The avian accessory optic nuclei receive direct projections from displaced retinal ganglion cells, and project directly to the oculomotor complex and the vestibulo-cerebellum (Brecha and Karten, 1979). It has been suggested that this system may play a specific role in visual control of head and eye stabilization. Fite, Reiner and Hunt (1977) found that lesions of the basal optic root or nucleus ectomamillaris (EM) virtually eliminated optomotor response (head nystagmus) to optokinetic stimulation.

The present study compared pre and post EM lesion behavior of pigeons to optokinetic stimulation presented both binocularly and monocularly. Single EM cell response characteristics were also investigated.

Before EMlesions, binocular head nystagmus was normal and characterized by a pursuit phase where head velocity matched stimulus velocity, both for clockwise and counterclockwise motion. In contrast, monocular head nystagmus approximated binocular conditions only with forward motion (posterior to anterior in the visual field). Backward monocular motion was far less effective in producing nystagmus and when it occurred head velocity was inappropriate. After unilateral EM lesions, head nystagmus was not elicited even by forward optokinetic stimulation of the contralateral eye. Post-lesion binocular nystagmus resembled pre-lesion monocular response.

Electrophysiological recordings of EM cells showed they were directionally selective, and preferred slow velocities of stimulus movement. Large single spots of light were generally ineffective, whereas very large textured patterns were optimal. These cells also exhibited a high spontaneous firing rate which was modulated up and down for preferred and null directions of motion respectively.

These observations lend support to the notion that the accessory optic system is involved in the processing of whole-field motion required for stabilization of the visual world.

2693 TWO-DIMENSIONAL SPATIAL FREQUENCY TUNING OF CAT STRIATE CORTICAL NEURONS. J. Anthony Movshon, Department of Psychology, New York University, New York, NY 10003.

Neurons in the striate cortex of cats and monkeys are selectively sensitive to the orientation and spatial frequency of sinusoidal grating patterns; this has been adduced as evidence in support of the idea that the visual system performs a kind of spatial Fourier analysis. But in order for the visual cortex to function as a global Fourier analyzer, the properties of striate neurons should permit them to signal the detailed harmonic structure of spatial patterns; this requires that the spatial frequency and orientation bandwidths of cortical neurons be inversely proportional to their optimal spatial frequencies.

Orientational half-width (in degrees) and spatial frequency bandwidth (in octaves) were determined for a large population of striate cortical neurons recorded from acutely prepared anesthetized cats. Optimal spatial frequencies ranged from 0.25 to 2.7 c/deg, and all receptive fields lay within 5 deg of the visual axis. Measurements on some cells taken over a matrix of orientations and spatial frequencies showed that within a cell the selectivity for one of these variables did not depend strongly upon the value of the other at which measurements were made.

Orientation selectivity and spatial frequency selectivity are well correlated with one another: orientation half-widths increase by about 10 deg for each octaves increase in spatial frequency bandwidth. Thus in a conventional two-dimensional frequency-space plot, the "receptive fields" of striate cells tend to be roughly round. Neither orientation half-width nor spatial frequency bandwidth, however, decreases strongly with spatial frequency: spatial frequency bandwidth decreases by about 0.5 octaves for each 1 c/deg increase in optimal spatial frequency, with considerable variability across the population. From these data it appears that if each striate neuron is to signal the presence of a particular spatial Fourier component, then only the first three or four spatial harmonics may be unambiguously extracted.

Thus the relations among the orientation tuning, spatial frequency tuning and optimal spatial frequency of striate neurons show these neurons to be ill-suited to performing a global Fourier analysis of the visual world. Their spatial properties are more consistent with a less extreme frequency analysis model which involves only the extraction of the first three or four spatial harmonics of images falling on a particular region of the retina.

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2694 DARK REARING DELAYS ONSET OF THE CRITICAL PERIOD IN KITTEN VISUAL CORTEX. George D. Mower*, Frank H. Duffy, James L. Burchfiel. Seizure Unit & Dept. of Neurology, Children's Hospital & Harvard Med. School, Boston, MA 02115.

The critical period in kitten visual cortex begins around the time of eye opening and ends at three months of age. We were interested in determining whether visual experience plays a role in the timing of the critical period or if it reflects a fixed maturational process. Kittens were visually deprived from birth to an age of 14-16 weeks by binocular lid suture or rearing in complete darkness. They were then given monocular experience for a period of up to 1 year.

Prolonged monocular experience following binocular lid suture resulted in little improvement of visual acuity for simple behavioral tasks (placing, tracking, obstacle avoidance, avoidance of a visual cliff). Recordings in area 17 revealed no dominance of the open eye. Twenty eight percent of the cells were driven only by the open eye, 27% only by the closed eye, and 21% were binocular. Twenty four percent of the cells were visually unresponsive. Many visually responsive cells, particularly binocular ones, had abnormal receptive fields. There were no significant differences in cell sizes between LGN laminae devoted to the opened or closed eye.

Dark reared kittens showed dramatic behavioral recovery during the course of monocular experience. Recordings in are 17 revealed clear dominance of the experienced eye. Eighty percent of the cells were driven exclusively by the open eye and these cells typically had normal receptive fields. Fourteen percent of the cells were binocular, and 6% were driven only by the closed eye. These cells typically had abnormal receptive fields. Anatomical studies of these kittens are in progress, but preliminary results suggest that only slight shrinkage (10%) occurs in geniculate laminae associate with the closed eye.

We conclude that in the total absence of visual stimulation considerable plasticity is maintained in visual cortex. Apparently there is sufficient visual stimulation through closed lids to produce permanent abnormal development.

2695 TECTAL AND CORTICAL AFFERENTS TO NUCLEUS LATERALIS POSTERIOR IN THE RAT. Sandra M. Nagel* and Charles M. Butter. Dept. of Psych., Univ. of Michigan, Ann Arbor, MI 48109.

Recent neuroanatomical investigations in various mammalian species have described a pathway from the superficial layers of the superior colliculus (SC), which receive retinal input, to particular thalamic nuclei, including nucleus lateralis posterior (NLP) in the rat. These nuclei project to visual cortical areas including prestriate cortex. However, the tecto-thalamic pathway in the rat has not been analyzed in detail; specifically, the characteristics of tectal cells which project to NLP have not been described. Likewise, other afferent projections to NLP have not been investigated. In the present study, horseradish peroxidase (HRP) was injected into NLP and adjacent diencephalic structures in the rat in order to describe these projections.

Following microiontophoretic injection of HRP confined to NLP, neurons containing HRP granules were found in the ipsilateral, and to a lesser extent contralateral SC. These neurons were confined to ventral stratum griseum superficiale and dorsal stratum opticum; they were identified as wide-field or narrow-field vertical cells, or stellate cells, as described by Langer and Lund. Following HRP injections into the dorsal lateral geniculate nucleus, labelled cells were also found in superficial layers of ipsilatersl SC. Pretectal injections resulted in labelling of superficial and intermediate tectal cells. Labelled pyramidal neurons were also found in layers IV - VI of ipsilateral prestriate (18a and 18b) and striate cortex, and in layer IV of temporal cortex (Krieg's area 20). These findings suggest that specific cell groups in particular sublayers of the dorsal SC project to NLP, which projects to visual cortical areas. These present findings also suggest that particular cell groups within these cortical areas project back to NLP.

2696 CHRONIC BLINDNESS FOLLOWING NONVISUAL CORTICAL LESIONS IN MONKEYS. Richard K. Nakamura and Mortimer Mishkin, Lab. of Neuropsychology, NIMH, Bethesda, MD 20205.

We previously reported that blindness can be produced in monkeys by a large cortical removal that preserves all areas known to be necessary for visual discrimination learning (Neurosci. Abs. 3:571, 1977). The large lesion spared the visual cortex (striate, prestriate, and inferior temporal) and limbic cortex (medial temporal, ventral frontal, and cingulate) but included all other cortical areas. Bilateral paralysis was prevented by placing the lesion in only one hemisphere, the other hemisphere having been left intact but visually deafferented by optic tract section and forebrain commissurotomy. Monkeys prepared in this way were behaviorally blind for periods ranging from 25 to more than 400 days, despite anatomical and physiological evidence that the retino-geniculo-striate pathway in the hemisphere with the ablation was preserved as intended.

The extreme variability in the duration of the blindness has not only been puzzling but has impeded analysis of the phenomenon. New studies indicate that this variability can be eliminated and a predictably long-term effect produced in two different preparations.

First, the original findings suggested a species difference that has now been confirmed with additional animals given the standard lesion. Thus, whereas a total of four rhesus monkeys (Macaca mulatta) have recovered from blindness after a median of only 28 days, none of four cynomolgus monkeys (Macaca fascicularis) has yet shown recovery despite a median postoperative survival period of 180 days.

Second, the rhesus monkey also will show prolonged blindness if the standard lesion is expanded to include most of the limbic cortex. Three animals prepared in this way have thus far remained blind for a median period of 100 days with no signs of recovery. It is important to note that the limbic lesion alone will not cause this effect as evidenced by two monkeys with such a limbic ablation that demonstrated accurate visual guidance immediately after surgery.

We have proposed on the basis of other evidence that the blindness results from a failure of visual processing within the intact visual system due to the loss of an essential activation normally provided, directly or indirectly, by the territory included in the ablation (Neurosci. Abs. 4:639, 1978). The present findings strengthen this hypothesis by rendering highly unlikely the alternative possibility that the blindness reflects some transient state such as surgical shock or diaschisis. We can now test our hypothesis directly by examining stimulus-evoked neuronal activity within the intact visual system of chronically blind animals.

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2697 REMOTE INHIBITORY AND FACILITATORY INPUT TO SIMPLE AND COMPLEX CELLS IN CAT STRIATE CORTEX. J. I. Nelson and D. M. Mitchell* Dept. Ophthalmology, NYU Med. Cntr., New York, NY 10016.

Large annular striped patterns (adapting fields) presented beyond the limits of conventionally-defined cortical receptive fields affect unit responsiveness. All but one of 49 simple cells showed greater than 10% inhibition of response to an optimal central stimulus upon presentation of the annular adapting field. 22 of 25 complex cells showed some effect, either facilitation, inhibition, or both. We have studied the spatial distribution, transfer across the visual midline, and interocular transfer of this effect in conventional acute cat preparations.

Interocular transfer was tested in 5 cells by presenting the adapting field to one eye and measuring a change in responsiveness to conventional (central) stimulation of the other. Transfer was always obtained; the observed inhibition was always stronger with adapting field stimulation of the dominant eye.

In 6 cells, the adapting field stimulus was presented on one side or the other of the vertical midline, making allowance for the inward cyclorotation of the eyes under paralysis and for the midline region of partial X-cell decussation. Thus we stimulated predominantly one cortical hemisphere or the other. Peripheral inhibition was equally strong, driven predominantly from contra- or ipsilateral cortical hemispheres for the one complex cell fully tested, whereas in the remaining simple cells, contralateral cortical stimulation was only 21 to 67% as effective as ipsilateral.

The spatial distribution of regions giving rise to remote interactions was studied with various adapting field sizes and shapes. The area of spatial integration for the remote inhibition of simple cells within 10° of the area centralis projection is about 8°. Many simple cells exhibit disinhibition for one sweep direction of an adapting field over a range of orientations closely matched to the optimal stimulus orientation of the cell under test. In one cell, response to a central stimulus was facilitated, not merely disinhibited, although the adapting field was unable to drive the unit directly. Disinhibitory regions are confined to areas along extensions of the receptive field's long axis. There are no preferentially inhibitory regions, though inhibition varies regularly across a broad range of orientations. Thus, simple cells may be said to display orientation domain inhibition which is general across space, and spatial domain facilitation which is specific to units sharing co-oriented and co-axially aligned receptive fields. Reports of remote influence upon complex cells have been variable, and indeed complex cells appear to display both inhibitory and disinhibitory regions. However, these interactions may be linked to a two-stimulus antagonism in complex cell responses which varies with response strength and not the peripheral region stimulated.

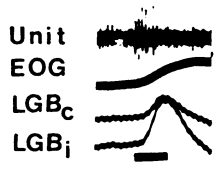
2698 PGO BURST NEURONS TRANSMIT EYE MOVEMENT INFORMATION TO THE VISUAL SYSTEM IN DESYNCHRONIZED SLEEP. John P. Nelson, Robert W. McCarley and J. Allan Hobson. Laboratory of Neurophysiology, Department of Psychiatry, Harvard Medical School, Boston, MA 02115

PGO waves are EEG macroelectrode spikes which are prominent in the pons, lateral geniculate bodies (LGB) and occipital cortex during the transition (T) from slow wave sleep to desynchronized (D) sleep, and during D when they are associated with rapid eye movements (REMs). Carefully placed bilateral LGB electrodes reveal variations in PGO wave amplitude during T and D--the larger wave occurs sometimes on one side, then on the other. During D, we have found, isolated saccades in one horizontal direction are virtually always associated with a larger PGO wave in the ipsilateral LGB (in one series, 88/90 in the predicted direction, chi square = 143, 1 df, $p < 10^{-4}$).

We have also described a class of neurons called PGO burst cells which are located in the midbrain in close apposition to the brachium conjunctivum and which discharge in stereotyped bursts of 5-6 spikes 12 msec before onset of PGO waves. A PGO burst cell (26 studied to date in 5 cats) fires in virtual 1:1 correspondence to larger PGO waves in the ipsilateral LGB and virtually never fires when the smaller wave is in that LGB. Their high correlation with PGO waves strongly suggests that PGO burst neurons are output elements in the system which generates PGO waves.

We conclude that PGO waves and the PGO burst neurons code eye movement direction, at least for horizontal saccades in D; they may represent the neuronal substrate for corollary discharge or efference copy of occulomotor signals which has long been speculated to be important for maintenance of perceptual stability of the visual world during saccades. The PGO burst neurons also fire single spikes before the eye movement potentials (EMP) seen in the visual system during waking (W). The possibility is thus raised that these cells may perform a similar function during W. Since the D state combines a highly activated brain with profound inhibition of sensory input, the functional characteristics of these cells may be more evident in D than in W.

The figure shows a characteristic discharge of a PGO burst cell, a large ipsilateral PGO wave, and a saccade to the ipsilateral side.
time bar = 50 msec



2699 EVIDENCE THAT CYCLIC GMP REGULATES MEMBRANE POTENTIAL IN VERTEBRATE ROD PHOTORECEPTORS. G.D. NICOL* and W.H. MILLER. Departments of Physiology, and Ophthalmology and Visual Science, Yale University School of Medicine, New Haven, CT 06510.

Injection of cyclic GMP (cGMP) intracellularly in dark-adapted rod outer segments (ROS) of the toad, *Bufo marinus*, causes a maximum depolarization of 40 mV within milliseconds. Based on two independent methods of estimation, this depolarization of the membrane pontential is *approximately* the Na^+ equilibrium potential; the data is consistent with the interpretation that the excess cGMP renders the ROS plasma membrane permeable to Na^+.

Injection of cGMP in the absence of illumination initiates a repolarization after a time lag that is proportional to the amount injected. A light flash delivered during the depolarization caused by excess cGMP initiates a repolarization of amplitude equal to the depolarization plus the control amplitude and of latency inversely proportional to the flash intensity but from 5 to 50 times control latencies. Previous light adaptation antagonizes the depolarization caused by injection of excess cGMP.

Our data is interpreted in terms of a model in which membrane potential is controlled by the intracellular concentration of cGMP which is in turn regulated by the resultant of cyclase and phosphodiesterase (PDE) velocities. The PDE velocity of hydrolysis in the dark is increased by additional substrate (injection of cGMP) up to a V_{max} orders of magnitude lower than that of the light activated enzyme. The high V_{max} caused by light activation of PDE is assumed in the model to decay with the time constant of dark adaptation so that subsequent injections of additional substrate shortly after prior light adaptation are capable of eliciting high enough velocities to antagonize the depolarization by causing a rapid repolarization. The data suggests that cGMP is an important factor in the regulation of the membrane potential of the ROS for both transduction and adaptation.

2700 A QUANTITATIVE MORPHOMETRIC ANALYSIS OF TRANSNEURONAL CHANGES FOLLOWING LONG-TERM DENERVATION OF THE OPTIC TECTUM IN XENOPUS. Jeanette J. Norden and John A. Freeman. Department of Anatomy, Vanderbilt University School of Medicine, Nashville, TN 37232.

The effects of denervation on post-synaptic tectal cells of metamorphic Stage 66 Xenopus laevis were investigated using standard anatomical quantitative methods and morphometric methods derived from stereology. Differences in the number of cells present and in the nuclear volume of cells in Layers 8 and 9 between denervated and control tecta were used to indicate transneuronal effects following short-term unilateral enucleation (30 days), long-term unilateral enucleation (120 days), and following a single optic nerve crush (150 days). Changes in the number of cells present were determined by computing an areal density (number cell nuclei/ μm^2) or by computing the volume fraction (V_V) of nuclei in Layers 8 and 9. Nuclear volumes were determined from measurements of the mean diameter of cell nuclei (under oil immersion optics).

Following long-term (120 days) enucleation, there is a decrease of 18% in the number of cells present in the tectum contralateral to the eye removal compared to the control (ipsilateral) tectum. Furthermore, there is a drop of 45% in the nuclear volume of the cells which remain in the denervated tectum, in addition to a substantial loss in total tectal volume. By contrast, no significant changes, except in tectal volume, could be shown at 30 days post-enucleation or following unilateral optic nerve crush and subsequent regeneration and reinnervation of the tectum. These results indicate that post-synaptic cells are capable of maintaining apparently normal cell functions, including the ability to synthesize proteins involved in the formation of new synaptic sites, for an appreciable time following denervation. The dramatic changes seen following long-term enucleation, however, suggest that a significant proportion of the products of nuclear gene expression (reflected in the volume of the nucleus) are involved in the maintenance of synaptic connections and that retinal input is necessary for the integrity of the majority of post-synaptic tectal neurons. (Supported by NIH Grant #EY 01117-07).

2701 MICROELECTRODE STUDY OF RETINAL AND CORTICAL AFFERENTS TO TREE SHREW SUPERIOR COLLICULUS. Thomas T. Norton. Dept. Physiological Optics, School of Optometry/the Medical Center, University of Alabama in Birmingham, Birmingham, AL 35294

The superior colliculus in tree shrew (Tupaia glis) appears to play an important role in the mediation of form vision following striate cortex ablation, perhaps by relaying visual information rostrally to the thalamus and, potentially, to the neocortex. In a recent study, Albano, et al. (1978) found an electrophysiological subdivision of the superficial gray layer (SGS) of the tree shrew superior colliculus. The upper SGS contains mostly SR (Stationary-Responsive) cells while the lower SGS contains fewer SR cells and an increased proportion of other cell classes. Anatomically, Graham and Casagrande (1979) reported that the upper SGS contains many afferent terminals from retina and few from striate cortex, while the lower SGS contains many terminals from both sources. These studies suggested that cells in the upper SGS, particularly SR cells, might receive their excitatory extracolliculus inputs primarily from retina, and might be only slightly, if at all, influenced by striate cortex. To functionally examine the excitatory inputs to the upper and lower SGS from these sources, I recorded extracellularly in acutely prepared, N_2O/O_2 anesthetized tree shrews. After identifying the receptive-field class, electrical stimulation was applied, in turn, through implanted optic chiasm (OX) and striate (VC) electrodes. Eighty-three percent of the cells sampled in the upper SGS were activated by OX stimulation (mean latency 3.5±1.4 msec). As expected, a similarly high proportion (89%) of the cells in the lower SGS were also activated by OX stimulation (mean latency 5.4±2.9 msec). Surprisingly, nearly half (47%) of the cells in the upper SGS were activated by VC stimulation (mean latency 4.0±2.1 msec). In the lower SGS, a significantly higher proportion (80%) of the cells were activated by VC stimulation (mean latency 6.2±2.4 msec). In both upper and lower SGS, SR cells were driven from retina and cortex in about the same proportion and at about the same latency as were the other cell classes. However, the mean latency of all cells, both to OX and VC stimulation, was slightly longer in the lower SGS. These results are consistent with the anatomical data in that there does appear to be less influence from striate cortex in the upper SGS. However, cortical activity clearly can affect cells in the upper SGS, including many SR cells. These results thus suggest that striate cortex activity in normal tree shrews, and striate cortex ablation, may affect the relay of visual information through the superior colliculus, both in the upper and lower SGS.
Supported by N.I.H. grant EY 02909.

2702 DIENCEPHALIC ORIGIN OF PULVINAR NEURONS IN NONHUMAN PRIMATES Marilee Ogren and Pasko Rakic. Yale Univ. School of Medicine Section of Neuroanatomy, New Haven, Ct. 06510.

In comparison with structures that comprise the primary visual pathways in primates (Rakic, 1976, Nature, 261), little is known about the ontogenetic development of the structures that comprise the secondary pathways including the pulvinar. Previous developmental analysis of human fetuses indicates that some pulvinar neurons may be of telencephalic origin, in part because this major thalamic nucleus attains its large size only after the proliferative zones of the diencephalon have been exhausted (Rakic & Sidman 1969, Z. Anat.Ent.-Gesch., 129). Also, dense bands of undifferentiated cells can be observed streaming to the dorsal thalamus from the cerebral ganglionic eminence during midgestation in human fetuses. Definitive evidence for a telencephalic origin of pulvinar neurons cannot be obtained from human histological material. We therefore determined the time of origin of pulvinar neurons in rhesus monkeys that had been exposed to ^{3}H-thymidine from embryonic day (E) 25 through birth (E165) and sacrificed 2-3 months postnatally. Heavily labeled pulvinar neurons were observed only in animals that had been exposed to ^{3}H-thymidine between E36 and E45, when diencephalic proliferative zones are still mitotically active; all of the dorsal lateral geniculate nucleus neurons are generated over roughly the same period (Rakic, 1977; J. Comp. Neurol., 176). Neurons that comprise the inferior and lateral subdivisions of the pulvinar are generated during the beginning and middle of this period while those destined for the medial pulvinar are generated at the end of this period. The site of origin and migratory pathways of these neurons were determined in a series of animals that had been exposed to ^{3}H-thymidine between E36 and E45 and sacrificed at various short intervals after injection. Neurons destined for the pulvinar arise only from the diencephalic ventricular and subventricular zones that border the 3rd ventricle. They migrate to positions medial and dorsal to the lateral geniculate where they remain during the first third of gestation. Subsequent expansion of the pulvinar occurs after all of its neurons have been generated and is probably attributable to ingrowth of afferents during the middle of gestation (Shatz and Rakic, 1978, Neurosci. Abs. 4), differentiation of neurons and elaboration of their processes, as well as the addition of glial cells. Although in rhesus monkeys, pulvinar neurons appear to have an exclusively diencephalic origin, the possibility remains that in man this same structure acquires an additional population of neurons of telencephalic origin which does not exist in nonhuman species. (Supported by NIH Grant EY02503).

2703 COMPARISON OF NEURONAL PROPERTIES IN AREAS 17 AND 18 OF THE CAT. Guy A. Orban, Henry Kennedy* and Hugo Maes*. Laboratorium voor Neuro- en Psychofysiologie, K.U.L., Campus Gasthuisberg, B-3000 Leuven, Belgium.

Single unit recordings were made in areas 17 and 18 in anaesthetized (N_2O/O_2) and paralyzed cats. Properties of neurons in areas 17 and 18 were compared over a wide range of eccentricities (0-45° in 17 and 2-28° in 18). Histological reconstruction, Receptive Field (RF) position and velocity sensitivity enabled us to determine whether the neuron belonged to area 17 or 18. Hand plotting allowed classification of units, and measurement of RF dimensions and orientation selectivity. Direction selectivity and velocity sensitivity were assessed quantitatively with a multihistogram technique. In both areas similar proportions of the four cortical families (A, B, C, S) were found. More area 17 neurons were end-stopped (H cells). In both areas the proportion of S and H family cells decreased with eccentricity. In area 17 orientation specificity decreased with eccentricity, this is not so in area 18. In central area 17 S cells were more narrowly tuned than the other cell types and more so than S cells of central 18. Only Area 17 S cells showed meridional differences with preferred orientation (oblique effect). In area 18 and not in area 17 direction selectivity decreased with eccentricity. The proportion of direction selective cells is significantly greater in area 18 than in area 17. Velocity sensitivity allowed classification of area 17 and 18 neurons into 4 types: velocity tuned, broad band, high pass and low pass cells. Area 18 has a substantial proportion of cells measuring velocity either in a discrete way (50% of central 18 neurons are velocity tuned) or in a continuous way (66% of peripheral 18 neurons are high pass cells). These velocity signalling units are scarce (10%) in area 17. Most (83%) cells of central 17 are low pass cells. The apparent RF width of these cells is almost invariant with velocity. These neurons thus carry more precise information on position. In both areas the latencies decrease with eccentricity and central 17 is on average slightly slower than central 18. Our results indicate that in both areas the peripheral neurons are less selective and that the two areas are involved in a parallel analysis of different aspects of the visual scene.

2704 DISTRIBUTION, SIZE, AND MORPHOLOGY OF RABBIT RETINAL GANGLION CELLS. Clyde W. Oyster and Ellen S. Takahashi. School of Optom. University of Alabama in Birmingham, Birmingham, AL 35294.

As determined from extensive cell counts in flat-mounted retinas, ganglion cell density can be shown to vary about ten-fold from peripheral retina to the region of highest density. Even so, the maximum density is only about 5000 cells/mm^2. The peak is more of a ridge, extending some 90^o along the center of the visual streak, the region within which the cell density is greater than 2000 cells/mm^2. Although there is some elevation in cell density in the center and temporal end of the streak, there do not seem to be any clearly defined local regions of especially high cell density. Measurement of the total retinal area and areas enclosed by isodensity contours allowed estimates of total cell population to be made; the mean estimate for three retinas was 406,000 ganglion cells, a figure which agrees well with recent counts of the number of axons in rabbit optic nerve. Like previous studies, we found that large ganglion cells form a large portion of the cell population in peripheral retina than in the streak, while the reverse was true for small ganglion cells. These differences were not large, however; for cells in a given size range, the proportions changed only 10% or so between streak and peripheral retina. The superior retina appears to be different; there are considerably more large cells here than in other retinal regions having comparably low cell density ($<$ 500 cells/mm^2). Golgi stains show the rabbit ganglion cells to have a number of characteristic dendritic ramification patterns. Some are predominantly unistratified, with dendrites confined to either the scleral or vitreal sublamina of the IPL. Others ramify throughout the thickness of the IPL and, like the unistratified groups, show several variations in the complexity of the branching patterns. An obvious group of ganglion cells are bistratified, a configuration which is consistent with the notion that they are on-off cells. Some one-fifth to one-fourth of the stained cells have the bistratified morphology, a proportion which is similar to the frequency with which on-off direction-selective cells have been recorded in rabbit retina. (Supported by USPHS Grant EY02207).

2705 RECEPTIVE FIELD STRUCTURE IN STRIATE CORTEX OF CAT. Larry A Palmer and Thomas L Davis* Department of Anatomy, University of Pennsylvania, Phila., Pa.

We have used 3 methods to define the receptive field structure of striate cortical neurons in the cat: PST response planes, moving narrow bars, and moving edges. Best insight into field structure were obtained with a combination of response planes and edge analysis.

The spatial and temporal distribution of excitatory and inhibitory domains in response planes of striate neurons allows for easy recognition and subdivision of simple and complex cells. Simple cells always have spatially non-overlapping regions of excitation and inhibition (spatially heterogeneous response planes). Complex cells, on the other hand, always exhibit the same sequence of excitation and inhibition at all points in the receptive field (homogeneous planes). Based on their planes, 2 distinct types of complex and 5 distinct simple cell types have been discerned.

Field structure as defined in the response plane can be used with most cells to quantitatively predict the results of moving slit or edge analysis. This was accomplished by staggered addition of the PSTs comprising the plane. On-times were chosen to correspond to particular velocities of moving slits or edges. Most complex cells show overlapping light and dark edge discharge centers which correspond to the 2 primary excitatory domains in their planes. Many simple cells show a single light edge discharge center corresponding to a single excitatory domain surrounded by inhibition. The most common simple cell has spatially offset light and dark edge discharge centers which correspond to two similarly offset excitatory domains, one at light off and one at light on. In all these cells, the strengths and positions of inhibitory domains modify responses to moving edges and slits. While staggered addition of PSTs of a response plane will usually quantitatively predict symmetrical or mildly asymmetrical responses to moving stimuli, it usually fails to predict direction selectivity of 5:1 or more.

We regularly find 8 distinct types of striate neurons based on receptive field structure alone. We anticipate that this physiological diversity will help to account for the variety of neuron morphologies recently described with serial electron microscopy (Davis and Sterling). NSF Grant BNS-78-25147.

2706 LIGHT GENERATED HEAT IN THE EYE: THE ROLE OF THE CHOROIDAL CIRCULATION AS A HEAT SINK. Leonard M. Parver*, C.R. Auker, and D.O. Carpenter. Georgetown University, Washington, DC, and AFRRI, Bethesda, MD 20014.

The choroidal circulation accounts for 85% of the total ocular blood flow. In comparison with the circulation in the renal cortex the choroid has four times the volume of blood flow per 100 grams of tissue. The question arises as to whether the high flow choroidal circulation is functioning solely to supply nutrients to the outer avascular retinal layers. The exceptionally high oxygen content of venous choroidal blood, about 95% of that found in arterial blood, suggests some other role. One such role might be the dissipation of heat generated by the absorbtion of focused light in the outer retinal layers and the retinal pigment epithelium.

To study this hypothesis temperature measurements were taken from the macula of the cynomologous monkey eye. A thermistor probe was inserted through the pars plana and positioned in the macula under direct ophthalmoscopic control. The animals were exposed to two light environments (1) a dimly lit room, or (2) a lamp delivering 1.09 mW/cm^2 at the corneal surface. Temperature measurements were taken under these two light conditions while choroidal blood flow was decreased by raising intraocular pressure.

Raising the intraocular pressure with the animals exposed to the illumination in a dimly lit room produced a decrease in temperature recorded in the macula. In comparison, those animals exposed to the 1.09 mW/cm^2 light source showed an increase in macular temperature when the intraocular pressure was elevated. Temperature measurements were also taken from a peripheral retinal site. Increasing the intraocular pressure here produced a decrease in temperature irrespective of the light source. The decrease in temperature was slightly less marked with the 1.09 mW/cm^2 light source, but ran a parallel course with the changes observed with only background illumination.

We conclude that in low light environments the choroidal cirdulation acts as a heat source, maintaining a constant temperature environment for the retina and the retinal pigment epithelium. In high light environments the choroidal vasculature switches to a "heat sink", dissipating the heat generated by the absorption of focused light at the macula.

2707 CORPUS CALLOSUM SECTION DECREASES BINOCULARITY IN CAT VISUAL CORTEX. B.R. Payne*, Andrea J. Elberger, Nancy Berman and E. Hazel Murphy. (SPON: T.J. Cunningham). Depts. Anat. and Physiol/Biochem., Medical College of Pennsylvania, Philadelphia, Pa. 19129 and (A.J.E.) Dept. Anat., Sch.Med., University of Pennsylvania, Philadelphia, Pa. 19174.

The property of binocularity in visual cortex neurons has been assumed to depend upon convergence of fibers originating in adjacent lateral geniculate laminae. This study indicates that the corpus callosum is essential for normal binocularity.

Following section of the posterior corpus callosum, single units were recorded from area 17 in cats two or more days after surgery. The response properties and ocular dominance of each cell were determined as were the size and location of the receptive fields. The extent of the lesion was verified histologically after the final recording session. One sham operated and five normal cats were studied for comparison.

In normal and sham-operated cats over 80% of the neurons are binocularly driven. In posterior corpus callosum sectioned cats the proportion of such cells decreases to 37%. Surprisingly this effect is not limited to cells which have receptive fields in a narrow band adjacent to the vertical meridian but can be found up to 30^o lateral.

Other properties such as the proportions of simple and complex cells sampled in area 17 were unaffected by the lesion.

A reduction in binocularly driven cortical cells has been shown in cats given surgically induced strabismus or monocular suture prior to the time of natural eye opening. Such cats also have abnormal callosal connections (Lund; Innocenti and Frost). These data and our evidence on the role of the corpus callosum in binocular integration suggest that disruption of the normal development of callosal connections may underlie the loss of binocularity following early visual deprivation and strabismus.

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2708 VISUAL RESPONSE PROPERTIES OF SINGLE NEURONS IN THE DORSOLATERAL CRESCENT (DL) IN THE OWL MONKEY: SELECTIVITY FOR STIMULUS SIZE, DIRECTION, AND ORIENTATION. S. E. Petersen*, J. F. Baker*, K. S. Rockland, and J. M. Allman. Div. of Biology, California Institute of Technology, Pasadena, CA 91125.

The Dorsolateral area (DL) is one of the five visual field representations that adjoin the anterior border of V-II and collectively constitute the third tier of visual areas in the owl monkey. DL wraps around the Middle Temporal area (MT) and relates to it topographically much as V-II does to V-I. We have quantitatively studied the response properties of 54 neurons in DL to stimuli of different size, direction of movement, and orientation. 23 of the 54 neurons showed a striking size selectivity. Many of these cells responded only to a narrow range of preferred sizes, and the preferred sizes were often smaller than the receptive field. Other units exhibited spatial summation up to a certain stimulus size, but were decreasingly responsive to larger stimuli. Neurons with similar size preferences were encountered sequentially in penetrations made normal to the brain surface. We also have observed qualitatively that many of these neurons were very responsive to expanding and/or contracting stimuli around the preferred size. The response properties of size-selective cells were largely independent of stimulus intensity and contrast.

DL neurons differ from cells in other extrastriate areas in their directionality and tuning to moving bars and spots. Directionality was quantified by comparing the response in the best direction to response in the opposite direction. Tuning was measured by comparing the response in the best direction to the responses in the surrounding directions within $\pm$ 90 degrees.

DL neurons were compared to neurons in MT and in a medial group of third tier areas: Dorsomedial (DM), Dorsointermediate (DI), and the Medial (M) areas. Cells in the medial group of areas are strongly orientation selective because they exhibit sharp tuning to bars and weak directionality. Medial group neurons are much more narrowly tuned to bars than to spots. MT neurons are strongly directional and only marginally more narrowly tuned to bars than to spots. DL neurons are significantly less directional than MT neurons but much more directional than neurons in the medial group. DL neurons are more broadly tuned to bars than MT neurons and much more broadly tuned than medial group neurons. DL neurons are only slightly more tuned to bars than to spots. In many cases, however, the most remarkable property of DL neurons is their selectivity for stimulus size.

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2709 THE ROLE OF VISUAL RECEPTORS IN MAGNETIC FIELD DETECTION: RETINULA 7 AND 8 CELLS, MAGNETORECEPTORS IN THE HOUSEFLY? John B. Phillips*. (SPON: P.G.Aitken) Neurobiology and Behavior, Cornell U., Ithaca, N.Y. 14850.

Behavioral tests of the compass orientation of houseflies (Musca domestica) under different lighting and magnetic field conditions have been carried out in order to test the theory of the mechanism of magnetic field reception proposed by Leask (Nature 267: 144, 1977). This theory implicates visual receptors capable of detecting the phosphoretic emission of the lowest triplet excited state of a functional molecule following light absorption. As a consequence, magnetic sensitivity is predicted to be dependent on the spectral and polarization characteristics of light absorption by the functional molecule. The 'optical/radio frequency double resonance process' proposed by Leask to mediate magnetic sensitivity is consistent with a dependence on (1) the axis, but not polarity and (2) a total intensity which must closely approximate that of the earth's magnetic field which has been demonstrated by Wiltschko (In: Animal Orientation and Navigation, NASA SP-262, 569, 1972) for the magnetic response of birds. In advanced flies, a subpopulation of the retinula 7 and 8 cells appears to be ideally suited to discriminate the phosphoretic emission of a single triplet substate of a pteridine pigment(s) present in the visual cells. Results of the present study on the orientation of houseflies indicate (1) the presence of a magnetic compass response, (2) a dependence on the axis, but not the polarity, of the magnetic field and (3) an influence of the wavelength of incident light.

2710 ROD VISION: SENSITIVITY OF INFANTS APPROACHES THAT OF ADULTS. Maureen K. Powers. Dept. Psychol., Univ. Wash., Seattle, WA 98195

Single rod photoreceptors can signal the absorption of a single quantum of light. That this must be so was demonstrated long ago in psychophysical experiments on adult humans. Are the rods born with such exquisite sensitivity, or does it develop after birth? The results of the present experiment suggest that the former is more likely to be the case, because the sensitivity of the infant eye approaches that of the adult by 3 months of age.

Dark-adapted infants, 11-13 weeks old, were held in front of a rear-projection screen in a totally dark room. The infant's face was illuminated with infrared light, to which a TV camera trained on the face was sensitive. Stimuli (17^{o} diameter, monochromatic spots) appeared on the screen, 20^{o} to the infant's left or right.

The forced-choice preferential looking (FPL) technique was used for data collection. An adult observer (O), who could not see the stimulus, viewed the infant's face on a TV monitor. Following each presentation O made a forced-choice judgment of whether the stimulus was on the left or right based on the infant's preferred direction of gaze. Several intensities were presented per session. At the highest, O was nearly always able to judge the position of the stimulus correctly. At the lowest, O was only able to guess correctly 50% of the time; i.e., performance dropped to chance. Sessions were 1 hour daily for 10 days, about 60 trials per session.

The intensity of 502 nm light that allowed the observer to guess the position of the stimulus correctly on 75% of the trials delivered about 100,000 quanta/sec to the retina; a conservative calculation shows that 1 isomerization occurred per 100 rods covered by the stimulus. Two adult subjects tested under similar conditions, but using the method of adjustment, required 1 isomerization per 1400 rods to barely detect the stimulus. A 610 nm stimulus yielded higher thresholds for both infants and adults, by nearly equivalent amounts, and the difference was close to that expected from the CIE scotopic sensitivity curve.

This is the first measurement of the absolute visual sensitivity of human infants. It shows that the sensitivity of the rod system at 3 months of age is no more than an order of magnitude lower than in adulthood when the infant's natural behavior is observed. We conclude that a single rod in the infant's eye can be stimulated by a single quantum, just as a rod in the adult eye can be. The infant eye is therefore similar to other vertebrate eyes in the sensitivity of individual rod receptors.

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2711 CONNECTIONS OF THE LATERAL POSTERIOR-PULVINAR COMPLEX WITH THE EXTRASTRIATE VISUAL CORTEX IN THE CAT. Denis Raczkowski*(Spon: Alan Rosenquist). Dept. Anat., Sch. Med., Univ. of Pennsylvania, Phila., Pa. 19104.

We have examined the projections from the lateral posterior (LP)-pulvinar (P) complex to several extrastriate visual cortical areas that were defined by electrophysiological mapping studies in this laboratory (Palmer, et al.,J.Comp.Neur.177:237-256,1978). Our method was first to record multiple unit activity through a micropipette during visual stimulation to confirm that the electrode tip was in the proper area and retinotopic locus. We then injected small quantities (0.1 ul) of a mixture of horseradish peroxidase (HRP) and tritiated amino acids into that cortical site. An analysis of our HRP results show first that there are at least three representations of the visual field in the LP-P complex that roughly correspond to parcellation schemes based upon cortical and subcortical afferent zones (Updyke, J.Comp.Neur.,173:81-122, 1977; Graybiel, Invest.Ophthal.,11:322-332, 1972). Using the terminology of Updyke, we find that small injections into the area centralis (AC) representation of both areas 21a and PLLS produced bands of labeled cells in the rostral portion of the LP-P complex. The label resulting from the area 21a injection was equally dense along the lateral border of P and the common border between LPl and LPi; after the area PLLS injection, the label was situated in the lateral half of LPi. Injections in the peripheral field representation of the horizontal meridian (HM) in area 20a led to labeling along the P-LPl border and the medial half of LPi throughout the rostro-caudal extent of the LP-P complex. Second, concerning the representation of the upper and lower fields in the LP-P complex, an injection in the lower field representation of areas AMLS labeled neurons in the ventral portion of LPi, whereas an injection in the representation of the upper fields (15^{o} above HM) in areas VLS produced equal amounts of labeled cells across the dorsal portion of LPl and LPi. Third, HRP results suggest that a great proportion of the LP-P complex is devoted to the AC and HM portions of the visual field. These experiments also refine our understanding of cortical subdivisions. Some cortical areas, such as PLLS, receive input from only one of the three thalamic zones (LPi), whereas other areas such as 20b receive from all three zones (LPl, LPi and P). Preliminary comparisons of thalamocortical and corticothalamic connection patterns indicate the presence of reciprocal connections in every case. Finally, every extrastriate visual cortical area thus far examined receives a bilateral input from the claustrum and an ipsilateral input from the central lateral nucleus. Supported by 1 R01 EY02654 and EY T32 07035.

2712 DORSAL LATERAL GENICULATE ORGANIZATION IN TURTLES, PSEUDEMYS SCRIPTA AND CHRYSEMYS PICTA. Wm. Todd Rainey. Dept. Anat., Univ. of Chicago, Chicago, IL 60637.

Dorsal lateral geniculate complex is the dorsal thalamic region that receives primary retinal input in pond turtles. This complex has two structural components: a cell lamina (CL) and a neuropil region (N). N is a cell-poor region medial to the optic tract (OT). Frequency histograms of soma areas from CL and N reveal significant differences in areas between regions, such that N neurons tend to be smaller. Autoradiographic materials reveal that optic optic nerve axons are distributed throughout N. When these axons are anterogradely filled with HRP, it appears that axon collaterals, as well as some axon shafts, can be traced within N. Most of the collaterals are so thin that their diameters cannot be resolved. CL is medial to N. Cortical and, possibly, tectal inputs terminate in this component. CL can be cytoarchitecturally divided into rostral and caudal regions. Rostral CL neurons have densely packed somata. Many of these contribute to tightly packed clusters. Caudal CL neurons have less densely packed somata. Analyses of Golgi material, and of neurons solidly filled with retrogradely transported HRP, reveal two cell types shared by both regions: CL neurons and N neurons. CL neurons display at least one of two types of dendritic arborizations. The characteristic arbor type consists of sparsely branching dendritic trunks that form large fields. The distal third to half of these fields is oriented parallel to the medial surface of OT. This region of the fields is found in the transitional region that consists of the medial edge of OT and the lateral half of N. Most dendritic segments in these fields are oriented perpendicular to OT axons. However, some distal segments turn and are oriented parallel to OT fibers. Both types of distal segments may bear one or more lobulated appendages that form tufted or ball-shaped arbors. Appendages with less complex arborizations are scattered sparsely along more proximal segments of dendrites within N. A second type of dendritic arborization is found, in addition, on many CL neurons. This type consists of one or more relatively thin dendritic segments that originate from the soma or proximal dendritic trunks and sparsely branch within CL. N neurons form the second type of geniculate neuron. Their dendriarborizations are sparsely branched and oriented parallel to optic tract fibers. These arborizations usually do not spread into CL. (Supported by PHS Grant GM-00094 and PHS Grant NS-12518.)

2713 DISTRIBUTION AND CENTRAL PROJECTIONS OF GANGLION CELLS IN THE RETINA OF THE GRAY FOX (UROCYON CINEREOARGENTEUS). David H. Rapaport, Michael A. Sesma*and Michael H. Rowe. Department of Psychology, University of California, Riverside 92521.

The overall pattern of retinal ganglion cell distribution in the gray fox, as seen in nissl-stained whole mounts, is quite similar to that of the domestic cat. The two most prominent features of ganglion cell isodensity maps are an area centralis, located above and temporal to the optic disc, and a visual streak which extends well into nasal retina approximately along the horizontal meridian. However, the area centralis of the fox seems somewhat less developed than in the cat. Ganglion cells in the fox retina range in soma diameter from 8-45 μm and appear to form 4 distinct size groups: 8-15, 16-23, 24-32 and 33-45 μm. Each of these 4 groups has a distinctive pattern of retinal distribution, so that cell size histograms from various retinal locations reveal striking differences between them in ganglion cell composition. Cells in the largest group form 2-5% of the total population in most areas, their absolute frequency being highest in the visual streak and superior temporal retina and falling off sharply in inferior regions. Cells in the 24-32 μm range form a high proportion of cells in temporal retina, but are much less frequent in nasal retina. Although the range of cell sizes in the area centralis is contracted due to the high density, it is clear that the cells in this group (24-32) are by far the most frequent type in the area centralis. Cells in the 16-32 μm range are relatively much more frequent in nasal than in temporal retina, and along with cells of the 8-15 μm group, concentrate to form the visual streak. Injections of HRP into one optic tract demonstrate that all groups in nasal retina project to the contralateral hemisphere. From temporal retina most cells in the 8-15 and 33-45 μm groups project to the contralateral hemisphere, but most cells in the 24-32 and 16-23 μm groups project to the ipsilateral hemisphere. An analysis of field potentials recorded around the perimeter of the optic disc in response to stimulation of the optic chiasm and the ipsilateral or contralateral tracts reveals at least 3 conduction velocity groups among retinal ganglion cell axons whose retinal origins and central projections suggest that they are the axons of the 3 larger cell size groups seen in retinal whole mounts. Thus, although the basic organization of the retinal ganglion cell population is similar in the cat and fox, the functional differentiation of this population into distinct groups seems more complete in the fox.

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2714 AN ASCENDING VESTIBULO-RETICULO-MLF PATHWAY IN CAT. R.S. Remmel & R. D. Skinner, Depts. of Physiology and Anatomy, Univ. of Ark. for Med. Sci., Little Rock, Ark. 72201.

This electrophysiological study indicates that functional excitatory pathways exist from both vestibular nerves (V-nerves) through the vestibular nuclei (synapse), then to the pontine reticular formation (PRF) rostroventral to the abducens nucleus (one or more synapses) and to axons ascending in the region of the medial longitudinal fasciculus (MLF) ipsilateral to the PRF neurons. Cats were either anesthetized with pentobarbital, or decerebrated under ether and anesthesia discontinued. The cerebellum was removed. Body functions were maintained. The right MLF was electrically stimulated 1 mm caudal to the trochlear nucleus to antidromically excite right PRF neurons, which were recorded extracellularly with glass micropipettes, as previously reported (Exp. Brain Res. 32 (1978) 31). The left medial vestibular nucleus (MVN) was stimulated near its ventral border in 15 experiments. Electrode locations were confirmed histologically. Stimuli were 0.1 msec cathodal shocks of < 50 μA (MLF) and < 300 μA (MVN). Of the neurons antidromically excited from the MLF, 77 could be orthodromically excited from the contralateral MVN (latencies: range, 1.15 to 7.4 msec; av. and std. dev., 3.2 ± 1.2).

In 5 of these experiments both V-nerves were also electrically stimulated by pins stuck into the round windows at < 1000 μA (< 4X threshold), 28 neurons could be excited from the ipsilateral V-nerve (latencies: 1.8 to 7.3; av., 3.8 ± 1.4) and 31 neurons from the contralateral V-nerve (latencies: 1.65 to 6.9; av., 3.6 ± 1.3). A large fraction could be excited from both nerves and the MVN. The extracellular technique made testing for inhibition difficult. If one takes into account the 0.3 msec nerve conduction time, the 0.5 msec vestibular synaptic delay, the > 0.4 msec conduction time for vestibuloreticular neurons (> 5 mm distance divided by < 14 m/sec conduction velocity (Peterson and Abzug, J. Neurophysiol. 38 (1975) 1421), and 0.5 msec for the PRF synaptic delay, then > 1.7 msec should be required for excitation. The shortest latencies were only slightly longer than this, suggesting no additional intervening synapses. Other units were excited at longer latencies with more intertrial variability, suggesting either a slowly-rising, varying EPSP or else an additional synapse(s). The data suggests that some vestibularly-related MLF fibers originate in the PRF. This vestibulo-reticulo-MLF pathway might participate in the vestibulo-ocular reflex, in accord with Lorente de No's finding (Arch. Neurol. Psychiat. 30 (1933) 245) that the vestibulo-ocular reflex could still be elicited after the direct vestibulo-ocular fibers were cut.

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2715 LATERAL INHIBITION, EXCITATION, AND THE CIRCADIAN RHYTHM OF THE LIMULUS COMPOUND EYE. G. H. Renninger and R. B. Barlow, Jr., Inst. for Sensory Research, Syracuse University, Syracuse, NY 13210.

Excitatory responses of the eye of Limulus exhibit a circadian rhythm. At night, nerve impulses in efferent fibers of the lateral optic nerve increase the sensitivity of each ommatidium of the eye. At dawn, the efferent activity stops and the sensitivity returns to the low daytime level. The nighttime sensitivity of a dark-adapted ommatidium can exceed daytime levels by as much as 10^5 (Barlow et al., Science 197, 86-89, 1977).

Lateral inhibitory interactions, on the other hand, are not significantly influenced by the circadian clock. When the eye is in the nighttime state, the strength of inhibition exerted by a small cluster of ommatidia on a neighboring ommatidium is equal approximately to that exerted in the daytime state.

Strong inhibitory interactions give rise to a striking "bursting" behavior of the response of the eye to diffuse illumination. Bursting is a sustained, periodic discharge of the optic nerve, in which impulses occur nearly synchronously in the individual nerve fibers. Depending upon the level of illumination, an ommatidium may respond with one or more impulses in each burst period. The bursting response was first observed only at high levels of light in the daytime state (Barlow and Fraioli, J. Gen. Physiol. 71, 699-720, 1978); we now find that bursting occurs over a wide range of light intensities in the nighttime state.

The burst period does not depend strongly upon the intensity of illumination. In the nighttime state, for example, the burst period is constant over a range of 3 log units above the threshold intensity for the bursting behavior. Interspike times in the response of an ommatidium during bursting do not depend strongly upon the level of sensitivity. In one preparation exposed to steady diffuse light, for example, the response of an ommatidium experiencing a fixed mean level of excitation consisted of a repetition every 0.14 s of two impulses, separated by approximately 0.03 s, in both the nighttime and daytime state.

The constancy of the burst period with increasing illumination and the negligible influence of the level of sensitivity upon the interspike intervals in the burst suggest that the temporal properties of lateral inhibition are not strongly affected by the circadian clock.

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2716 DIFFERENCES IN THE IPSILATERAL RETINOCOLLICULAR PROJECTION IN ALBINO VERSUS FULLY PIGMENTED RABBITS. Michael Rezak, Dept. of Anatomy, University of Illinois at the Medical Center, Chicago, Illinois 60680.

It has been well established in a wide variety of mammalian species that the lack of retinal pigment is closely related to aberrant retinofugal projections. In general, it has been shown that the number of optic fibers decussating in the chiasm is abnormally increased in animals lacking retinal pigment. Although much detailed work has been done on the retinogeniculate organization relatively few studies have focused upon the retinocollicular pathway. The present study examines the differences in the ipsilateral optic projection to the superficial layers of the superior colliculus in albino and fully pigmented rabbits. Three albino and three wild type (fully pigmented) rabbits received intravitreal injections of 3H-proline and allowed to survive for three days. Upon sacrifice, the brains were removed and processed for autoradiography. Although the fully pigmented rabbits received a heavier ipsilateral retinal projection than the albino rabbits, in both types of animals the the grains formed distinct clumps or patches and were restricted to the ventral portion of the stratum griseum superficiale. In the fully pigmented rabbit the patches appeared either in isolation or in aggregates of two to three. In the albino rabbit, on the other hand, only two distinct and widley spaced patches appeared. In contrast to an earlier report using the degeneration method, the present results indicate that the ipsilateral projection in the fully pigmented rabbit occurs throughout the medial-lateral extent in the anterior portions of the colliculus. The patches in the ipsilateral colliculus of the albino rabbit occur in a ventrolateral position and in a mid-dorsal position within its anterior portion. It appears then that a large portion of the ipsilateral projection to the colliculus present in the fully pigmented rabbit decussates in the albino rabbit and terminates in the contralteral colliculus. It is worthy to note a recent report demonstrating a similar pattern in retinotectal projection in another albino animal, the Siamese cat (Weber et al., '78). Furthermore, it is of interest to know whether aberrant input in the albino rabbit colliculus is handled in a fashion similar to that in the Siamese cat, e.g., via a suppressive mechanism or by some other means.

2717 RECEPTIVE FIELD PROPERTIES OF LUXOTONIC UNITS IN MACAQUE STRIATE CORTEX. Ronald R. Riso*, Héctor Brust-Carmona, John R. Bartlett† and Robert W. Doty. Center for Brain Research, University of Rochester School of Medicine and Dentistry, Rochester, N.Y. 14642.

Taking strict precautions to assure the animal's comfort (as described in J. Neurophysiol. 37: 621, 1974), initial methohexital anesthesia was allowed to subside while chronically prepared monkeys were immobilized with pancuronium, thus allowing study of receptive fields (RFs) and of luxotonic properties of single units in striate cortex. General anesthesia, or even N_2O, produces severe disruption of luxotonic activity. In its absence about 30% of 244 units encountered were luxotonic, i.e., their average maintained discharge for 2 min of diffuse, patternless illumination vs. darkness showed at least a two-fold difference. Photergic units (those discharging fastest in light) were 2.5 times more common than the scotergic type (firing fastest in dark). Detailed study of 46 luxotonic and 85 nonluxotonic units, for which demarcated fields could be demonstrated, revealed that 72% of the luxotonic units were indifferent to the orientation of either a rectangular or edge stimulus, whereas 28% were selective for stimulus orientation. The latter type included both "simple" and "complex" units. In contrast, nonluxotonic units were about equally likely to be orientation selective or not. Some luxotonic units had small, punctate RFs with no antagonistic surround, while others had intermediate sized fields which were either entirely homogeneous in responsivity or had a centrally more responsive area surrounded by a synergistic, less sensitive region. Many luxotonic units were driven according to the total luminous energy present in their RFs irrespective of the geometrical distribution of the luminous energy. This was determined by comparing the response to a pattern, having discrete light and dark elements, to the response for a uniform grey stimulus having the same total luminous flux as the pattern. Several pairs of patterns and their respective flux-matched, neutral density equivalents were tested, covering a 2 $\log_{10}$ unit range. Other luxotonic units were driven primarily by the intensity of the illumination and were relatively insensitive to changes in stimulus area. The large majority of luxotonic units showed luminance properties. Some units showed monotonic luminance functions over the full 4 $\log_{10}$ unit range tested, and others showed much more restricted operating ranges. A class of luxotonic units had exceptionally large RFs (>60°) which extended well into the ipsilateral visual hemifield, thus raising the question as to how this information achieves the striate cortex. About 66% of luxotonic units were binocular, and these revealed a variety of binocular interactions. For half of them a diffuse light stimulus to one eye was excitatory, while for the other eye it was inhibitory. (†Deceased)

2718 CONNECTIONS FROM PRESTRIATE CORTEX TO THE SUPERIOR TEMPORAL SULCUS IN THE RHESUS MONKEY. Kathleen S. Pockland and Deepak N. Pandya. Boston University School of Medicine; Neurological Unit, Beth Israel Hospital, Boston, MA; V.A. Medical Center, Bedford, MA.

Various subdivisions of the occipital lobe project to the superior temporal sulcus (STS) in rhesus monkey. Recently the projections from striate cortex (area 17) to STS have been shown to be topographically organized (Ungerleider and Mishkin, Anat. Rec., 1978). In regards to the connections from peristriate cortex (area 18 and 19) to STS considerably less information is available. In present study using the autoradiographic technique with horseraddish peroxidase (HRP), the connections of peristriate cortex to STS are investigated in the rhesus monkey. A series of tritiated amino acid injections in different parts of area 18 (striate-recipient cortex) and area 19 indicated that the connections from these two regions also have distinct organization. The connections from area 18, like those from area 17, are directed to the caudal third of the STS and are topographically organized. Projections from the dorsomedial part of area 18 terminate in the caudal most part of the sulcus, predominantly in its lower bank, while projections from the lateral and ventral surfaces are directed to progressively more rostral portions of the STS. Connections from the ventral part of area 18, like those from the ventromedial portions of area 17, terminate dorsal to those from more lateral and medial parts of area 18. The apparent overlap of connections from area 17 and 18 was confirmed by an injection of horseradish peroxidase (HRP) in the STS. This injection resulted in HRP-filled neurons in visuotopically corresponding parts of area 17 and 18. A somewhat different organization was observed for the connections of area 19 to the STS. These terminate mainly rostral to the 17- and 18-recipient zone in the STS. Furthermore, connections from lateral as well as ventral portions of area 19 are directed to similar regions of the STS, where they both terminate in the lower bank and depth of the sulcus. Differences were also observed in the laminar arrangement of these connections. The connections from areas 17 and 18 terminate mainly in layer IV of the STS, whereas those from area 19 terminate in vertical bands in layers I through IV. HRP injections in areas 17, 18, or 19 consistently resulted in HRP-filled neurons in the STS. After injections of areas 17 or 18, these are predominantly in the infragranular layers (VI and Vb), with some located superficially in layer III. Neurons projecting to area 19 are more evenly distributed between the supra- and infragranular layers in the STS.

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2719 **FIRING CHARACTERISTICS OF NEURONS IN THE VENTROLATERAL AND ORBITAL PREFRONTAL CORTEX IN MONKEYS DURING COGNITIVE BEHAVIOR.** **Carl E. Rosenkilde, Richard H. Bauer and Joaquin M. Fuster. Brain Res. Inst. and Dept. Psychiat., UCLA, Los Angeles, CA 90024.**

Extracellular activity of 258 single units from 3 rhesus monkeys performing two short-term memory tasks was analyzed with computer. The tasks, spatial delayed response and visual delayed matching to sample, have been described in a progress report of the present research (Neuroscience Abstracts, 1978, p80). The cells were located in the ventrolateral prefrontal cortex, including the lower bank of the principal sulcus (n=118), and in the orbital cortex medial to the lateral orbital sulcus (n=140).

One of two discharge patterns was observed during presentation of the cue in 86% of the cells. About half of these cells responded in a uniform manner to all four types of stimuli (red, green, left and right white lights) independent of the animal's subsequent behavior. The remainder of the cells displayed differential activity related to the color or position of the cue. However, this activity was either abolished or replaced by new firing components in those trials where the animal with a later and incorrect choice indicated that the cue was deficiently processed.

Increased or decreased activity was observed, equally often, in 48% of the neurons during the delay period interposed between cue and choice; two thirds of these cells differentiated between the qualities of the no longer present stimuli. So far, we have found no indication of regional differences within the prefrontal cortex in activity during cue or delay.

Forty-seven percent of the cells altered firing after the animal, with its choice, indicated which stimulus had been presented prior to the delay. This activity was related to the delivery of the liquid reinforcement. Thus, type I cells changed activity after reinforced trials, and showed no change, or a change in opposite direction, after unreinforced trials. In type II cells, post-trial alterations were observed only after unreinforced choices, but opposite frequency changes in some units followed unconditional delivery of the reward. Type III cells displayed comparable post-choice firing changes after all trials. A chi-square test showed that these cell types did not have a uniform topographic distribution. Type I cells were most common in lateral cortex, whereas type II cells predominated in orbital areas. No clear differences were revealed in distribution of type III neurons.

These results indicate participation of the prefrontal cortex in visual processing and in short-term memory. The post-trial activity of some neurons may represent reaction to interoceptive and motivational information; in other cells, this form of discharge may reflect their role in suppression of prior mnemonic traces (Supported by the Danish Medical Research Council and NSF grant BNS 76-16984)

2720 LECTIN-BINDING TO ISOLATED CELLS FROM TURTLE RETINA. P. Vijay Sarthy*, C. David Bridges*, Francis L. Kretzer* and Dominic M.K. Lam. Cullen Eye Institute, Baylor College of Medicine, Houston, TX 77030.

The presence of specific saccharides on the surface of retinal cells was examined by reacting FITC-labeled lectins with cells dissociated from papain-treated retinas. The pattern and intensity of binding was found to vary among the cells examined. With Con A (α-mannopyranosides, α-glucopyranosides and oligomers of mannose) there was strong surface binding to both rods and cones, with an intense ring of fluorescence just above the nucleus. Bipolars also showed strong surface labeling with intensification on the soma and at the synaptic ending. The pattern seen with Muller cells was quite striking in that intense fluorescence was observed in the apical, microvillous region. In contrast, the horizontal cells were devoid of any surface label. These observations are in agreement with the binding of ferritin-Con A seen in sections of turtle retina. When Ricin-60 (N-acetyl galactosaminyl and β-galactosyl residues) or Ricin-120 (β-galactopyranosides) or wheat germ agglutinin (oligomers of β(1 → 4) linked N-acetyl glucosamine) were incubated with photoreceptors (devoid of outer segments), bipolars or horizontal cells, little or no fluorescence was visible. However, all the three lectins bound strongly to the apical portion of the Muller cells. Asparagus pea lectin (α-L-fucopyranosides) did not bind to any of the cells examined, although patches of amorphous fluorescent material were often seen. In all cases examined, lectin-binding was inhibited by the appropriate haptene sugar: Me-α-Man for Con A, GalNAc for RCA 60, Gal for RCA 120 and GlcNAc for WGA. Furthermore, prior treatment of cells with neuraminidase did not significantly alter lectin-binding to any cell type. While the specificity of lectin labeling must be viewed with caution, the abundance of lectin receptors on the apical surface of Muller cells suggests the presence of membrane glycoproteins or glycolipids containing mannose, galactose, N-acetylgalactosamine and N-acetylglucosamine residues. Some of these sugars are probably present in the terminal, non-reducing position. In contrast, the horizontal cells have few accessible receptors for the lectins tested, while Con A binding to the bipolars and photoreceptors suggests the presence of surface saccharides containing mannose residues.

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2721 PHYSIOLOGICAL PROPERTIES OF PHOTORECEPTORS IN THE COMPOUND EYE OF DAPHNIA MAGNA. Robert Schehr* and E. Macagno (SPON: E. Holtzman). Dept. Biol. Sciences, Columbia University, New York, N.Y. 10027.

Intracellular recordings have been made for the first time from the photoreceptors of the compound eye of the branchiopod crustacean Daphnia magna. These cells, 176 of which form 22 fused rhabdoms, are about 10μm in diameter and 30μm in length. Their axons project some 100μm to the optic ganglion, where they synapse onto 110 laminar neurons. These synaptic projections have been mapped in detail (PNAS 70: 57, 1973).

The photoreceptors have resting potentials in the range of -30 to -50 mV and slowly depolarize when illuminated. These depolarizations are composed of a transient phase and a maintained phase and are generally similar to other invertebrate photoreceptor potentials. Both the size and shape of the potential and its latency are strongly affected by the state of adaptation of the receptor and by the intensity of illumination. The transient phase is especially reduced in duration in the light-adapted state, revealing a spike-like response. There is, however, no evidence of impulse activity nor can the spike-like response be evoked by current injection in the cell body. Although Daphnia have been reported to show wavelength discrimination, our initial measurements of spectral sensitivity have detected only photoreceptors with a peak response in the blue.

2722 THE NATURE OF THE GENICULO-STRIATE INPUT TO THE SUPERIOR COLLICULUS. Peter H. Schiller*, Joseph G. Malpeli, and Stanley J. Schein (SPON: B.M. Dawson). Dept. Psyc., MIT, Cambridge, MA 02139 and Dept. Psyc., U. Ill., Champaign, Ill. 61820.

In the rhesus monkey the superior colliculus (SC) receives a visual input both directly from the retina and indirectly via the visual cortex. We have shown previously that inactivation of the indirect pathway by cooling visual cortex disrupts all visually driven activity in the SC except for the cells in the superficial laminae which receive the direct retinal input (Schiller, Stryker, Cynader, and Berman. J. Neurophysiol. 37: 181-194, 1974.)

The aim of this study was to determine to what extent the color-opponent (X) and the broad-band (Y) channels contribute to the indirect visual pathway to the SC. We reversibly inactivated these channels at the level of the lateral geniculate nucleus (LGN) by small (15-30 nanoliter) injections of 2% Lidocaine into either magnocellular or parvocellular LGN laminae while recording from single cells in topographically corresponding areas of the SC. The effects of these injections were directly compared with the effects of cooling visual cortex.

Our results were straightforward. Inactivation of magnocellular LGN laminae disrupted the activity of collicular cells the same way as did cortical cooling: superficial SC cells were unaffected while cells in the deeper laminae became unresponsive to visual stimulation. By contrast, inactivation of the parvocellular LGN laminae had no discernible effect on collicular cells.

These results suggest that in the monkey the indirect geniculostriate pathway to the superior colliculus is activated only by the broad-band (Y) system. (Supported by NSF grant #BNS 76-82543, NIH grants 5 R01 EY00676 and 5 F32 NS05799, and the Sloan Foundation grant #BR-1895).

2723 MOTOR SIGNIFICANCE OF VISUAL RESPONSES IN INTRALAMINAR THALAMUS OF CATS. J. Schlag and M. Schlag-Rey. Dept. Anat. and BRI, Sch. Med. UCLA, Los Angeles, CA 90024.

The saccade-related activity of most eye-movement (EM) neurons in the thalamic internal medullary lamina (IML) of alert cats is direction-specific. More than 90% of these cells also respond to visual stimuli. Thus, upon presentation of a target that produces a saccade in the preferred direction, they fire twice: first, when the target appears and, second, starting slightly before the targeting movement. The initial visual response and the EM discharge have the same direction specificity. This led to the hypothesis that the stimulus time-locked pattern of discharge already contains information about the anticipated motor response. The notion of "adequate motor response" in proposed as a counterpart to the classical notion of adequate stimulus, to characterize the conditions in which a given visual-oculomotor neuron would fire. To test this hypothesis, we sought to create experimental situations in which the predictions of responding would be different on the basis of stimulus spatial (receptive field) and on the basis of movement spatial parameters (direction of targeting). Fast, linearly moving targets were used for this purpose. Unable to seize the target at the site of its appearance on the screen, the animals eventually would try to capture it further along its trajectory. IML units were activated even in the absence of saccades; they respond to fast moving stimuli well before the latter penetrated into the receptive field as determined with stationary stimuli. The visual responsiveness was better accounted for in terms of direction of eventual saccades appropriate for catching up with the targets than in terms of receptive field. Distinguishing between sensory and motor neuronal events appears meaningless when, time-wise, such events are related to the sensory input while, information-wise, they are related to the motor output.

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2724 DISTRIBUTION OF NEURONS CONTAINING CYTOPLASMIC LAMINATED BODIES (CLB) IN THE dLGN OF MONOCULARLY DEPRIVED (MD) AND DARK REARED (DR) CATS. Marie Luise Schmidt. Union College, Schenectady, N.Y. 12308.

Large samples of cells of the cat's dLGN including the projection of the area centralis in lam.A, A1 and the monocular segment (MS) were examined for the presence of CLBs in paraffin sections. CLBs were stained with Luxol fast blue and nerve cells with Carbol fuchsin or Cresyl violet.

Considerable variation in the proportion of CLB-containing cells among nearby regions of the same dLGN as well as among corresponding regions from different normal cats had been described (Schmidt, Neurosc.Abstr. 1977, 576). The mean percentage ofCLB-containing neurons in four cats vary between 40-61% in lam.A(medial), 40-53% in lam. A1 and 36-47% in the MS.

Similar distributions were observed in four dLGNs of MD cats: lam.A(medial) 46-66%; lam.A1 42-53%; MS 33-53%. The proportion of CLB-containing neurons in lam. A1 of the individual dLGNs was either equal or lower than in lam. A(medial) regardless which lamina had input from the deprived eye. A high or low proportion of neurons containing CLBs in the MS corresponded to a high or low percentage of these cells in the medial portions of lam.A and A1 in the particular dLGN. The percentage of nerve cells containing CLBs in two dark reared animals are in the range found in normal animals: lam.A(medial) 62 and 58%; lam. A1 55 and 50%; MS 42 and 48%.

A very small percentage of neurons in the dLGN of normal cats contained two CLBs. As a group deprived animals had a higher percentage of these cells. There may be a true increase in the number of neurons containing two CLBs in visually deprived animals. However it is possible that cells containing two CLBs are observed more frequently in these cats since their nerve cells may be smaller due to deprivation.

These data do not indicate a significant change in the proportion of CLB-containing nerve cells in the dLGN after visual deprivation as would be expected when CLBs serve as markers for X-cells (LeVay & Ferster, J.Comp.Neur.,172(1977),563-584). However fewer Y-cells have been recorded in the dLGN of visually deprived cats. Three interpretations are possible: (1) CLBs are not found in either X- or Y-cells exclusively or(2) only a very small fraction of Y-cells are lost or (3) Y-cells are not lost due to visual deprivation but the probability of recording from these cells is decreased.

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2725 COLUMNAR PATTERN AND SPATIAL FILTERING IN VISUAL CORTEX: SEGMENTATION OF THE VISUAL SCENE BY COLOR,DEPTH AND TEXTURE Eric L. Schwartz, Brain Res. Dept. Psychiat. N.Y.U. Med. Ctr.

In recent work, it has been shown that several visual illusions (Mackay after-image,fortification illusion) may be explained in terms of the cortical representation of the visual field (Schwartz,1979). In previous work, it had been shown that the global retinotopic map, and the size of cortical ocular dominance columns, were sufficient to provide a prediction of rhesus monkey binocular disparity tuning (Schwartz,1977) which was subsequently verified (Schwartz,1979). The implication of this work is that neuronal trigger features such as binocular disparity tuning may be viewed as epi-phenomena of underlying anatomical structure. In the present paper, this work is extended to show that regular interlacing of discrete receptor input is sufficient to encode the difference map of the receptor mosaics as a spatial frequency modulated signal. Binocular disparity and color opponency are examples of visual sub-modalities which are represented by columnar structure at the cortex, which by definition are difference maps of discrete receptor systems, and which are known from psychophysical work to have differential spatial frequency sensitivity. It is shown by analytic calculation, and by digital and optical simulation, that columnar structure and spatial filtering are sufficient to provide segmentation (feature extraction) of the visual scene based on spatial texture of the cortical map, for color and depth channels.Visual texture may be extracted by means of lateral inhibition between spatial frequency channels, which is shown to provide spectral estimates of the higher order moments of the visual scene.Thus, the spatial pattern of cortical activity is capable of encoding color,depth,projected form, and texture without reference to "labeled lines", contingent only on the existence of columnar pattern and spatial frequency filtering. The existence of well defined neuronal trigger features is consistent with this analysis, and in fact, may be quantitatively derived. However, segmentation (feature extraction) may be conceptualized entirely in terms of spatio-temporal pattern, with no reference to the discrete cellular properties of the nervous system, beyond the receptor level. This provides a field theory of vision which is anatomically,physiologically, and psychophysically consistent.

Schwartz, E.L. Bio. Cybernetics 28 1-14 (1977)
Schwartz, E.L. Vision Research (1979)

2726 INTERHEMISPHERIC CONNECTIONS OF RETINOTOPICALLY DEFINED VISUAL CORTICAL AREAS IN THE CAT. Mark A. Segraves. Dept. Anat., Univ. of Pa., Phila., PA 19104.

The distribution of visual cortical cells whose axons pass through the corpus callosum was examined using HRP/o-dianisidine. In 2 cats, the posterior 2/3 of the callosum was cut and a cotton pledget soaked in 50% HRP placed between the cut ends of the callosum for a survival period of 48 hours. HRP labelled cells were found along the entire 17/18 border and the lateral half of area 19. A comparison with the visuotopic maps of Palmer, Rosenquist, and Tusa indicated that labelled cells in 17 were confined to within an estimated 5° from the vertical meridian (VM), cells in 18 to about 10° from VM, and about 20° from VM in 19. In contrast, extensive label was present in nearly all portions of lateral suprasylvian (LS) cortex, areas 20, and 21. Furthermore, callosal cells in these areas appeared to convey information from a more widespread portion of the visual field, out to as much as 40° from VM. Callosal cells at the 17/18 border and in 19 were primarily restricted to lower layer III (60%) and upper layer IV. Medium to large pyramids were found in both laminae and a few stellate cells were labelled in IV of 17 and 18. A small percentage of label in 19 consisted of pyramidal and fusiform cells in layers V and VI. In LS areas, medium to large pyramids in III still accounted for about 60% of labelled cells, but there was a decrease in the number of cells in IV and a substantial increase in the number of labelled cells in V and VI.

A number of small, combined HRP/tritiated leucine injections were made from a recording micropipette at electrophysiologically defined cortical injection sites. Injection sites included a range of receptive fields from loci on VM to about 40° from VM. We found that injections restricted to specific LS areas, area 20, and 21 always received the heaviest callosal input from the homotypic cortex of the contralateral side; thereby connecting visual field representations that were mirror symmetrical in the case of injection sites located away from VM. In addition, there was a convergence of projections to the injection site from other visual areas known to contain callosal cells in visual field representations that were mirror symmetrical to the receptive field of the injection site. (Supported by 1R01 EY02654 & 5T01 GM00281)

2727 VISUAL-VESTIBULAR INTERACTION IN HEMIDECORTICATE HUMANS. James A. Sharpe and Alex W. Lo*. Div. Neurology, University of Toronto, Toronto M5T 2S8, Canada.

Visual modulation of the vestibulo-ocular reflex (VOR) was quantitated in 5 subjects 8 to 12 years after cerebral hemidecortication for the control of epilepsy. The results were compared with data from 14 control subjects. Horizontal vestibular smooth eye movement velocity/head velocity gains were measured during passive sinusoidal whole body rotation at frequencies from 0.3 to 1.0 hertz.

While attempting fixation of a target moving with the head, hemidecorticate subjects had abnormally high gains of vestibular smooth eye movement contralateral to the side of cortical removal at each frequency. During attempted fixation of a stationary target, the VOR gain toward the side of cerebral damage was abnormally low at low rotational frequencies so that eye velocity failed to match head velocity. The impaired visual suppression of the contralateral VOR while fixating targets moving with the head and the defective augmentation of the ipsilateral VOR while fixating stationary targets were compensated by foveating saccades toward the side of cortical ablation. In darkness, VOR gains were horizontally symmetrical and normal.

With the subjects' heads immobilized to eliminate vestibular eye movements, smooth pursuit of 20° amplitude target motion was examined at 8 frequencies, from 0.125 to 2.0 hertz. During tracking toward the decorticate hemisphere smooth pursuit velocity/target velocity gains were subnormal at target frequencies over 0.125 hertz. During tracking away from the side of cerebral cortical ablation, gains exceeded unity at target frequencies up to 0.75 hertz. With the head immobile each subject had primary position jerk nystagmus toward the decorticate hemisphere. The nystagmus stopped in darkness.

Symmetry of the horizontal VOR in darkness and failure of the nystagmus slow phase velocity to increase in darkness implicated the horizontal pursuit system imbalance in the genesis of the fixation nystagmus. The unidirectional impairment of smooth pursuit correlated with the impaired visual augmentation of the ipsilateral VOR and with the impaired visual suppression of the contralateral VOR. These enduring manifestations of hemidecortication demonstrate roles of the cerebral hemisphere in visual-vestibular interaction.

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2728 AXONAL TRANSPORT OF LECTINS FROM CAT VISUAL CORTEX DEMONSTRATED WITH AN IMMUNO-PEROXIDASE METHOD. H. Sherk* and S. LeVay. Dept. of Neurobiology, Harvard Medical School, Boston, Mass. 02115.

Certain plant lectins, proteins with high affinities for specific carbohydrate groups, have recently been shown to be transported retrogradely with great efficiency by CNS neurons (Schwab et al, Brain Res. 152: 145-150, 1978.) An injection of one such lectin, wheatgerm agglutinin (WGA), labeled as many cells as a control injection of horseradish peroxidase (HRP) that was several 100 to 1000 fold more concentrated. These studies used autoradiography of ^{125}I-lectins. We have investigated whether axonally transported lectins can instead be visualized with an indirect immuno-peroxidase method.

Antibodies to WGA and concanavalin A (Con A) were raised in rabbits. 0.1 ul of a 2% solution of WGA was injected into the left visual cortex of a cat, and a similar amount of Con A into the right cortex. After 24 hours, the cat was perfused with 4% paraformaldehyde. Alternate frozen sections were incubated with rabbit anti-WGA and rabbit anti-Con A, followed by HRP-coupled goat anti-rabbit IgG. Sections were then reacted in a diaminobenzidine-H_2O_2 solution at pH 5.1.

The injection sites in the cortex and cells in the lateral geniculate nucleus (LGN) were clearly labeled using this method. Each injection site was labeled only by the appropriate antiserum. Compared to the results of equivalent injections of HRP, these injection sites were remarkably small, dense, and well-defined. Presumably the diffusion of these lectins is limited by their high affinity binding to cell surface sugars.

Sections of the thalamus incubated with anti-WGA showed labeled cells in the left LGN, while those incubated with anti-Con A showed labeled cells in the right LGN. Cells were limited to a small part of the nucleus, indicating that the effective injection site was indeed restricted to the dense region of reaction product visible with this method. Reaction product in these neurons took the form of dense cytoplasmic granules similar to those resulting from HRP transport. In addition, many neurons containing granular reaction product were visible in the vicinity of the cortical injection sites.

This method has two potential advantages over conventional HRP tracing techniques. First, the restricted nature of the injection site should facilitate the study of local connections. Second, it offers the possibility of demonstrating multiple projections from a single region by the injection of several different lectins in one animal. Compared with the autoradiography of ^{125}I-lectins, the immuno-peroxidase method is faster and, since the reaction product is contained within cells, allows them to be unequivocally identified throughout the section thickness. (Supported by 1 F32 EY 05296-01 and EYRO-1960.)

2729 THE ORGANIZATION OF VISUAL INPUTS TO THE INFERIOR OLIVARY COMPLEX. Barbara A. Smythe*, Joseph T. Weber, Michael F. Huerta and John K. Harting. (SPON: P.B. Schechter) Dept. of Anatomy, University of Wisconsin, Madison, WI 53706

We have used the horseradish peroxidase (HRP) tracing method to identify visually related cell groups which project upon the inferior olivary complex. Subsequent to the identification of these cell groups, autoradiographic tracing procedures were used to define the specific termination sites of these inputs.

In our studies we have used three mammals, ie; the rat, the tree shrew and the cat. Thus far, the results of our experiments reveal that four visually related nuclei project upon the inferior olive. These are: (1) the superior colliculus, (2) the pretectal complex, (3) the nucleus of Darkschewitsch, and (4) the interstitial nucleus of Cajal.

The tecto-olivary pathway arises primarily from cells within layer IV of the superior colliculus. This pathway is predominantly crossed, and terminates within the caudal medial accessory olive of all three mammals. In the cat, an identifiable ipsilateral projection is apparent which also terminates in the medial accessory olive.

The pretecto-olivary pathway has also been studied. This pathway is entirely uncrossed. In the tree shrew, HRP experiments reveal that only the nucleus of the optic tract (NTO) projects upon the inferior olive. In contrast, HRP experiments in the rat and the cat show that in addition to the NTO, the anterior and the posterior pretectal nuclei also project upon this region. As determined from the autoradiographic data, the pretecto-olivary pathway in all three mammals terminates within the dorsal cap of Kooy. This projection is especially dense in the tree shrew and the rat. Two additional pretectal olivary projections were observed in the rat and the cat, ie, one to the rostral portion of the dorsal accessory olive and one to the beta nucleus.

Our findings regarding the olivary projection zone(s) of the nuclei of Darkschswitsch and Cajal are as yet not complete. We can, however, report observations made following large injections of HRP placed within the inferior olivary complex of all three animals. The results of these experiments show that the inferior olive receives an ipsilateral input from the nucleus of Darkschewitsch. On the other hand, the pathway to the inferior olive from the interstitial nucleus of Cajal is predominately ipsilateral, however, a small contralateral component is present.

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2730 EFFECTS OF STRIATE CORTEX AND/OR SUPERIOR COLLICULI ABLATIONS ON ACCURATE REACHING BY MONKEYS. Stephen J. Solomon*, Tauba Pasik and Pedro Pasik. Dept. Neurol., Mount Sinai Sch. Med., CUNY, New York, N.Y. 10029.

Ten monkeys (M. mulatta) were trained on a spatial localization task before and after sequential total ablation of the striate cortices with partial damage to circumstriate cortices, and bilateral aspiration of the superior colliculi in balanced order. Lesions were histologically verified. The task, given under normal room illumination, required the animals to reach for a target randomly placed in one of 8 equal segments of a 46 cm diameter white disc located frontally at 20 cm from the monkey's eyes. An apple cube, affixed to the center of the target served as the reward for accurate reaching. A correct response consisted of an initial contact with either the target or the apple cube directly. The animal was trained sequentially to a criterion level of performance using black discs of decreasing diameter (90, 55, 35 and 15 mm). Finally only the apple cube of about 10 mm was presented.

Six of the monkeys with only bilateral striatectomies could be retrained to reach accurately for all targets but with marked deficits on the first test. In general, error scores were higher in the upper quadrants. No defect was noted on subsequent targets. An additional decrease in performance of similar magnitude occurred when the apple cube alone was presented. This time errors were greater in the lower quadrants. Four animals with bilateral colliculectomies performed just as accurately as before the lesion. Three of these subjects were operated in two unilateral stages. In one of them, there was a transient increase in errors to targets on the right side after initial removal of the left colliculus. This monkey, however, had also some accidental damage to the left occipital lobe.

The ablations were combined in six of the subjects. Four of these monkeys, with almost total collicular destruction and/or degeneration, failed to reach criterion in 6,000 trials. The presence of a minimal remnant on one side correlated with higher number of correct responses to contralateral target locations. The other two monkeys with as much as 60% of the collicular tissue destroyed showed a minimal deficit only in the first test.

Conclusions: Results confirmed previous studies which showed that monkeys with total bilateral striatectomy can reacquire the ability for visually guided accurate reaching. Moreover, the present findings indicate that the superior colliculi are dispensable structures for this capacity. They become critical, however, in the absence of striate cortex. These results together with previous findings do not support a sharp dichotomy between two visual systems in monkeys, one subserving discrimination capacities (cortex) and the other localization functions (colliculi). Aided by NIMH Grant #MH-02261.

2731 SUBCORTICAL PROJECTIONS TO THE PULVINAR-LATERALIS POSTERIOR COMPLEX IN THE CAT. R. Spreafico*, C. Kirk*, S. Franceschetti* and G. Avanzini*, Depts. of Anat. and Physiol., UNC, Chapel Hill, NC 27514 and Dept. of Physiol., Neurological Inst. "C. Besta", 20133 Milan, Italy. (SPON: R.L. Montgomery)

The Pul-LP complex is considered part of the extrageniculate visual system and receives its major ascending afferents from tectal and pretectal regions. Since discrepancies exist in the literature about which subdivisions of the pretectal area project to the Pulvinar Lateralis (Pul (lat)), the present study was undertaken to elucidate this issue and at the same time to verify other structures which also project to the Pul-LP complex in the cat. In adult cats under barbiturate anesthesia, 0.2 to 1.0 ul of 30% HRP were injected stereotaxically in the Pul-LP complex. After 24-48 hours all animals were perfused with mixed aldehydes. After overnight wash in 30% sucrose buffer, brains were cut frozen in 40 um thick sections and reacted with either 3,3' diaminobenzidine (DAB), 3,3', 5,5' tetramethylbenzidine (TMB; Mesulam 1978) or Hanker-Yates substrate (H-Y; Hanker et al., 1977). Labelled cells were charted with an X-Y plotter, and in some cases their distribution was reconstructed tridimensionally with the aid of a PDP11/40 computer. With DAB or TMB, neuronal perikarya were intensely labelled, and proximal dendrites were visualized as well; with H-Y, cells were generally less intensely labelled and sometimes difficult to detect. Labelled cells were found in all subdivisions of the pretectal area after injection in Pul(lat) although their largest number was in NTO and NPP (Kanaseki and Sprague, 1974). Throughout the pretectal area, fusiform and triangular neurons were identified in the HRP material. When impregnated by the Golgi technique, both types of neurons showed long dendritic branches with only sparse ramifications. The majority of labelled neurons after LP injection were found in the second layer throughout the entire antero-posterior extent of the superior colliculus (SC). After more restricted injections, computer reconstruction of serial sections demonstrated that the medial part of the SC projects to the posterior LP, while more lateral parts of the SC project to the antero-ventral LP. Neurons from intermediate collicular layers also project to this portion. The parabigeminal nucleus also projects to LP, and other projections to Pul(lat) arise from locus coeruleus, subcoeruleus, reticular formation, periaqueductal gray, and hypothalamus.

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2732 THE SPATIOTEMPORAL ORGANIZATION OF X-, Y-, AND W- CELLS. Alan Stein*, Walter H. Mullikin*, John K. Stevens. Depts. of Anatomy and Physiology, Univ. of Pennsylvania, Phila., Pa. 19104

Stevens and Gerstein have described two types of receptive fields (RFs) in cat retina: one group has a spatially homogeneous distribution of excitation and inhibition, and the second group has a spatially heterogeneous distribution. In contrast, many other investigators have described three basic groups: X-, Y-, and W-RFs. We have repeated the conventional tests for X-, Y-, and W-RFs and compared them directly to the heterogeneous/homogeneous characterization.

All units classified as X-cells by conventional methods had heterogeneous-RFs. Mean latency from the optic chiasm (OX) was 2.20 msec. (s.d.0.35,n=37). The velocity preference, using small bright bars, ranged from 0.5 to 64 deg/sec with the mode at 16 deg/sec. The spatial frequency at which these heterogeneous cells stopped their modulated response to drifted gratings was 1.94 c/deg or higher. Mean center size was 0.94 deg. (s.d.0.35, n=44). Cells with strong surrounds showed a clear null position to gratings.

All units classified as Y-cells had homogeneous-RFs. Mean latency from the OX was 1.16 msec. (s.d.0.23,n=54). Twenty-two of these cells could be activated from the superior colliculus (SC) and their OX latency was 1.12 msec. (s.d.0.18). When a bar 1 by 0.25 deg. was used as a stimulus, these cells had velocity preferences ranging from 32 to 256 deg/sec, with the mode at 128 deg/sec. The spatial frequency cutoff for modulated responses was 1.33 c/deg or below. Mean center size was 1.92 deg. (s.d.0.57,n=57). A null position could never be demonstrated in homogeneous-cells.

Units which were classified as W-cells had a mean latency of 3.47 msec. (s.d.0.80,n=33). Ten of these cells could be activated from the SC. The OX latency for this subgroup was 3.51 msec. (s.d.0.98). Mean center size was 1.24 deg. (s.d.0.65,n=33). These cells gave very poor responses to all but the slowest moving stimuli (0.5-1 deg/sec). Eleven of these cells had the same spatiotemporal organization as heterogeneous cells. The remaining 23 units had RFs which could be synthesized from standard heterogeneous and/or homogeneous components, and we call these hybrid RFs.

These data demonstrate that all X-cells have heterogeneous RFs; all Y-cells have homogeneous RFs; and all W-cells have heterogeneous or hybrid RFs. Most importantly, these data suggest that all retinal RFs can be constructed from a basic set of homogeneous and heterogeneous "building blocks". (Supported by NIH EY01832).

2733 SUPERIOR COLLICULUS INFLUENCES ON EYE MOVEMENT IN THE NEONATAL CAT. Barry E. Stein, H. Peter Clamann and Stephen J. Goldberg. Departments of Physiology and Anatomy, Medical College of Virginia, Richmond, Virginia 23298

The present experiments were initiated to (a) determine whether eye movements can be generated via the superior colliculus even before colliculus neurons are activated by visual stimuli (7 days of age) and (b) compare the movements to those evoked in adult cats. Twenty-six kittens, 2-77 days of age, and six adult cats were studied. A steel chamber was implanted over a cranial opening in each animal and a small mirror was glued to each anesthetized eye (the eyelids were surgically opened when necessary). Electrical stimulation of the superior colliculus consisted of 70 msec. trains of .1 msec. pulses at 200 pps. delivered through glass insulated tungsten microelectrodes. The electrodes were lowered to the colliculus through the visual cortex and eye movements were measured using the displacement of light beams which were reflected off each mirror onto a translucent hemisphere positioned in front of the animal. Adult cats exhibited many spontaneous conjugate saccadic movements, as well as some conjugate drifts of the eyes. Evoked eye movements, however, were always contraversive and conjugate. Neonates (2-12 days of age), on the other hand, showed many spontaneous slow drifts of the eyes and few ballistic-like movements. Surprisingly, the eyes often moved independently of one another spontaneously. With electrical stimulation, eye movements, as well as ear, neck and vibrissae movements, could be evoked in even the youngest animals studied. However, the eye movements elicited in kittens were of smaller amplitude, sometimes nonconjugate and required higher stimulus currents than those evoked in adult cats. Thus, in some cases only one eye moved or the eyes moved in different directions and/or had different amplitudes of displacement. Consequently, while eye movements can be generated by the superior colliculus in neonatal (and perhaps prenatal) kittens, this control system is not fully organized until after visual cells normally develop in the colliculus.

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2734 DISSECTION OF A NEURONAL CIRCADIAN OSCILLATOR SYSTEM IN THE EYE OF APLYSIA: INTRACELLULAR RECORDING FROM SINGLE DISCONNECTED PHOTORECEPTORS IN CELL CULTURE. Felix Strumwasser, D. P. Viele* and J. M. Scotese*. Division of Biology, California Institute of Technology, Pasadena, CA 91125.

The isolated eye of Aplysia californica produces a circadian rhythm (CR) in the frequency of optic nerve compound action potentials (CAPs), under conditions of total darkness, whose period is temperature compensated. The CR of CAPs is entrainable, in vitro, by light as well as chemical agents. Thus this eye contains all the cellular machinery for a temperature-compensated, entrainable circadian oscillator (CO).

In an attempt to dissect the CO further we have produced primary cultures of disconnected eye cells. We incubate eyes in a neutral protease (2.5% w/v) for 12 hr (15°C), followed by 7 hr (23°C) and gently triturate to dissociate cells. (After this enzyme treatment of intact eyes, the CR is not abolished.) Photoreceptors (PRs) and monopolar (MP) neurons are easily recognized. Our best cultures have yielded approximately 800 single PRs and 1000 MPs from a single eye. These cells as well as others attach to plastic culture dishes, produce webs and, in the case of MPs, grow long neurites in either modified MEM or L15 medium.

PRs can be recognized by a microvillous bush (rhabdomere) which marks the portion of the cell oriented toward the lens. Dimensions of selected PRs, from scanning electron microscopy (EM) of fixed, dehydrated specimens (n=4), reveal a cell body $\sim$ 30x14 µm with microvillous extensions ranging between 4-30 µm. Sections of PRs from cell culture viewed with transmission EM exhibit a normal morphology with three distinct parts, including a rhabdomere forming the apical portion, a cluster of pigment granules in the mid-portion, and a nucleus and a dense population of photic vesicles in the basal portion.

Stable intracellular recordings from isolated PRs have lasted 36-48 hr in exceptional cases but can normally be maintained for over 12 hr. PRs respond to light with graded depolarizations and do not produce action potentials. During the light response, there is a conductance increase, as measured by the steady state membrane voltage obtained in response to constant current pulses. Some preparations exhibit "spontaneous" quantum bumps whose frequency increase (up to $\sim$ 20/min) during dark adaptation and are suppressed after a light pulse. Light sensitivity in dark adapted cell cultures extends over at least 5 decades of intensity with $\sim$ 25 mV responses to 10^{-3} lux (5 second pulse). We find, using a programmer to scan the dynamic range of a PR, that light responses, after dark adaptation, are stable and have no circadian components thus eliminating the PR as a source of the CO in the eye of Aplysia. [Supported by NIH (NS 13896) and NASA (NSG-7387) grants.]

2735 VISUAL CORTICAL INPUT TO AREA 20 OF THE CAT: ANATOMICAL EVIDENCE FOR SUBDIVISION OF THIS REGION INTO TWO AREAS. Laura L. Symonds and Alan C. Rosenquist, Dept. of Anatomy, School of Medicine, University of Pennsylvania, Phila., PA. 19104.

Area 20 of the cat visual cortex was first defined by Heath and Jones on the basis of the patterns of connections with cortical and thalamic regions. Recently, our laboratory has electrophysiologically mapped this region of the visual cortex and subdivided it into two areas called 20a and 20b. We have shown that areas 20a and 20b are somewhat unique among other cortical areas because they receive projections from three separate subdivisions of the lateral posterior complex. We have studied the cortical connections of areas 20a and 20b to determine the degree of retinotopy, the pattern of cortical connectivity, and whether anatomical evidence could be found to support the division of this region into two functional areas. Our method was to make small combined horseradish peroxidase (HRP)/^{3}H-leucine injections from a recording micropipette at electrophysiologically defined regions of either 20a or 20b. The results reported here are based on the HRP data providing information about the cortical afferents to 20a and 20b. First, our results show that after injections into physiologically defined regions of 20a or 20b the labelled cells in other cortical areas are located only at similar retinotopic loci. Second, both areas 20a and 20b receive projections from many of the same cortical areas, including areas 17, 18, 19, 21b and 7. Third, areas 20a and 20b receive projections from different lateral suprasylvian visual areas. 20a receives input from PLLS and DLS on the lateral bank; 20b receives input from PMLS and VLS on the medial bank.

Our data thus provide additional evidence for the existence of two separate cortical visual areas within area 20 of the cat brain, confirming the electrophysiological findings. In addition, although areas 20a and 20b are regions which receive converging input from the posterior thalamus, they also receive input from separate cortical areas. (Supported by NIH Grant 1R01EY02654 and NIH Training Grant 5T32EY0703503).

2736 SIZE AND DISTRIBUTION OF RETINAL GANGLION CELLS PROJECTING TO THE RABBIT MEDIAL TERMINAL NUCLEUS. Ellen S. Takahashi, Clyde W. Oyster, John I. Simpson and Robert E. Soodak. School of Optometry/the Medical Center, University of Alabama in Birmingham, Birmingham, AL 35294 and Department of Physiology & Biophysics, New York University Medical Center, 550 First Avenue, New York, NY 10016.

Recent evidence from extracellular recording studies indicates that the medial terminal nucleus (MTN) of the rabbit diencephalon receives major, if not exclusive, inputs from a specific class of retinal ganglion cells, the on-type direction-selective cells. These ganglion cells have been selectively labeled by the retrograde transport of horseradish peroxidase (HRP) injected into the MTN. The number of labeled cells, their distribution over the retina, and their soma areas were determined. In one animal, in which the HRP injection completely filled the nucleus, some two thousand ganglion cells were labeled in the contralateral eye. This number agrees with previous estimates of the number of retinal axons terminating in the MTN. Unlike results in other species, none of the ganglion cells were displaced (Dogiel's cells). The density of labeled cells was highest in the visual streak and, overall, the distribution of labeled cells corresponded with the regional distribution of on-type direction-selective cells as previously determined in electrophysiological studies. Cells labeled by the HRP injection were among the 20% largest cells in the retina. This result, in conjunction with conclusions from other studies, leads to the prediction that on-type direction-selective cells can be characterized morphologically as having large cell bodies, very extensive dendritic spreads, and dendrites which ramify in the vitreal sublamina of the inner plexiform layer.

Although the ipsilateral retinal projection to the MTN in pigmented rabbit, as demonstrated by autoradiography, is considerably less dense than the contralateral projection, the number of labeled cells (seven) in the ipsilateral retina was lower than expected. This difference may reflect the relative sensitivities of the retrograde HRP method and the orthograde autoradiographic technique. (Supported by NIH Grants EY 00771 and EY 02207).

2737 MONOAMINE OXIDASE ACTIVITY IN RETINA—DISTRIBUTION AND DRUG INHIBITION. Thomas N. Thomas, David L. Sparks*, Neil S. Buckholtz, and John W. Zemp. Departments of Biochemistry and Psychiatry, Medical University of S.C., Charleston, S.C. 29403

Dopamine (DA) and serotonin (5-HT) are putative neurotransmitters of the retina.[1,2] Monoamine oxidase (MAO) catalyzes the enzymatic degradative deamination of these monoamines. Evidence points to the existence of at least two different forms of MAO (type A and B) having different substrate specificities and inhibitor sensitivities in the brain and other tissues. In brain, norepinephrine and 5-HT seem to be MAO-A specific whereas DA can be deaminated by both MAO-A and B. There is very little information available regarding the types of MAO and drug sensitivities of MAO in retina. This report deals with the distribution of MAO A and B in bovine retina and their inhibition by a number of drugs known to affect brain MAO activity. Fresh bovine retinas were homogenized in ice-cold 0.32 M sucrose and fractionated to give two synaptosomal fractions. One fraction (P_1) was enriched in photoreceptor terminals and the second fraction (P_2) contained synaptosomes derived from the inner plexiform layer. Type A MAO activity in these fractions was assayed using 1.5 μM [^{3}H]-serotonin as substrate and type B with 4 μM [^{14}C]-β-phenylethylamine. The distribution of MAO activity (nmoles/hr/μg protein) in retinal fractions is shown in the table below (mean ± SEM).

Fraction	Type A	Type B
Homogenate	0.67 ± 0.23	7.22 ± 0.21
P_1	0.79 ± 0.26	9.53 ± 1.11
P_2	2.29 ± 0.26	27.41 ± 0.84

The ratio of MAO-A to B activity is approximately equal in all fractions. Monoamines in retina have been shown to be localized in the inner plexiform layer and the P_2 fraction derived from this region contains most of the MAO activity. Therefore, the P_2 fraction was used to determine the IC_{50} values of a number of known MAO inhibitors. Pargyline and nialamide were of about equal potency in inhibiting both MAO A and B. 6-Methoxy-1,2,3,4-tetrahydro-β-carboline and clorgyline were more potent inhibitors of type A, whereas deprenyl was more effective in inhibiting type B. Similar actions of these drugs have been demonstrated on brain MAO except that, in brain, pargyline is a more potent MAO-B inhibitor. Drugs that inhibit MAO have proven useful in the treatment of depression and mild to moderate hypertension. Our observations raise the possibility that these drugs, by inhibiting retinal MAO activity, may alter retinal neurotransmission and possibly vision.

Supported in part by P.H.S. Grant MH26712 (N.S.B.) and South Carolina General Medical and Faculty Research Appropriation 1978-79 (T.N.T., N.S.B.).

1 Thomas, T.N. et al. Brain Res. 155 (1978) 391-396.
2 Thomas and Redburn Exp. Eye. Res. 28 (1979) 55-61.

2738 PHYSIOLOGICAL EFFECT OF UNEQUAL ALTERNATING MONOCULAR DEPRIVATION. David G. Tieman*, Maureen A. McCall*, and Helmut V.B. Hirsch (SPON: H. Ghiradella). Neurobiology Research Center, State University of New York at Albany, Albany, NY 12222.

Kittens reared with equal periods of normal monocular vision in each eye but no binocular vision (alternating monocular deprivation or AMD) show a reduction in the number of binocular units in the visual cortex and a loss of stereopsis. In this study we report that unequal stimulation of the two eyes of an AMD kitten produces a shift in the distribution of ocular dominance for cells recorded in the visual cortex (Area 17).

Four experimental kittens (AMD-8/1) were reared in the dark from birth to 4 weeks of age, after which they were brought out for daily periods of exposure with one eye occluded. One eye was exposed for 8 hr and the other for 1 hr on alternate days. In control kittens each eye was exposed for either 8 hr (AMD-8/8) or 1 hr (AMD-1/1) on alternate days. In control cats, most cells were monocular and the numbers of cells dominated by either eye were approximately equal. In contrast, in experimental kittens the cells were more likely to be dominated by the 8-hr eye (79%) than by the 1-hr eye (16%). The distribution of ocular dominance of the cortical cells, thus, resembles that for monocularly deprived cats. However, unlike cells driven by the deprived eye of a monocularly deprived cat, many cells driven by the 1-hr eye exhibited well-defined receptive fields and normal tuning for orientation. Some of the cells were excited through one eye and inhibited through the other.

Therefore, even though both eyes received repeated patterned input which is sufficient to maintain or induce normal properties for receptive fields of cells in Area 17, more cells are dominated by the eye which was exposed for a longer period of time. Behavioral tests of these AMD-8/1 cats showed the 1-hr eye to have a restricted visual field (Tumosa, S. Tieman and Hirsch, 1979). The behavioral and physiological results suggest that a difference in the duration of stimulation may place one eye at a competitive advantage (supported by Alfred P. Sloan Foundation Fellowship BR1677 and USPHS Grant RO1EY01268).

2739 LOSS OF GENICULOCORTICAL TERMINALS FROM DEPRIVED LAMINAE IN MONOCULARLY DEPRIVED CATS. Suzannah Bliss Tieman, Neurobiology Research Center, State University of New York at Albany, Albany NY 12222.

In monocularly deprived (MD) cats, few cells in the visual cortex respond to input from the deprived eye, while most cells in the lateral geniculate nucleus (lgn) respond relatively normally. This suggests that the connections to visual cortex from the deprived geniculate laminae may have been disrupted. I have examined these connections in MD cats using both light (LM) and electron (EM) microscopic autoradiography of visual cortex after injections of tritiated lysine into single laminae of lgn. The placement of isotope was guided by recording cellular activity with the same micropipette used for injection. At the LM level, I found a shrinkage of ocular dominance patches from the deprived laminae (Tieman, 1977; cf Stryker and Shatz, 1976; Shatz and Stryker, 1978). At the EM level, after injections into either deprived or experienced laminae, I have found label over terminals which contain mitochondria and round synaptic vesicles and which make asymmetric contacts with dendritic profiles. However, the percentage of grains lying over these terminals was much higher when the experienced lamina was injected than when the deprived lamina was injected. To correct for variations in injection size and for a probable reduction in protein synthesis by cells in the deprived laminae, I have computed the ratio of labeled terminals to labeled myelinated axons. When the experienced lamina was injected, slightly more than three terminals were labeled for every labeled myelinated axon, whereas when the deprived lamina was injected, fewer than two terminals were labeled for every myelinated axon. These results suggest that geniculocortical axons from deprived laminae have fewer terminals than geniculocortical axons from experienced laminae. This reduced number of terminals could help to explain the reduced effectiveness of the deprived eye in driving cortical cells (supported by 1 F22EY01277, NS11614 and RO1EY01268).

2740 VISUAL ACUITY IN CATS FOLLOWING SURGICAL ROTATION OF ONE OR BOTH EYES. Brian Timney* and Carol K. Peck. Psych. Dept. Univ. Western Ontario, London, Canada, N6A 5C2 and Pomona Coll., Claremont, CA 91711.

It has been observed that, following surgical rotation of an eye, cats show substantial evidence of visual functioning in the rotated eye (cf Mitchell et al. Exp. Brain Res. 1976, 25, 109-113; Peck et al. Exp. Brain Res. 1979, 34, 401-418). Visuomotor behaviour is adequate, the animals can learn simple pattern discriminations and they appear to be able to compensate for their rotated visual world. The present experiments measured the visual acuity of cats which had undergone surgical eye rotation. Rotations were accomplished by sectioning the extraocular muscles and repositioning the globe within the orbit. Acuity was assessed using the jumping stand technique Mitchell et al., 1976, 6, 181-193), in which the cats were trained to discriminate a high contrast grating pattern from a gray field matched for luminance.

One group of kittens underwent unilateral rotations of about 90° at approximately the time of natural eye opening. In all cases, when each eye was tested separately, the acuity of the rotated eye was significantly worse than that of the normal eye by a factor of 2 or more, even though visuomotor coordination through the rotated eye seemed quite adequate. Kittens which were given smaller degrees of rotation suffered correspondingly less severe deficits. A second group of kittens received unilateral rotations at 2 months of age or older. Those cats with very large rotations (around 90°) also showed severe deficits in the rotated eye and those with smaller rotations were less impaired. A final group underwent bilateral rotations of approximately equal magnitude. Although there was some indication that acuity was reduced in both eyes of these cats, there was little difference between the two eyes.

Control experiments, and, in some cases, anatomical and physiological observations, suggest that the deficits are not due primarily to physical disturbance of the visual pathways. It seems more likely that the reduction of vision is a form of ambylopia produced by discordant binocular stimulation.

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2741 THE EFFECT OF STIMULUS RATE AND STIMULUS PATTERN ON 14C-DEOXYGLUCOSE UTILIZATION IN THE VISUAL SYSTEM OF THE RAT. Arthur W. Toga, Simon Horenstein and Robert C. Collins, Dept. of Neurology, St. Louis Univ., and Dept. of Neurology, Wash. Univ. Med. Sch., St. Louis, MO 63110

The metabolic response to visual stimuli has been previously studied with the 14C-deoxyglucose autoradiographic technique (DG) in several species. There is still little known about the relationship between the site and intensity of metabolic response to changes in stimulus rate or stimulus pattern. We have studied these issues in albino rat.

The day prior to experiment, we enucleated the right eye and inserted an intravenous line. Animals were fasted overnight. To study the effect of stimulus rate, 4 to 6 animals each were assigned to one of six groups: total darkness, ambient light and full screen photo flash at 1, 4, 16.5 or 33/sec. White noise was used to mask auditory signals. During stimulation 14C-DG, 50 μCi/kg, was injected i.v. and animals were killed 45 minutes later, perfused-fixed, frozen, and brains cut at 20 μm thickness for autoradiography on Kodak SB-5 film. Since 90% of retinal fibers cross at the chiasm the visual system contralateral to the enucleated eye was used as a blind control. With visual stimuli the greatest changes in metabolism occurred in superior colliculus, with a 40% increase with either darkness or ambient light, and a linear 60 to 190% increase with full screen flash from 1 to 33/sec. By comparison the linear increase in geniculate was 50% as great and did not occur in visual cortex. Changes in metabolism were also studied during on/off pattern flash and pattern reversal (4 mm black/white squares) at 16.5/sec. There was only a small increase (10-20%) in metabolism during pattern reversal compared to total darkness control. During pattern flash there was 57% increase in superior colliculus, 27% in lateral geniculate and none in cortex. These changes did not differ compared to full screen flash at 16.5/sec when controlled for luminance flux. The results of this study provide evidence that different anatomical structures of the visual system have different metabolic requirements in processing visual information. The role of the superior colliculus in visual attention may be responsible for the greater increase in glucose utilization observed in this structure.

2742 EFFECTS OF BINOCULAR DEPRIVATION ON RESPONSES OF CELLS IN THE CAT'S LATERAL SUPRASYLVIAN VISUAL CORTEX. Lillian Tong*, Peter D. Spear, and Carol Sawyer*. Dept. of Psychology, U. Wisconsin, Madison, WI 53706.

Eight cats were raised with binocular lid suture (BD), and single cells were recorded in the binocular and monocular segments of the lateral suprasylvian visual area (LS area). Only about 25% of the cells could be driven by visual stimuli in BD cats, compared to 87% of the cells in normal cats. In addition, among 165 responsive cells that were studied in BD cats, 50% had diffuse, ill-defined receptive fields and responded best to whole-eye illumination. Cells with well-defined receptive fields generally responded better to stationary flashing stimuli (39%) than to stimulus movement (11%), and none of the cells were direction selective. Less than 5% of the cells had inhibitory receptive field surrounds. In contrast, 93.5% of the responsive cells in normal cats have well-defined receptive fields and over 80% are direction selective. In addition, nearly 40% of the cells in normal cats have inhibitory receptive field surrounds. Thus, binocular deprivation produces severe abnormalities in the response properties of LS area neurons. In addition, cells in the binocular segment showed somewhat abnormal ocular dominance. Only 48% of the cells could be driven by the ipsilateral eye in BD cats, compared to 70% of the cells in normal cats. There was no difference in the effects of BD on receptive field properties of cells in the monocular and binocular segments of LS cortex.

Many of the abnormalities observed in the LS area of BD cats resemble those that are present in normally reared cats after visual cortex (areas 17, 18, and 19) has been removed (e.g., loss of direction selectivity, increased response to stationary flashing stimuli, and reduced input from the ipsilateral eye), while others differ (e.g., loss of responsive cells, increase in cells lacking well-defined receptive fields, and loss of inhibitory surrounds). To investigate the extent to which the changes following BD are due to abnormal cortico-cortical inputs, five additional BD cats were studied after visual cortex had been removed. There was no difference between the results in these animals (69 responsive cells were studied) and those in BD cats with visual cortex intact. Thus, efferents from visual cortical areas 17, 18, and 19 have lost (or failed to develop) their influence on LS area neurons following rearing with binocular lid suture. In addition, the differences between LS area response properties in BD cats with visual cortex removed and those in normally reared cats with visual cortex removed indicate that binocular deprivation produces abnormalities in the thalamic-LS cortex pathway independent of the geniculo-striate system.

2743 PROJECTION OF THE SUPERIOR COLLICULUS UPON THE DORSAL LATERAL GENICULATE NUCLEUS IN THE CAT. F. Torrealba*, G.D. Partlow[1] and R.W. Guillery. Dept. Pharmacolog. & Physiolog. Sci., Univ. of Chicago, Chicago, IL 60637; [1]Dept. Biomed. Sci., Univ. Guelph, Guelph, Ont. N1G2W1, Canada.

The cat's dorsal lateral geniculate nucleus (LGN) is known to receive afferents from the superior colliculus (SC) as well as from the retina. It was our interest to study the precise relationship between these two pathways. Fink-Heimer (FH) and autoradiographic methods confirm that SC projects to the most ventral laminae in the LGN (Niimi, et al., 1970; Graybiel, 1972; Graham, 1977). The terminals extend broadly in the nucleus, from the most caudal regions to the anterior fourth of the nucleus, where the layers bend ventrally. This connection is in register with the known retinotopic organization of the LGN and of the SC. In addition, there is a thin band of terminals just medial to the medial interlaminar nucleus and also the more extensive thalamic projections described previously.

By combining an intraocular injection of radioactive proline on one side with a tectal lesion on the other side it has been possible to show that tectal connections go mainly to C_3 (a layer of small cells to which a retinal input has not been demonstrated) and that both retinal and tectal afferents may overlap in layer C_2. This overlap was not complete, but rather patchy, especially in the medial regions of LGN. In some cases at the level of the optic disc representation in LGN, there is a gap in the band of tectal terminals, but no evident rarefaction of cell bodies. This gap is wider than the one in the A and C layers.

In separate experiments, HRP was injected into the brachium of the SC, and this gave Golgi-like staining of tecto-LGN axons and terminals. This method and the FH show that these axons are of fine caliber, and travel dorsally in the optic tract (OT). They give off terminals near to the main branches, so that terminals from one axon occupy a thin leaflet about 100μ in the medio-lateral plane and less than 40μ in the dorso-ventral dimension. The endings can be linked in a short chain (boutons en passant); they may form small clusters of 3-5 swellings, or they may be individual swellings joined by a thin stem to a branch. Some of these axons end among small islands of 2-6 neurons in the OT, where the ventral part of C_3 is invaded by OT fibers.

EM studies of these HRP filled axons reveal that tectal terminals in the LGN are small in diameter, have round vesicles and form asymmetrical contacts on smooth dendrites.

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2744 QUANTITATIVE STUDIES OF VISUAL RECEPTIVE FIELDS IN FROG TECTUM. Evangelia Tzanakou*, Richard J. Michalak* and Erich Harth.
Physics Department, Syracuse University, Syracuse, NY 13210.

The receptive fields of retinal fibers and tectal cells in the frog have been re-examined in a quantitative way. The method used is computer controlled, and the stimuli are square spots of preselected size and luminance generated on a CRT screen. The positions of the spots were varied in a random manner in order to minimize systematic errors. The responses of the unit monitored are recorded extracellularly as a function of the spot location. Spot sizes could be selected conveniently subtending angles from about 1° to 15° at the frog's retina. In this way the available field represented by the CRT screen could be scanned at different resolutions, with the finest scan consisting of 1024 field elements. The response matrices are translated into intensity patterns, and the global responses to these patterns are recorded. In some cases the patterns were modified by use of clustering techniques while observing the resulting responses. Data will be presented of over 900 independent runs of about 70 cells. Most of the fields found were approximately circular with angular widths between 1° and 20°. Others were either elongated or showed irregular shapes. The majority of the cells showed a preference for dark stimuli on bright background rather than the opposite. When different resolutions were used, the results showed consistently that the responses of the different parts of the receptive field did not add linearly. Non-linear behavior and interactions between different areas of the receptive field were examined for some cells, by scans in which two spots were presented simultaneously with randomly changing locations. The temporal characteristics of the responses were also examined; successive runs show that good stability and reproducibility can be achieved over long periods of time.

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2745 THREE CORTICAL PROJECTION FIELDS OF AREA 17 IN THE RHESUS MONKEY. Leslie G. Ungerleider and Mortimer Mishkin. Lab. Sensorimotor Research, NEI and Lab. Neuropsychology, NIMH, Bethesda, MD 20205.

Although the projections of lateral striate cortex, the part representing central vision, have been extensively studied in the rhesus monkey, little information exists regarding the projections of posterior and medial striate cortex, parts representing peripheral and far peripheral vision, respectively. We therefore investigated the cortical efferents from all parts of area 17, with the aim of defining the locus, extent, and topographic organization of the entire striate-prestriate projection system.

One series of monkeys (Macaca mulatta) was prepared with unilateral lesions of lateral, posterior, or medial striate cortex, such that collectively the lesions included all of area 17 with little or no invasion of area 18; their brains were processed for terminal degeneration by the Fink-Heimer procedure. In a second series, selected striate sites were injected with tritiated amino acids, and the brains processed for autoradiography; representations of the injection sites ranged from 4° to 25° from fixation in either the upper or lower visual field. The results indicate that striate cortex projects to at least three separate and topographically organized visual areas within prestriate cortex.

The largest projection field is a circumstriate cortical belt which corresponds remarkably closely to area OB of von Bonin and Bailey. It completely surrounds area 17 along the 17-18 border except at the representation of fixation. Within this visual area, the representations of the upper and lower visual fields are entirely separate. Progression from central to far peripheral vision is represented: a) in the lower field, by a progression into the posterior bank and depth of the lunate sulcus, medially along the surface of the buried annectent gyrus into the parieto-occipital sulcus, and then rostrally along the upper lip of the calcarine fissure; b) in the upper field, by a progression into the inferior occipital sulcus, ventromedially into the occipitotemporal and collateral sulci, and then rostrally along the lower lip of the calcarine fissure.

A second, smaller projection field is located in area OA along the caudal portion of the superior temporal sulcus; here, progression from central to far peripheral vision is represented by a progression down the posterior bank of the sulcus and continuing along its floor (Ungerleider & Mishkin, Anat.Rec. 190:568,1978).

Finally, a third, even smaller projection field, also located in area OA, begins in the anterolateral part of the annectent gyrus and extends forward to occupy the depth of the lateral bank of the intraparietal sulcus. Despite its small size, evidence of a complicated topographic organization within this field raises the possibility that it may contain more than one visuotopic map.

We thank Dr. E.G. Jones for assistance with the autoradiography.

2746 EVIDENCE FOR ADDITIONAL EXTRASTRIATE CORTICAL PROJECTIONS TO THE DORSAL LATERAL GENICULATE NUCLEUS IN THE CAT. B. V. Updyke. Department of Anatomy, Louisiana State University Medical Center, New Orleans, Louisiana 70112

Substantial cortical projections onto the dorsal lateral geniculate nucleus arise from visual areas 17, 18, and 19 in the cat. Areas 17 and 18 project onto all of the laminae and the intralaminar zones of the nucleus, and area 19 projects to the parvocellular C laminae (Updyke, J. Comp. Neur., 163: 377, 1975). These connections appear to reciprocate the known projections originating from the geniculate laminae (Gilbert and Kelly, J. Comp. Neur., 163: 81, 1975).

In the course of investigating the projections arising from lateral suprasylvian areas, it was noted that certain of these areas also project onto the dorsal lateral geniculate nucleus. In these experiments, individual cortical areas - as defined by Tusa et al. (Soc. for Neurosci. Absts., 1: 52, 1975) - were identified by electrophysiological mapping, and then injected with tritiated proline. Projections onto the thalamus were identified autoradiographically.

No projections to the dorsal lateral geniculate nucleus were evident from areas ALLS, PLLS, and DLS occupying the lateral bank of the suprasylvian sulcus, or from area AMLS on the medial bank of the sulcus.

Areas PMLS on the medial wall of the suprasylvian sulcus, and area 21a on the crown of the posterior suprasylvian gyrus do project sparsely onto the parvocellular C laminae of the dorsal lateral geniculate nucleus. In addition, sparse terminal labeling of these laminae occurs after injections made more posteriorly on the suprasylvian gyrus. Because of the complex juxtaposition of areas 20a, 20b, VLS, and 21b in this region, it has not yet been possible to unequivocally identify the exact source of these corticogeniculate fibers.

The results indicate that a strip of the lateral suprasylvian cortex extending from the medial wall of the middle suprasylvian sulcus over the surface of the posterior suprasylvian gyrus contributes a sparse projection to the parvocellular C laminae of the dorsal lateral geniculate nucleus. These observations suggest that a strict reciprocity of connections may not be an essential feature of corticogeniculate projections in the cat.

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2747 AREAL BOUNDARIES AND TOPOGRAPHIC ORGANIZATION OF VISUAL AREAS V2 AND V3 IN THE MACAQUE MONKEY. D. C. Van Essen, J.H.R. Maunsell* and J. L. Bixby*. Division of Biology, California Institute of Technology, Pasadena, CA 91125

The organization of areas V2 and V3 in the macaque was studied by analyzing cortical myeloarchitecture, projections from striate cortex (V1), and interhemispheric connections. Results were displayed on two-dimensional cortical maps that permit accurate measurements of spatial relationships within the cortex. 1) All of V1 projects to V2, but only dorsal V1, representing inferior visual fields, projects to V3. Thus, V3 may contain an incomplete representation of the visual field and be restricted to dorsal extrastriate cortex; alternatively, it may extend further but have a highly asymmetric pattern of inputs. 2) We found no evidence for projections from foveal V1 to V4, as claimed by Zeki (J. Physiol. 277, 1978). 3) V2 is 10-15 mm wide along much of its extent, except near the representations of the far periphery (medially) and the fovea (laterally). In the region of far peripheral representation, near the anterior tip of the calcarine sulcus, there is a complete gap of about 1 cm between dorsal and ventral subdivisions of V2; the intervening strip of cortex (the prostriate area of Sanides) is characterized by its distinctive architecture and by an absence of interhemispheric connections along the border with V2. Laterally, near the foveal representation, there is a distinct transition in myeloarchitecture at the border between V2 and V4. The minimum width of V2 in this region varies from 2 mm in one case to less than 1 mm (with a possibility of a complete gap) in two other cases we have examined. 4) The detailed organization of inputs to V2 was examined by making lesions and ^{3}H-proline injections focally (up to several mm in extent) within V1. As reported by others, dorsal V1 projects to dorsal V2, ventral V1 to ventral V2, and the horizontal meridian representation of V1 to both subdivisions of V2. Within each V2 subdivision, however, the pattern of inputs from V1 is variable. In some cases the projection terminates as a single patch 2-4 mm across, but in other cases there are two distinct patches, each 2-3 mm across and separated by a gap of 0.5-1 mm (cf. Weller et al., Arvo Abstr., 1979). Moreover, the inputs to individual patches are arranged as irregular clusters that may reflect a specific pattern of organization at a sub-millimeter level. Altogether, these experiments indicate several significant differences in the organization and connections of V2, V3 and V4 compared to previous descriptions.

2748 SELECTIVE ATTENTION IN MIRROR IMAGE DISCRIMINATION BY MONKEYS. Betty A. Vermeire* and Charles R. Hamilton, Division of Biology, California Institute of Technology, Pasadena, CA 91125.

Normal animals often find left-right mirror images (LR) harder to discriminate than up-down mirror images (UD). One popular interpretation ascribes the difficulty to confusion between the normal topographic representation in the cortex and a duplicate but reversed projection through the interhemispheric commissures. In support of this, optic chiasm sectioned monkeys taught to discriminate LR monocularly, reversed their performance when tested through the other eye. This paradoxical transfer, however, may be interpreted as a selective attention to cues on the side of the stimulus contralateral to the eye being tested, which occurs because of the temporal hemianopia present when using each eye. Selective attention caused by stimulus masking successfully predicts the otherwise uninterpretable result that split-chiasm monkeys find LR easier to learn than UD.

A more generalized concept of selective attention to stimulus cues could interpret confusion of left and right in normal animals. For example, animals could code stimulus orientation relative to asymmetries in the world or they could attend to cues near the place of response, often at the top or bottom of the stimuli. To see if an obvious asymmetry or place of response is a significant factor, split-chiasm monkeys were trained monocularly on UD or LR in a WGTA equipped with a vertical panel containing planimetric patterns and a peg for responding located near one edge of the patterns. With the peg aligned with the axis of symmetry of the stimuli, discriminations were easier than with an orthogonal placement, as predicted by selective attention. In addition, because of the split chiasm, LR were learned more easily than UD, as previously found. In the aligned peg condition interocular transfer tested with the peg position changed 180° led to reversed transfer of both UD and LR. Testing LR showed, in addition, increased magnitude of reversed transfer associated with masking by the retinal scotoma. Reversal associated with manipulating the place of response was half the magnitude of that associated with masking. A similar experiment with normal monkeys showed that they, too, reversed their performance on LR and UD discriminations when the place of response was reversed. Thus it appears that attention to cues near the place of response, or coding orientation relative to asymmetries in the world, can occur during discrimination of mirror images. Therefore in conjunction with the prominent up-down asymmetries in the world and the tendency for animals to respond to the upper or lower edges of test stimuli, mechanisms of selective attention can explain confusion of left and right by normal animals.

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2749 POTASSIUM RELEASE AND CURRENT FLOW IN THE FROG RETINA: A TEST OF THE MÜLLER CELL HYPOTHESIS OF ELECTRORETINOGRAM (ERG) B-WAVE GENERATION. David A. Vogel and Daniel G. Green. University of Michigan.

The electroretinogram is an electrical potential that develops across the retina in response to a flash of light. One of the components of the ERG, the b-wave, has been used by many investigators as a measure of responsiveness of the retina. Despite its common use, the exact origin of the b-wave remains unknown.

It has been postulated that extracellular accumulations of potassium ions may act to locally depolarize the membrane of the Müller cell. This depolarization should then be the site of the sink for the extracellular current flow which causes the b-wave. A strong test of the Müller model is to compare the distributions and the time courses of development of the known K+ increase and the b-wave current sources and sinks.

The light adapted eyecup preparation from Rana pipiens was used for this experiment. Local ERG's and K+ concentration were measured simultaneously using a double barrel microelectrode. The extracellular sources and sinks were calculated by computer.

The prediction from the Müller cell model is that the ERG current sinks should occur at the same locations as the K+ increases. Further, the time courses of the development of the K+ increase and the ERG current sinks should be identical. It is quite apparent from the results that the profiles of the K+ increase and the b-wave sinks are not identical. The potassium profile has only one peak which occurs at a depth of 60μ and 1.5 seconds after the light was presented. The source/sink surface has two peaks, the proximal one being somewhat larger than the distal one. The time courses of development of the two profiles are also dissimilar.

The above findings are a direct contradiction to the results predicted by the Müller cell hypothesis. Therefore, if the Müller cell plays any role in b-wave generation, it must be in conjunction with other retinal cells.

2750 THE EFFERENT CONNECTIONS OF THE PRETECTAL COMPLEX OF THE TREE SHREW (TUPAIA GLIS). Joseph T. Weber, Michael F. Huerta and John K. Harting. Dept. of Anatomy, Univ. of Wisconsin, Madison, WI 53076.

The efferent connections of the pretectal complex of the tree shrew (Tupaia glis) have been analyzed using anterograde (autoradiography) and retrograde (horseradish peroxidase) tracing techniques.

The autoradiographic data reveal that the targets of the pretectal complex can be grouped into several categories. First, there are ipsilateral, descending pathways which terminate within the dorsal cap of Kooy of the inferior olivary complex, the dorsolateral and dorsomedial griseum pontis, the mesencephalic reticular formation dorsal and lateral to the red nucleus, the medial terminal nucleus, and the superficial layers of the superior colliculus. Rostrally located targets include the ipsilateral lateral and reticular nuclei of the thalamus, the zona incerta, and the central lateral and paracentral intralaminar nuclei. A bilateral projection to the ventral lateral geniculate nucleus is also present. A third category of targets includes nuclei which are closely associated with cranial nerve nuclei related to eye movements. Thus, fibers arising from the pretectum terminate ipsilaterally within the nucleus of Darkschewitsch, bilaterally within the nucleus of the posterior commissure and the interstitial nucleus of Cajal, and contralaterally with the somatic cell column of the oculomotor and trochlear nuclei. There are also commissural projections to contralateral pretectal cell groups.

Injections of horseradish peroxidase (HRP) were placed within eleven pretectal targets. These data confirm and extend our autoradiographic findings. For example, they show that (1) most pretectal targets receive input from several pretectal nuclei, and (2) the size of pretectal neurons, rather than their location with the pretectum, indicates the target of their axons, i.e., the cells of origin of descending pretectofugal pathways are larger than the cells of origin of ascending pretectofugal pathways.

Our findings reveal several important points regarding overall pretectal connectivity. First, the pretectum does not project directly upon the cells of origin of the preganglionic parasympathetic outflow involved in the pupillary light reflex. The pretectum does, however, project upon the nucleus of the posterior commissure, which in turn sends a projection to the visceral cell column of the oculomotor complex. Second, the pretectum has direct connections with several precerebellar relay nuclei. Finally, the pretectum projects upon a region of the dorsal thalamus, called the lateral nucleus, which others have shown projects upon visually associated cortical regions.

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2751 C57BL/6J-c^{2J} MOUSE OPTIC NERVE: ELECTRON MICROGRAPHIC AXON COUNT. I. S. Westenberg. Neurobio., Barrow Neur. Inst., Phoenix, AZ 85013.

Baseline data on optic nerve axonal composition are fundamental for studies on visual systems, e.g., the effects of neurological mutants. The inbred C57BL/6J mouse strain is valuable in this area, because of the availability of various neurological mutants with normal littermate controls that are otherwise genetically identical, e.g., albino and black C57BL/6j-c^{2J} mice. To resolve small axons in mouse optic nerve, electron microscopy is required.

In early estimates, C57BL/6J mouse optic nerve axon counts ranged to over 60,000; axon densities ranged to 1/μm^2. However, the axon count and density were estimated recently to be lower by as much as a factor of 4. The goal of the present study was to resolve the discrepancy between the earlier, higher estimates and the later, lower estimates.

The left optic nerve of a black, 111-day-old, male C57BL/6J-c^{2J} mouse was fixed (aldehyde), stained (osmium tetroxide), dehydrated (ethanol) and embedded (Epon 812). A thin whole-nerve cross section was stained (uranyl acetate, lead citrate) on a Formvar-coated slot grid. A photomontage of the section was made from serial electron micrographs. Axons were counted in 25 sample areas (each representing 10μm X 10μm, magnification = 3,750 X) evenly distributed across the entire section.

The section's shape was round-to-oval. Its longest diameter was 400μm; its shortest diameter was 325μm. The section's area was about 100,000μm^2. Thus, the total sample area of 2,500μm^2 (25 samples, 100μm^2 each) represented about 2.5% of the section. The total number of axons counted in this small sample was 2,650. This leads to an estimated axon count on the order of 100,000, with a density of about 1/μm^2.

The present data suggest that the earlier, higher estimates of axon count and density were more accurate than the recent, lower estimates. Further, the present data suggest that even the earlier estimates, though high, were conservative. This conclusion is supported by data from a recent complete axon count, albeit in a different mouse strain. On the other hand, the data from the complete axon count suggest that the present estimated axon count may be too high.

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2752 DISSECTION OF A NEURONAL CIRCADIAN OSCILLATOR SYSTEM IN THE EYE OF APLYSIA WITH X-RAYS. John C. Woolum* and Felix Strumwasser. (SPON: B. Peretz). Division of Biology, California Institute of Technology, Pasadena, CA 91125.

The rhythm of compound action potentials (CAPs) from the isolated eye of Aplysia has been shown to exhibit all the properties of a classical circadian oscillator. The number, location and mechanism of the oscillator(s) are not as yet known. We have used X-rays to "dissect" the oscillator function of the eye from its other functions. Irradiation of the eye with 50 kvp X-rays at a dose of 40 krad will stop the expression of the circadian oscillation of CAPs. However, the size and shape of the CAPs, the CAP average rate and bursting rhythm, and the response to light are not changed at this dose rate. These results indicate that the circadian oscillator mechanism is unlikely to be associated with the actual generation of the CAPs, the bursting pattern, or the light response mechanisms. Irradiation at lower doses (e.g., 16 kr) allows a circadian rhythm of smaller amplitude to be expressed than in the unirradiated control eye. This result seems to imply that there are probably a number of oscillators in the eye. At large doses all oscillators are inactive but at lower doses some oscillators are active so a rhythm of smaller amplitude is observed. Results of irradiating parts of the eye by using lead shields reveal that irradiation of the anterior (corneal) part of the eye decreases the amplitude of the rhythm slightly while irradiation of the posterior (optic nerve) part decreases the rhythm greatly (perhaps totally) implying the largest concentration of oscillators is in the posterior part of the eye. Of the 82 eyes irradiated with doses between 2 and 500 kr, none showed any sign of an ultradian rhythm--as previously reported in eyes with the front part cut away (Jacklet and Geronimo, 1971). This X-ray "dissecting" technique appears to be superior to the technique of cutting away parts of the eye as far as separating the oscillator function of the eye from its other functions. These X-ray results are similar to the Actinomycin D effects in blocking the circadian rhythm (Rothman and Strumwasser, 1977) and imply that RNA transcription may be important in generating the CR. [This work was supported by NIH grant NS-07071 to F.S.]

2753 FUNCTIONAL ORGANIZATION AND LAYERING OF VISUAL INPUT TO THE OPTIC TECTUM OF AMBYSTOMA. Daniel F. Wunk* (SPON: L. Aulsebrook). Vanderbilt University, Nashville, TN 37232.

Visual input to the optic tectum of the larval Tiger salamander, Ambystoma tigrinum, is being studied using field potential analysis, extracellular unit recording, and intracellular recording from postsynaptic tectal neurons. In response to optic nerve shock, surface-negative field potential waves are recorded which reverse polarity deeper in the tectum. Laminar current source-density analysis of these waves indicates the existence of three major current sinks located approximately 150, 75, and 50 μm. below the tectal surface, and occurring 4-7, 7-10, and 30-50 msec., respectively, after optic nerve shock. These depths correspond to layers of coarse terminal degeneration following enucleation (Riss & Jakway, Brain Beh. Evol., 5; 401, 1972). Current sources are located adjacent to the sinks. Since very few cell bodies are located in this region of the tectum, these results suggest that the retinal fibers make mostly excitatory synapses onto the distal dendrites of deeper-lying tectal cells.

Visual stimulation using flashed spots of light also reveals a functional layering of retinal afferents in the superficial tectal neuropil. ON-OFF and OFF unit responses are localized to the same depths as the two deeper current sinks evoked by optic nerve shock. On-going experiments are attempting to associate visual afferent units with the most superficial and slowest current sink. These responses are also being correlated with postsynaptic potentials elicited by similar stimuli and recorded intracellularly in deep tectal cells that are being recovered after dye injection. The presence in most cells of inhibitory postsynaptic potentials of a transient ON-OFF nature and large visual receptive field indicate that the ON-OFF retinal afferents participate, either directly or indirectly, in a global tectal inhibitory system.

2754 THE GABA SYSTEM IN GOLDFISH RETINA: A COMPARATIVE ANALYSIS WITH ^{3}H-GABA AND ^{3}H-MUSCIMOL: Stephen Yazulla* and N. Brecha (SPON: P. Witkovsky). Depts. of Biology and Psychiatry, SUNY, Stony Brook, NY 11794.

Muscimol, a GABA analogue with a reported low affinity for GABA uptake sites, was used initially to study synaptic binding to GABA receptors in goldfish retina. In retinal homogenates ^{3}H-muscimol (M*) shows saturable binding with a Kd of 10^{-8}M, a receptor concentration of 160 fM/mg-protein and is blocked by GABA at an IC_{50} of $4x10^{-7}$M. Localization of M* labeling by autoradiography shows heavy label over 4 distinct lamina in the inner plexiform layer (IPL) and over a population of amacrine cell bodies and lightly over cone horizontal cell bodies (HC). GABA (1mM) abolished M* labeling over HCs and the proximal IPL but had little inhibitory effect on amacrine cells and the distal IPL. Since the M* labelling pattern appeared partially due to uptake and was differentially inhibited by GABA, we performed parallel incubations in M* and ^{3}H-GABA (G*) under a variety of conditions designed to block uptake mechanisms. The normal G* pattern differs from that of M* and includes intense label over cone HC bodies and axon terminals (HAT), some amacrine cells and in the proximal IPL rather than throughout. Incubation in the presence of 1mM muscimol does not inhibit G* uptake. The specific GABA-uptake blocker DABA (0.2mM) slightly suppresses M* label in the IPL and strongly inhibits G* label in the proximal IPL while sparing the remaining patterns. Incubation in Na^{+}-free Ringers or in the presence of 0.4mM oubain eliminates somatic label of M* leaving a reduced but obvious pattern in the IPL. Oubain similarly affects the G* pattern, while in Na^{+}-free Ringers, considerable G* uptake is seen in amacrine cells and the distal IPL (i.e., resembles the GABA block on M*). From these results we conclude that the binding of M* at GABA synaptic receptors can be measured biochemically in the retina. Localization of M* binding by ARG is severely limited by uptake contamination, although with proper controls M* binding is seen in the IPL but not in the OPL. There are at least 3 "species" of GABA uptake sites: 1. HC and proximal IPL, 2. amacrine cells and distal IPL, and 3. HAT. Finally there is some, as yet undetermined, relation between M* labeling and Na^{+}-independent GABA uptake. These results are not unique to goldfish but have been duplicated to a large extent in chick retina.

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2755 PUPILLARY RESPONSES REVEALING RECEPTOR CHARACTERISTICS IN WILD-TYPE AND MUTANT DROSOPHILA. H. Zuidervaart*, D. G. Stavenga*, W. S. Stark 1 and G. D. Bernard*2. Dept. of Biophysics, Rijksuniversiteit, Groningen, the Netherlands (1 Johns Hopkins University; 2 Yale University).

The pupil mechanism in flies is a migration of intra-retinula-cell pigment granules elicited by a change in illumination. We studied the pupillary response by measuring the reflection in the deep pseudopupil which is maximal around 550 nm in Drosophila (see Franceschini, Thesis, Grenoble, 1972, CNRS (Paris) #A03802).

We determined intensity and spectral responses in wild-type flies. Univariance was shown since pupillary responses as a function of intensity were parallel across wavelengths. The dynamic range from threshold to saturation is 10^{10}-10^{13} quanta/cm^{2}s at 500 nm. The action spectrum for a criterion reflection increase is the same as the electrophysiological sensitivity of the predominant photoreceptor type, R1-6; the curve is like one obtained by measures of antidromic transmission in the deep pseudopupil of the white-apricot (w^a) mutant (Franceschini, ibid.)

Bright short wavelength irradiation induces a post-stimulus Prolonged Pupillary Response (PPR) which is very long-lived (several hours). This is like the Prolonged Depolarizing Afterpotential (PDA) induced after intense blue light substantially converts the 480 nm absorbing rhodopsin to its 580 nm absorbing stable metarhodopsin. The PPR is extreme at $\lambda<530$ nm and is absent at $\lambda\geq550$ nm. The PPR becomes noticeable after delivering $I\cdot t \cong 8x10^{13}$ quanta/cm^2 and saturates around $I\cdot t = 2x10^{15}$ quanta/cm^2 (λ=441 nm). Both PPR induction and suppression are determined by the total number of quanta delivered. Thus, the PPR is an integrating phenomenon as is the PDA. The PPR is eliminated by vitamin A deprivation as is the PDA.

The mutant transient receptor potential (trp, discovered by Cosens and Manning, Nature 224, 285, 1969) exhibits a transient pupillary response upon light adaptation. The full response is reached only after a long dark adaptation time (5-10 min.). This is slightly longer than the several minutes for receptor potential recovery in trp (Minke, Biophys. Struc. Mech. 3, 59, 1977). The PPR induced by blue light in trp is only a partially closed pupil in accordance with trp's receptor potential's PDA. Long wavelength light is necessary to renew the pupil's excitability (after a dark adaptation period). The sensitivity of the trp pupil has the same dynamic range as the wild-type pupil (after long wavelength illumination then dark adaptation).

Pupil and receptor potential mechanisms in Drosophila have very similar characteristics. Thus, with the pupil, one can actually witness manifestations of electrophysiological events using non-invasive optical techniques.